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EDITORIAL

Potential applications of 7 Tesla magnetic resonance imaging in paediatric neuroimaging: Feasibility and challenges

Arosh S Perera Molligoda Arachchige, Letterio S Politi

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Abstract

The integration of 7 Tesla magnetic resonance imaging (7 T MRI) in adult patients has marked a revolutionary stride in radiology. In this article we explore the feasibility of 7 T MRI in paediatric practice, emphasizing its feasibility, applications, challenges, and safety considerations. The heightened resolution and tissue contrast of 7 T MRI offer unprecedented diagnostic accuracy, particularly in neuroimaging. Applications range from neuro-oncology to neonatal brain imaging, showcasing its efficacy in detecting subtle structural abnormalities and providing enhanced insights into neurological conditions. Despite the promise, challenges such as high cost, discomfort, and safety concerns necessitate careful consideration. Research suggests that, with precautions, 7 T MRI is feasible in paediatrics, yet ongoing studies and safety assessments are imperative.

Key Words: 7 Tesla magnetic resonance imaging; Pediatric imaging; Feasibility; Challenges

Core Tip: 7 Tesla magnetic resonance imaging has the potential to revolutionize paediatric neuroimaging, offering unparalleled clarity and precision given that the necessary safety precautions and challenges are addressed. Its heightened sensitivity reveals subtle structural abnormalities, aids in epilepsy diagnosis, and provides deeper insights into conditions like neuro-oncology. Harnessing the power of ultra-high-field imaging may provide access to a new era of diagnostic accuracy and therapeutic planning for paediatric patients.

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INTRODUCTION

In the ever-evolving landscape of medical imaging, the integration of cutting-edge technologies holds immense promise for advancing diagnostic precision and patient care. One such groundbreaking innovation is the 7 Tesla magnetic resonance imaging (7 T MRI) which received United States Food and Drug Administration (FDA) and European Medicines Agency approval for clinical use in 2017[1]. In particular, the FDA has approved 7T MRI in infants of 1 month and older. The limit of the main static field in neonates is 4T MRI while the specific absorption rate limits are the same for both adults and neonates[2]. Traditionally reserved for research settings due to its unprecedented image resolution, the feasibility of employing 7 T MRI in routine clinical practice for paediatric cases could present a transformative prospect. According to a team of experts at King's College London, the current emphasis is on scanning infants at a high risk of brain injuries that may go unnoticed by other scanners, such as those born prematurely or with congenital heart conditions[3].

However, the transition to 7 T MRI in infants is not without its intricacies and hurdles. This article delves into the current state of the art in 7 T MRI for paediatric patients, exploring both its feasibility as a diagnostic tool and the unique challenges encountered in its application. By navigating the nuances of this technological frontier, we aim to provide a brief overview that contributes to the ongoing discourse surrounding this technical innovation in paediatric radiology.

APPLICATION

Most of the applications of 7 T MRI in the paediatric population so far have been focused on neuroimaging and holds substantial promise in this realm, presenting a spectrum of opportunities and challenges. 7 T MRI offers superior signal-to-noise and contrast-to-noise ratios, elevated spatial resolution, and enhanced tissue contrast, making it an appealing tool for in-depth examination of the paediatric brain and spinal cord, see Figure 1.

In the last years, there have been several ultra-high-field MRI studies demonstrating diagnostic benefits in neuroimaging. The high spatial and contrast resolution enable the detection of subtle structural abnormalities, particularly relevant in epilepsy cases where imaging at conventional field strengths may yield negative results[4]. Studies on adult and paediatric population with epilepsy have demonstrated that the higher spatial resolution, signal-to-noise ratio and altered contrast behaviour of 7 T MRI can increase detection sensitivity and delineation of potential epileptogenic lesions as well as the detection of epileptogenic focal cortical dysplasia (FCD) not clearly visible at conventional field strengths. In a prospective study, 7 T MRI revealed distinct lesions in some patients previously considered MRI-negative. For example, 7 T MRI helped diagnose FCD in paediatric patients who underwent surgical resection[5]. Also, FCD is very common among paediatric patients and can make epileptogenic lesions seen in focal epilepsy radiologically subtle. A pilot study suggested that among paediatric patients, those who are 3 T MRI-negative, but positron emission tomography-positive in particular could benefit from MRI at 7 T[6]. Similarly, at 7 T imaging could reveal more anatomic details of polymicrogyria compared with 3 T conventional sequences.

Furthermore, 7 T MRI has been shown to enhance MR angiography, offering improved image quality and the ability to detect smaller vessels. This capability, together with the increased contrast is especially pertinent in neurovascular assessments, for example allowing better identification of subtle vascular malformations due to increased susceptibility-weighted imaging (SWI) contrast, as well as delineation of cavernomas[7-9].

Structural and functional connectivity studies, facilitated by 7 T MRI, empower presurgical planning for deep brain stimulation surgery and hold promise for unravelling alterations in neuronal networks associated with various neuropsychiatric disorders. Neuro-oncology applications also benefit significantly from the fine structural detail attainable at 7 T. To provide an example of the level of detail achievable at 7 T, a recent study investigated childhood maltreatment (CM) related changes using 7 T MRI. The study suggested that experiencing emotional abuse or neglect in childhood affects morphology of brain regions involved in cognition, emotional processing, and memory. Further investigations of CM related morphological brain changes with 7 T MRI and greater sample size will be needed to confirm these findings and to be conducive to better understanding of the neurological effects of emotional events[10].

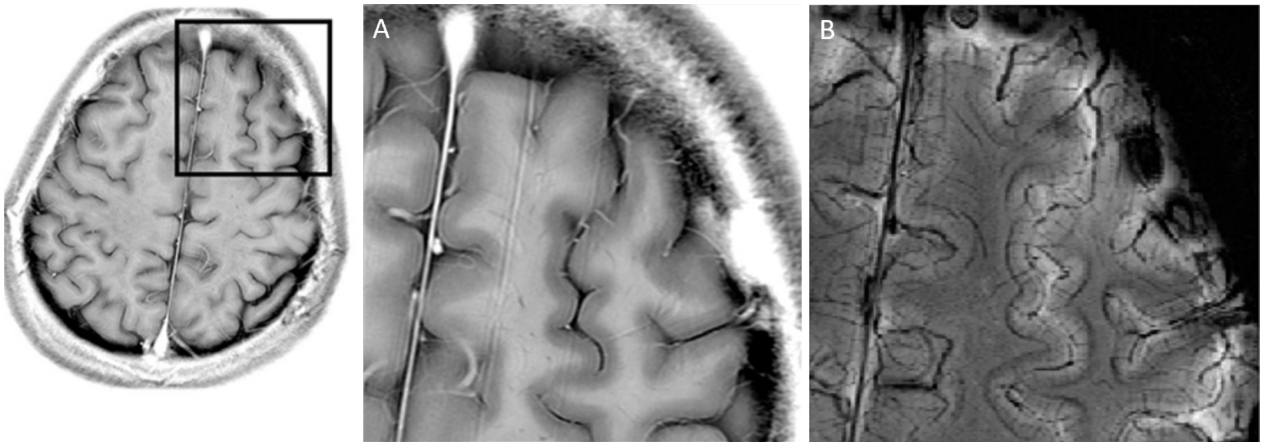

Figure 1 Comparative display of structural magnetic resonance images utilizing 3 Tesla and 7 Tesla systems. A: Displaying an axial fast spin echo image and its magnified view in the left frontal region with the 3 Tesla system. The resolution is field of view (FOV) 200 mm/512 pixel = 0.39 mm/pixel, and the scan duration is approximately 5 min; B: Presenting an axial fast spin echo image and susceptibility-weighted image in the same slice using the 7 Tesla system. The resolution is FOV 80 mm/512 pixel = 0.16 mm/pixel, and the imaging time is approximately 4 min. A and B: Citation: Yamada K, Yoshimura J, Watanabe M, Suzuki K. Application of 7 tesla magnetic resonance imaging for pediatric neurological disorders: Early clinical experience. *J Clin Imaging Sci* 2021; 11: 65. Copyright© The Authors 2021. Published by Scientific Scholar. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms. The authors have obtained the permission for figure using from Kenichi Yamada (Copyright permission).

Another area of immense promise is magnetic resonance spectroscopy (MRS) at 7 T. Due to the increased signal-to-noise ration and increased spectral resolution, this advanced technique could take advantage from the increased magnetic field strength allowing for the quantification of numerous neurometabolites with higher precision, presenting diagnostic and prognostic opportunities for conditions involving disrupted neurotransmitter balances, such as mood disorders, autism spectrum disorder, and epilepsy. Additionally, MRS can serve as a valuable tool for monitoring therapeutic responses, aiding in the personalized treatment of neurological conditions. The advantages of 7 T MRI extend beyond MRS. For example, chemical exchange saturation transfer imaging at 7 T provides opportunities to probe tissue chemistry in unprecedented detail, potentially unveiling insights into conditions like stroke, tumors, and neurodegeneration by monitoring pH and metabolite levels. Even spinal cord imaging, historically challenging at high field strengths, shows promise at 7 T, enabling research into conditions like multiple sclerosis[4].

In addition, the neonatal period is a critical phase of rapid brain development, making it an opportune time to study brain anatomy and potential injuries. However, conventional MRI systems at 1.5 T and 3 T have limitations in terms of tissue contrast and resolution. In contrast, 7 T MRI offers enhanced signal-to-noise ratio and tissue contrast, potentially improving sensitivity to both anatomical and pathological features in neonatal brain imaging. In a study by Fuente *et al* [11] involving 17 neonatal scans with a median corrected age of 40 + 3 wk, all conducted with ethical approval, revealed that 7 T MRI provided additional anatomical and pathological details compared to 3 T MRI in the same neonates. These improvements were confirmed through neuroradiology reviews and included enhanced visualization of structures like the hippocampus, cerebellar vermis, and occipital cortical folding. Moreover, 7 T imaging offered better insights into conditions like periventricular leukomalacia, providing clearer visualization of cystic septi and the hemorrhagic origin of cystic lesions in preterm infants[11].

Furthermore, 7 T MRI has demonstrated diagnostic value in various neurological conditions, including polymicrogyria, moyamoya disease, hippocampal sclerosis, brain tumors, stroke, and multiple sclerosis. Likewise, outside applications in the nervous system, a study by Kolb *et al*[12] evaluated the clinical feasibility of ultrahigh field 7-T SWI to visualize vessels and assessed their density in the immature epiphyseal cartilage of human knee joints. The study concluded that the use of SWI in conjunction with 7-T MRI makes the *in vivo* visualization of vessels in the growing cartilage of humans feasible, providing insights into the role of the vessel network in acquired disturbances. While there exist only few studies discussing the applications of the 7T MRI outside the CNS in paediatric patients, we can speculate that its use will expand to other areas in the near future.

As also demonstrated through feasibility studies, the enhanced quality of both SWI and single-shot T2-WI (T2WI) at 7 T is ascribed to a shorter T2-relaxation time, superior spatial resolution, and heightened susceptibility, potentially facilitating microstructural assessments. In contrast, T1WI quality at 7 T in infants has been observed to be inferior to that at 3 T, attributed to prolonged T1-relaxation time and the elevated water content in neonatal brains. However, the extended T1-relaxation time holds promise for future improvements in angiography quality[13].

CHALLENGES AND SAFETY CONSIDERATIONS

While studies at 7 T on adults are numerous, the literature about application and safety in paediatric population is scarce. It is imperative to highlight the certain considerations before conducting ultra-high field MRI studies in paediatric

patients. Challenges associated with 7 T MRI include high cost, susceptibility artifacts, image inhomogeneity, and patient comfort and safety concerns including tissue heating, consideration with protocol to reduce specific absorption rate, implants heating, *etc.* Increased radiofrequency power deposition, dizziness, nausea, vertigo, visual disturbances, and metallic taste are potential discomforts associated with 7 T MRI[5].

In the context of exploring the feasibility of employing 7 T MRI in paediatric settings, Chou *et al*[14] have shed crucial light on the comfort and practicality of this high-field imaging technique for children. Notably, the prevalence of noisiness as a source of discomfort across all age groups underscored the importance of addressing the sensory experiences of paediatric patients undergoing 7 T MRI scans. Additionally, the age-related differences in discomfort levels, with younger children reporting higher discomfort, highlighted the need for age-appropriate approaches to ensure the feasibility of 7 T MRI in paediatrics. While general discomfort was more frequent in adults during 7 T scans, the majority of adult respondents believed that children aged 12-17 years would tolerate 7 T scans well. These perceptions emphasize the potential psychological factors influencing pediatric patients' experiences. In light of these findings, cautious enrolment of younger children in 7 T MRI studies is recommended, prioritizing their comfort and well-being, and underscoring the necessity for further research and strategies to enhance the feasibility and acceptance of 7 T MRI in paediatric populations. Nonetheless, the study concluded that the tolerability of 7 T MRI in children, particularly those above 8 years of age, appears to be comparable to lower field strengths, suggesting its potential viability for use in this demographic[14].

In addition, specific considerations are needed when using 7 T MRI in paediatric patients due to differences in size and development. Thus, optimizing the system and ensuring safety, particularly with regards to the magnetic susceptibility effect, relaxation times, and participant comfort[15]. Fuente *et al*[11] performed a paediatric 7 T imaging study and to ensure the safety and comfort of the neonates during the 7 T scans, various precautions were taken, including modifying the scanner software to address potential temperature instability, swaddling the infants using vacuum-evacuated bags, providing hearing protection, and continuously monitoring vital signs such as heart rate and temperature using 7 T-compatible equipment. A range of MRI sequences were acquired, including high-resolution T2WI in multiple planes, susceptibility-weighted images, T1 and T2 quantitative maps, functional MRI, and spectroscopy. The results of this study demonstrated the feasibility of conducting 7 T MRI scans on neonates, with all infants tolerating the procedure well. Vital sign monitoring remained stable throughout the scans, which lasted for an average of 52 min.

Furthermore, despite the advantages, there are limited reports of the clinical application of paediatric 7 T MRI. Thus, further research and experience are needed to determine the full potential of 7 T MRI in this context and therefore, rigorous safety screening and assessment are imperative before any paediatric patient enters the magnetic resonance scanner[5,14].

Incorporating the findings from Annink *et al*[13] into our discussion on preparations and safety measures for infant 7 T MRI enhances our understanding of specific considerations for this vulnerable population. According to Annink *et al*[13], positioning the infant precisely in the isocenter of the coil is paramount for Specific Absorption Rate (SAR) safety. This emphasizes the importance of meticulous patient positioning to optimize safety profiles. Additionally, they highlighted the significance of acoustic noise protection during 7 T MRI examinations. The study reported that the background noise in the sound booth was 28 dB, and the 7 T hood attenuated this noise by 8.5 dB. Using hearing protection devices such as the Alpine Muffy Baby and Natus Minimuffs further reduced the acoustic noise levels, ensuring a safer and more comfortable environment for infants. Importantly, the study found that global and peak SAR levels in the infant model did not exceed those in the adult model when positioned correctly, reaffirming the feasibility of 7 T MRI in infants when appropriate safety measures are implemented. Integrating these findings, it is evident that specific preparations, including precise positioning and acoustic noise protection, are essential for ensuring the safety and efficacy of 7 T MRI in infants, aligning with the overarching goal of minimizing risks while maximizing the potential benefits of this advanced imaging modality[13].

Moreover, recently, a patient has been scanned for the first time in the world under general anaesthesia in the highly powerful MAGNETOM Terra 7 T MRI scanner from Siemens Healthineers. This was performed at King's College London's Advanced MRI Centre, located St Thomas' Hospital, London. This innovative anesthetic approach aims to enable patients, particularly young children who struggle to remain still in the 7 T MRI scanner, to access the technology. The procedure has the potential to benefit children dealing with conditions like epilepsy, tumors, and movement disorders, including dystonia-induced muscle spasms. Additionally, it could contribute to advancing innovative research projects[16].

CONCLUSION

In conclusion, as the investigation into the feasibility of implementing 7 T MRI in paediatric settings continues, the undeniable allure lies in its potential advantages concerning diagnostic precision, treatment monitoring, and research capabilities. The heightened image quality achieved through enhanced resolution and tissue contrast holds promise for elevating diagnostic sensitivity, shedding new light on the pathophysiological intricacies of conditions, especially those impacting neonates, such as neurodevelopmental disorders[17-19]. Nevertheless, the realization of these benefits necessitates a delicate balance with safety considerations and optimization efforts. The distinct challenges posed by high magnetic fields, particularly in the paediatric population, underscore the importance of ongoing research and expert oversight to ensure the judicious application of this advanced imaging technology.

FOOTNOTES

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EDITORIAL

Unveiling childhood asthma: Exploring biomarkers, zinc, and beyond

Amit Agrawal

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Abstract

This editorial discusses a case-control study by Ibrahim *et al,* published in the recent issue of the *World Journal of Clinical Pediatrics*. Childhood bronchial asthma is a chronic inflammatory respiratory disease. It was found that an increase in oxidative stress leads to a decrease in antioxidants causing oxidative damage to mitochondrial respiratory chain complexes resulting in the inflammation of the airway, hypersecretion of mucus causing a cascade of clinical manifestations ranging from recurrent episodes of coughing, wheezing, and breathlessness to shortness of breath. Since oxidative stress mediates the inflammatory response in asthma, the supplementation of anti-oxidants can be one strategy to manage this disease. Zinc is one such antioxidant that has attracted much attention about asthma and airway inflammation. Zinc is a crucial trace element for human metabolism that helps to regulate gene expression, enzyme activity, and protein structure. Apart from zinc, free serum ferritin levels are also elevated in case of inflammation. Several previous studies found that ferritin levels may also help determine the pathology of disease and predict prognosis in addition to tracking disease activity. However, this study's results were different from the findings of the previous studies and the zinc levels did not show a significant difference between asthmatic children and non-asthmatic children but ferritin levels were significantly high in asthmatic children as compared to the controls. Hence, the possible role of the biochemical nutritional assessment including zinc and ferritin as biomarkers for asthma severity should be assessed in the future.

Key Words: Asthma; Biomarker; Children; Zinc; Ferritin; Immunoglobulin E

Core Tip: Asthma is a chronic inflammatory disorder of the respiratory system, more common in children than adults. The disorder is the most common cause of emergency department visits, absenteeism from school, and hospitalization in children. The etiology of asthma is not clear but various triggering agents including environmental, nutritional, and genetic factors may have their roles. Previous studies found that trace elements with antioxidant properties such as zinc can be effective in the treatment of asthma. Apart from zinc, serum ferritin, and Ig E levels are also elevated in such children. Hence, further studies assessing these biomarkers are needed in the future.

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INTRODUCTION

Bronchial asthma is a chronic inflammatory disease of the respiratory tract system affecting more than 300 individuals worldwide[1,2]. In children, asthma causes significant morbidity and mortality and its prevalence has been rising rapidly in recent times. Although the exact cause of asthma is unknown, several environmental and nutritional factors have an important role in triggering asthma[3]. In the case of inflammation, several immune cells are activated which release several harmful mediators such as reactive oxygen species and free radicals. An increase in oxidative stress leads to a decrease in antioxidants causing oxidative damage to mitochondrial respiratory chain complexes. This damage causes inflammation of the airway, hypersecretion of mucus, contraction of smooth muscle, and epithelial shedding resulting in limitation of the air to the lungs causing a cascade of clinical manifestations ranging from recurrent episodes of coughing, wheezing, and breathlessness to shortness of breath. It was also found that different cytokines and chemokines contribute to the pathogenesis of asthma[2].

Since oxidative stress mediates the inflammatory response in asthma, the supplementation of anti-oxidants can be one of the strategies to manage this disease. It was discovered that a decline in the dietary intake of antioxidants contributes to the increased incidence of asthma. Among all antioxidants, zinc has garnered attention about asthma and airway inflammation due to its immune-modulating properties. Zinc is a crucial trace element that contributes to the maintenance of the immune system, growth and development, oxidative stress, tissue repair and regeneration, and inflammatory reactions[1]. It is the second most abundant trace metal in the human body after iron which is present in the approximate concentration of 2-4 g. The disruption of zinc homeostasis causes Th1/Th2 balance to shift towards a Th2 response, which leads to a hyper-inflammatory response, a fundamental abnormality in asthma[3].

Furthermore, zinc is a crucial part of antioxidant enzymes, which are needed to prevent the creation of free radicals, which are thought to exacerbate asthma[4]. Due to its antioxidant and anti-apoptotic effects and the presence of several growth co-factors, zinc safeguards the respiratory system and contributes to the synthesis of proteins, DNA, as well as, RNA, the production of energy, and the growth of the cell. Hence, a decreased dietary intake of anti-oxidant substances or increased consumption of oxidative substances may raise the incidence and severity of asthma. In addition to zinc, the levels of free serum ferritin tend to rise in case of inflammation as it has a protective role in redox biology and iron homeostasis. Immunoglobulin E acts as a highly sensitive immunological amplifier by triggering rapid pathological reactions and recognizing antigens.

In the recent issue of the *World Journal of Clinical Pediatrics,* an interesting article titled 'Childhood asthma biomarkers including zinc: An exploratory cross-sectional study' has been published[5]. It is a case-control study carried out at the Children's Hospital, Cairo University, investigating children with bronchial asthma (n = 40) and healthy children (n = 21). Data on sociodemographic and clinical characteristics such as body mass index and degree of asthma severity were collected. The study addresses an important issue wherein the effect of serum zinc levels on bronchial asthma was assessed. Additionally, other laboratory parameters such as serum ferritin and IgE levels, were studied. They found that the mean ± SD serum zinc level for asthmatic *vs* non-asthmatic children was 94.4 ± 24.7 and 85.2 ± 19, respectively, with no significant difference. Similarly, no significant differences were observed regarding serum iron, Hb, and albumin levels in cases *vs* controls. In contrast, serum levels of IgE and ferritin showed significant differences between cases and controls (P = 0.001 and 0.006, respectively).

In line with this study, a meta-analysis conducted by Ghaffari *et al*[3] found that there was no significant difference between the standard mean differences in zinc levels among asthmatic children as compared to the control group. Elsayed *et al*[6] found higher mean serum zinc levels among asthmatic children than in the healthy group. In contrast to the present study, Abdelaziz *et al*[4] showed a significant improvement in decreasing the frequency of attacks (P = 0.036), nocturnal symptoms (P < 0.001), clinical control of asthma symptoms (P < 0.001) after 8 wk of zinc supplementation and a significant improvement was found in pulmonary functions in forced expiratory volume in 1 s (P < 0.001) and forced vital capacity (P = 0.002). Another study conducted by AbdulWahab *et al*[7] also showed a positive correlation between serum zinc levels and bronchial asthma in children.

In a meta-analysis of 21 articles including 2205 children, Xue *et al*[8] found a statistically significant association between circulating zinc and risk for childhood asthma and wheezing. These conflicting results between this study and the other studies may be explained by the differences in study designs, genetic, and environmental differences among the participants. It was found that the frequency of zinc deficiency is higher in Middle Eastern countries, mainly due to the

presence of high concentrations of dietary phytates in cereals and legumes[8]. The presence of phytates in beans, unrefined grains, and nuts is the main reason for the inhibition of dietary zinc absorption resulting in its deficiency. Hence, pre-existing zinc deficiency in children can be correlated with the diverse study results.

The strength of this study was the assessment of not only zinc but also the levels of serum ferritin, as well as, IgE among asthmatic children. Also, the sample size is adequate for a clinical study. In our view, a few limitations exist in this study as they have not assessed the relationship between serum zinc levels and the severity of asthma. They have assessed only the relationship between these two parameters but not in terms of disease severity. Also, on what basis the children were classified as mild, moderate, and severe asthmatic was not specified.

CLINICAL IMPLICATIONS

Zinc is a potent immunomodulator and an important cofactor of more than 2000 transcription factors and more than 300 enzymes which helps to maintain the oxidative balance in the body by maintaining various cellular processes such as cell proliferation, differentiation, apoptosis, DNA and RNA synthesis, tissue maintenance, immune function, and glucose and lipid metabolism. This anti- and pro-oxidative balance is disrupted in asthma which can be maintained using zinc supplements. Due to these properties, it has been used as a medication for various allergic diseases like chronic rhino-sinusitis and atopic dermatitis[9]. Zinc supplements have been shown to reduce the nocturnal exacerbation of asthma, wheezing, cough, and other symptoms in children with bronchial asthma[6,10,11]. The use of zinc supplements decreases antibiotic consumption and the incidence of respiratory tract infection, particularly in children. Hence, the patients and their parents need to be educated about the importance of zinc in their diet.

FUTURE PERSPECTIVES

Zinc dyshomeostasis has been linked with several lung diseases including asthma. The comparison of zinc levels among asthmatics and healthy children can help to formulate the exact role of zinc among such patients. With further and more extensive studies on varied populations, a gold standard value of appropriate zinc level can be obtained. The zinc levels below this value can be considered a risk factor for bronchial asthma. However, more research on zinc homeostasis is required which could pave the way for new therapeutics targeting the use of zinc supplementation in inflammatory lung diseases.

CONCLUSION

In conclusion, we can say that zinc contributes to many biological functions and acts as an anti-inflammatory, antioxidant, antiviral agent, and immunomodulator. Zinc supplementation can be used for treating various respiratory diseases including asthma. However, the correlation between asthma and zinc levels among asthmatic children is not found significant in this study. We need more studies with bigger sample sizes and powerful methodology to confirm this relationship. Even though these studies do not have powerful methodologies; zinc supplementation could be effective in the prevention and treatment of asthma in children as an additive.

FOOTNOTES

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EDITORIAL

Prediabetes in children and adolescents: A ticking bomb!

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Abstract

Prediabetes in children and adolescents is on the rise which has drawn significant attention over the past decade. It is an early warning sign of the underlying pathophysiological changes which in due course of time might compound into type II diabetes mellitus. The incidence of prediabetes in adolescents ranges from 4%-23% which is alarmingly high and requires active intervention from the system. We have discussed early identification of high-risk patients, prompt screening and active intervention to manage this growing problem.

Key Words: Prediabetes; Children; Adolescents; Glucose metabolism; Complications

Core Tip: Prediabetes is an early warning sign of an underlying pathophysiological change in glucose metabolism which in the due course of time might advance to type II diabetes mellitus. There has been a recent global upsurge in prediabetes in children and adolescents. Notwithstanding, there is still a dearth of sufficient literature regarding this very pertinent issue. Prediabetes status must be identified at the earliest to prevent further medical complications. American Diabetes Association and the International Society for Paediatric and Adolescent Diabetes recommend the screening of high-risk children for developing prediabetes. At present, the possible ways of managing this crisis are the prevention of childhood obesity, early identification and screening of high-risk patients, and active intervention.

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INTRODUCTION

Prediabetes in children and adolescents is on the rise which has drawn significant attention over the past decade. It is an early warning sign of the underlying pathophysiological changes which in due course of time might compound into type II diabetes mellitus (T2DM). The incidence of prediabetes in adolescents in the United States ranges from 4%-23% which is alarmingly high and requires active intervention from the system[1]. Studies have found that there is a likelihood that 8% of prediabetic adolescents will develop T2DM over the next three years[1]. Juvenile onset or the youth- onset T2DM has been found to have a more aggressive course with early organ system involvement[2]. Therefore, it becomes the need of the hour to have a proper understanding of the disease process with the goal aimed at prevention, early diagnosis and management of the disease.

In the present era, it becomes imperative to discuss the role of childhood obesity in outgrowing the number of prediabetics in this cohort. Obesity is a multifactorial disorder which directly or indirectly affects most organ systems. From 1980 till the next three decades, the number of obese patients has nearly doubled in 70 countries worldwide, of which the majority of the countries belong to developed nations[3,4]. As per the survey done in 2015, there are more than 107 million obese children worldwide which cannot be ignored in any healthcare system. Obesity has been linked with insulin-resistance which lays the fertile ground for dysglycaemia leading to prediabetes, diabetes and other cardiovascular sequelae[3]. National Health and Nutrition Examination Surveys (2005–2016) done in United States reported that the incidence of T2DM in adolescents aged 12 to 18 years is likely to quadruple by the year 2050[5]. A recent systematic review and meta-analysis pooled in the data from 48 studies and found that the overall prevalence of prediabetes in adolescents is 8.84%[6].

While we deliberate on prediabetics in children and adolescents, there is still a dearth of sufficient literature regarding this very pertinent global issue. This patient cohort is majorly diagnosed and managed primarily on the lines of the adult population[1]. The criteria for prediabetes (in children and adolescents) are the same as that for adults *i.e.* fasting blood glucose between 100-125 mg/dL or post-prandial (after 2 h of glucose) between 140-199 mg/dL or glycosylated haemoglobin between 5.7%-6.4%[7]. Based on the American Diabetes Association (ADA), the prevalence of prediabetes in obese children ranges from 21%-40% which varies with race, sex and ethnicity. As there are various criteria for diagnosing prediabetes, the prevalence varies owing to differences in their sensitivity[8,9].

It is essential to understand the basic pathophysiology of prediabetes, not only to prevent future generations from this growing epidemic but also to help in the successful management of those who have been diagnosed with it. Prediabetes is diagnosed based on blood glucose levels and therefore it's difficult to predict future outcome as T1DM or T2DM. The pathophysiology of prediabetes and T2DM is very similar which includes insulin resistance, impaired insulin secretion and damaged beta cells[10]. Even puberty has been linked with insulin resistance which can be synergistically with factors leading to T2DM[11].

Before planning for any intervention against prediabetes, prediabetes status must be screened at the earliest to prevent further medical complications. In a survey of the adult population, it was found that active intervention in the form of lifestyle modification over a period of 4 years decreased the disease progression to T2DM from 33% to 20%[12,13]. However, it is not practically feasible to screen all obese children for prediabetes and therefore ADA and the International Society for Paediatric and Adolescent Diabetes recommend the screening of children who are at high risk of developing prediabetes[14,15]. These include children with a history of gestational diabetes to mother, those with a history of T2DM in 1st or 2nd-degree relatives, race and ethnic groups with high prevalence rates, and medical conditions which are known to be associated with insulin resistance (*e.g.* primary hypertension, dyslipidaemia *etc.*)[16].

MANAGEMENT OF PREDIABETES

Efficient and timed management of prediabetes plays a pivotal role in the prevention of T2DM. These include: (1) Lifestyle modification in the form of increased physical activity and dietary control play very crucial in successfully treating prediabetes[17,18]. Physical activity has been found to have a role in increasing insulin sensitivity and the Endocrine Society Clinical Practice Guidelines recommend a minimum of 30 min of moderate to vigorous physical activity while aiming at 60 min per day[19]. In a meta-analysis including 15 trials, it was found that physical activity brought a 40% improvement in oral glucose tolerance test results while increasing insulin sensitivity[20]. In another study performed on children and adolescents (6-17 years), physical activity decreased adiponectin and waist circumference[21]. They also observed increased insulin sensitivity in the pubertal age group[21]. Regarding dietary intake, the American Academy of Pediatrics and World Health Organization (WHO) recommend increased intake of fruits and vegetables, decreased intake of saturated fat and avoiding sugar-containing beverages[22,23]. The role of roughage has been extensively studied wherein it has been shown to increase insulin sensitivity[24]. WHO advocates preventing obesity *via* individual and community-based programs[25]; (2) Metformin, an oral hypoglycaemic agent (OHA) acts by decreasing glucose absorption, preventing gluconeogenesis and increasing peripheral uptake of glucose[26]. It has been found to delay or stop the progression of prediabetes to T2DM. In a meta-analysis, it was observed that metformin resulted in decreased body mass index (-1.3 kg/m^2) with one-fourth of trials showing an improved insulin sensitivity with the drug [27,28]. However, the Paediatric Obesity Clinical Guidelines from The Endocrine Society and ADA recommend the use of metformin in addition to lifestyle modification in high-risk patients[29]; (3) Glucagon-like peptide analogs are a class of OHA which act by increasing glucose-dependent insulin secretion while suppressing glucagon secretion and decreasing gastric emptying[30]. Liraglutide is approved for the treatment of obesity in children above 12 years of age. In 2019, the Food and Drug Administration approved its use for the treatment of T2DM in children above 10 years of age although it

lacks sufficient data regarding its role in the management of prediabetes[31]; (4) Other drugs like sibutramine and orlistat have been shown to improve insulin sensitivity in children although their use in this patient cohort is still controversial. Also, there is some evidence to support the role of peroxisome-activated receptor gamma agonists in increasing insulin sensitivity in children[32]; and (5) Bariatric surgery helps in controlling obesity which is an important factor responsible for insulin resistance leading to prediabetes and T2DM. In the Teen-Longitudinal Assessment of Bariatric Surgery study, 20 adolescents had T2DM while 17 had prediabetes at the time of bariatric surgery. These patients were followed for a period of 3 years. They showed a remission rate of 76% for prediabetes and 95% for T2DM respectively[33].

Despite the above-mentioned modalities of treatment, the focus should be on the timely diagnosis of the problem. Childhood obesity is a very important factor which aids in the development of prediabetes. Despite realising the problem, the data based on the paediatric population is very limited. The majority of the criteria and treatment protocols are based on the adult population. Although the aforementioned interventions have shown moderate improvement, there is still no generalised consensus on the line of management. Monitoring the improvement with these interventions requires the development of new biochemical markers such as a single-point insulin sensitivity estimator or new score systems such as metabolic syndrome severity score[34]. These tools should be developed and validated on the paediatric population to help in the medical management of this grave problem. Owing to different prevalence rates in different races and ethnicities, research is required concerning race/ethnicity-specific management protocol[35]. Also, the role of gut microbiome in future treatment of prediabetes or T2DM cannot be overlooked. Prevention of childhood obesity, early identification of high-risk patients, prompt screening and active intervention are the only ways of managing this growing problem.

CONCLUSION

To conclude, prevention of childhood obesity, early identification of high-risk patients, prompt screening and active intervention are the only ways of managing this growing problem.

FOOTNOTES

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REVIEW

Microphallus early management in infancy saves adulthood sensual life: A comprehensive review

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Abstract

Microphallus/Micropenis is a rare condition with significant physical and psychological implications for affected individuals. This article comprehensively reviews micropenis, its etiology, epidemiology, and various treatment options. We conducted a thorough literature review to collect relevant information on micropenis and microphallus, as well as related disorders. Our primary databases

were PubMed, Medline, and Google Scholar. We searched for articles published in English between 2000 and 2023. Our analysis included 67 review articles, 56 research studies, 11 case reports, one guideline, and one editorial. Our search terms included "microphallus", "micropenis", "congenital hypogonadotropic hypogonadism", "androgen insensitivity syndrome", "pediatric management of micropenis", "testosterone therapy", and "psychosocial implications of micropenis". We focused on diagnosing micropenis and related conditions, including hormonal assessments, medical and surgical treatment options, psychosocial and psychological well-being, sexual development of adolescents, and sociocultural influences on men's perceptions of penile size. Additionally, we explored parenting and family dynamics in cases of micropenis and disorders of sex development, implications of hormonal treatment in neonates, and studies related to penile augmentation procedures and their effectiveness. The article highlights the importance of early diagnosis and intervention in addressing the physical and psychological well-being of individuals with micropenis. Surgical procedures, such as penile lengthening and girth enhancement, and non-surgical approaches like hormonal therapy are explored. The significance of psychological support, education, and lifestyle modifications is emphasized. Early management and comprehensive care are crucial for individuals with micropenis, from infancy to adolescence and beyond. A multidisciplinary approach involving urologists, endocrinologists, and mental health professionals is recommended. Regular assessment of treatment effectiveness and the need for updated guidelines are essential to provide the best possible care. Healthcare professionals should prioritize early diagnosis, and neonatologists should measure stretched penile length in neonates. A collaborative effort is needed among professionals, parents, and affected individuals to create a supportive environment that recognizes worth beyond physical differences. Continuous research and evidence-based updates are crucial for improving care standards.

Key Words: Microphallus; Micropenis; Hypogonadism; Children; Adults; Sensual life; Testosterone therapy

Core Tip: Early diagnosis is crucial in identifying isolated micropenis, especially when gonads are non-palpable, as it may indicate gonadotropin deficiency. Measuring stretched penile length in neonates is an important step towards early detection. A comprehensive approach involving urologists, endocrinologists, and mental health professionals is recommended to address both the physical and psychological aspects of micropenis. Psychological support is particularly important during childhood and adolescence to help individuals cope with self-esteem, body image, and potential bullying concerns. Regular assessments of treatment effectiveness and guideline updates are encouraged, adapting to evolving evidence and medical technologies. Collaboration among healthcare professionals, parents, and affected individuals is essential.

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INTRODUCTION

Micropenis, also known as microphallus, is a medical condition where the penis size is abnormally small, usually below the average for a given age group. This condition is defined when the length of the stretched penis falls below 2.5 standard deviations (SDs) beneath the mean for the age group, which may vary slightly depending on the population studied. In newborn males born at full-term, the average stretched penile length is 3.5 cm. Micropenis is defined when it measures less than 2 cm-2.5 cm (2.5 SDs beneath the mean)[1,2]. The term microphallus is used when there is an associated hypospadias or some degree of ambiguity. Severe forms of microphallus are considered ambiguous genitalia [3]. The presence of a well-developed scrotum and adequately sized, palpable testicles suggest a high likelihood of a normal male karyotype. However, the absence of palpable testicles and penile urethra may indicate ambiguity, requiring karyotyping and counseling for sex developmental disorders[4].

The appearance of micropenis and microphallus during infancy, adolescence, or adulthood presents significant questions about embryogenesis, hormonal signaling, and possible causes[5]. Microphallus is a challenging condition that significantly impacts the lives of infants and their families, so exploring this critical and often overlooked aspect of male reproductive health is essential[6]. The focus should be on managing microphallus during infancy and its consequences for adult sensual life. This comprehensive review demonstrates the critical importance of early intervention and management strategies to mitigate potential negative impacts on adult sensual life[7]. This review aims to provide a better scientific understanding of microphallus and micropenis and emphasize the importance of a holistic, multidisciplinary approach to managing these conditions. We aim to pave the way for improved clinical care, better patient outcomes, and a deeper understanding of the human phallic spectrum through heightened awareness and a comprehensive approach.

We conducted a thorough literature review to gather relevant information on micropenis and microphallus, including related disorders. Our primary databases were PubMed, Medline, and Google Scholar. We searched for articles published in English between 2000 and 2023. We included 67 review articles, 56 research studies, 11 case reports, one guideline, and one editorial. Our search terms were "microphallus", "micropenis", "congenital hypogonadotropic hypogonadism", "androgen insensitivity syndrome (AIS)", "pediatric management of micropenis", "testosterone therapy", and "psychosocial implications of micropenis". We extracted data from peer-reviewed scientific articles, case reports, and clinical studies. The study flow chart is shown in Figure 1.

We focused on diagnosing micropenis and related conditions, including hormonal assessments, medical and surgical treatment options, psychosocial and psychological well-being, sexual development of adolescents, and sociocultural influences on men's perceptions of penile size. We also looked into parenting and family dynamics in cases of micropenis and disorders of sex development, implications of hormonal treatment in neonates, and studies related to penile augmentation procedures and their effectiveness. We systematically reviewed, summarized, and synthesized the data extracted from the selected articles to provide a comprehensive overview of the diagnosis, management, and psychosocial considerations related to micropenis.

Our research indicates that micropenis is diagnosed based on penile length measurements. According to the included studies, micropenis is defined as an erect penile length of less than 9.3 cm (3.66 inches) in adults or more than 2.5 SDs below the mean in neonates and children. Figure 2 shows the rate of increase in penile length in both antennal, postnatal, and adulthood. Micropenis is defined when the penile length is below -2 SD. The prevalence of micropenis varies between 0.6%-0.7% of male infants. Various underlying conditions, including congenital hypogonadotropic hypogonadism and AIS, can cause micropenis. These conditions can result in micropenis due to hormonal imbalances or insensitivity to androgens. The different causes of micopenis/microphallus are shown in Table 1. The penile growth is affected by different factors. Table 2 shows the different factors that affect the stretched penile length at birth, while Table 3 shows the factors affecting penile length from birth to adulthood. Figure 3 shows the prenatal and postnatal penile development and its relation to the testosterone surge. Hormonal assessment of micropenis is of critical importance for proper management. Figure 4 shows the flow chart for this assessment. Hormone therapy is often used to treat micropenis in neonates and young children. The administration of testosterone or human chorionic gonadotropin (hCG) can lead to increased penile growth, especially if initiated during the early stages of life. The treatment's success may depend on the underlying cause of micropenis. Surgical interventions, such as penile lengthening procedures, were identified as options for adults with micropenis who sought to increase penile length. Surgical interventions were generally considered a last resort due to associated risks and complications. Studies on penile augmentation procedures reported varying outcomes. Surgical interventions aimed at increasing penile length showed mixed results and patient satisfaction was influenced by individual expectations. Long-term implications of hormone therapy in neonates with micropenis are not always well-documented.

Micropenis can have a psychological impact on individuals. Adolescents and adults with micropenis often experience lower self-esteem and increased anxiety regarding their body image and sexual performance. Psychological support is essential to help individuals cope with these issues. Parents play an essential role in managing micropenis in infants. Studies noted the importance of family support in making decisions regarding treatment options and providing emotional support to their children. Societal perceptions of masculinity and penis size can exacerbate psychological distress related to micropenis. This influence has been shown to contribute to decisions for penile augmentation.

PENILE DEVELOPMENT

The male phallus is a remarkable example of biological diversity, showcasing the intricacies of embryonic development, endocrine regulation, and genetic influences. The process of penile development is a complex and highly regulated one that begins during early embryogenesis and continues throughout fetal growth, childhood, and puberty[8]. It is a well-orchestrated symphony of genetic signaling, hormonal cascades, and tissue differentiation, forming the male external genitalia and the mature penis. Understanding the intricacies of penile development is crucial in comprehending the causes of anomalies, such as microphallus and micropenis, appreciating the normal variations in penile size, and identifying potential areas of disruption in this delicate process[9].

Penile development begins during the embryonic phase and is mainly driven by genetic and endocrine influences. At around the sixth week of gestation, the bipotential genital tubercle, which is initially undifferentiated and a precursor to the external genitalia, undergoes sex differentiation in response to the genetic instructions encoded within the sex chromosomes[10]. This results in the formation of either a male or female form. The presence or absence of the sex-determining region Y (*SRY*) gene on the Y chromosome determines male or female development, respectively. In males, the *SRY* gene triggers a series of events that lead to the formation of the genital tubercle into the phallus, thus beginning penile development[11]. At the same time, the genital folds (also known as urethral folds) develop on either side of the genital tubercle. The genital tubercle, which is now committed to male development, undergoes a series of complex morphogenetic changes[8]. During this phase, androgens, primarily testosterone and dihydrotestosterone (DHT), play a pivotal role. By the seventh week of gestation, testosterone, produced by the Leydig cells in the developing testes, acts on the genital tubercle, stimulating its growth and elongation. Subsequently, the enzyme 5-alpha-reductase converts testosterone into DHT, a more potent androgen, which further amplifies penile growth and differentiation[12].

During fetal development, the genital tubercle elongates, and the urogenital folds, which flank the developing urethral groove, fuse together in the middle to form the penile urethra[13]. As the urogenital folds unite, the scrotal raphe begins to appear, creating the characteristic midline seam on the scrotal skin in males. This fusion not only separates the penile

Table 1 The causes of Microphallus and Micropenis

Factor		Description
Genetics	Specific genes mutations	Genes related to testosterone production and androgen receptor function. Genes associated with the genital tubercle development
	AIS	
	Genetic deficiencies in Enzymes Involved in Hormone Production *e.g.*, 5 alpha-reductase deficiency	
	Genetic abnormalities affecting hormonal signalling	
	Genetic disorders affecting pituitary gland function	
	Genetic causes of congenital adrenal hyperplasia (deficiency in steroidogenic acute regulatory protein (*STAR* gene mutation), 3 beta-hydroxysteroid dehydrogenases (salt-wasting, non-salt-wasting, and non-classic types), 17 α-hydroxylase (mutations in the *CYP17A1* gene located on chromosome 10q24-q25)	
	Chromosomal Abnormalities *e.g.*, Klinefelter syndrome (XXY), Trisomy of the chromosomes 8, 13, 18 or 21	
	Inherited syndromes	Bardet-Biedl syndrome, Prader-Willi syndrome and Kallman syndrome (hypogonadotropic hypogonadism, osteoporosis, hearing impairment, and anosmia). Noonan syndrome (hypertelorism, short neck, low-set ears, skeletal malformation, bleeding disorders, and pulmonary valve stenosis). Others: Charge syndrome, Silver Russel syndrome, Rud syndrome
Hormonal and endocrinal causes	Primary Hypogonadism: Either congenital or acquired *e.g.*, Anorchia, Klinefelter and poly-X syndromes, gonadal dysgenesis (incomplete form), luteinizing hormone receptor defect (incomplete form), testosterone steroidogenesis (incomplete form), Noonan syndrome, Trisomy 21, Robinow syndrome, Bardet-Biedl syndrome, Laurence-Moon syndrome	
	Secondary Hypogonadism: Secondary to pituitary gland or hypothalamus disorders, resulting in decreased secretion of luteinizing hormone and follicle-stimulating hormone	
	AIS	
	Enzyme deficiencies affect testosterone synthesis or its conversion to the more potent dihydrotestosterone	
	Growth hormone deficiency or abnormalities in IGF-1	
	Hypothyroidism	
	Adrenal gland disorders such as adrenal hyperplasia	
Anatomical and structural abnormalities	Penis agenesis, cloacal dystrophy, hypospadias or chordee, Peyronie's disease, corpus cavernosa and corpus spongiosum hypoplasia, vascular abnormalities, ligaments or connective tissue abnormalities, and inadequate penile shaft length	
Environmental factors	Antenatal exposure	Endocrine-disrupting chemicals, including phthalates, bisphenol A, and certain pesticides
		Anti-androgenic drugs
		Maternal substance abuse, including alcohol, drugs, or tobacco
		Ionizing radiation
		Antenatal infections
		Inadequate nutrition and a poor maternal diet
		Pollutant exposure such as heavy metals and dioxins.
		Antenatal exposure of antifungal
	Postnatal exposure	Improper or excessive use of antibiotics or hormonal medications
		Hormonal treatments for conditions like precocious puberty or delayed puberty
		Surgical interventions or treatments for disorders affecting the genitalia
Idiopathic	Unknown cause	

AIS: Androgen Insensitivity Syndrome; *STAR*: Spliced transcripts alignment to a reference; *CYP17A1*: Cytochrome P450 17A1; IGF-1: Insulin-like growth factor-1.

urethra but also marks the scrotum, establishing male external genitalia[14]. Hormones and genetic factors regulate penile growth during fetal development, which follows a linear pattern until birth. This results in a newborn male having an adequate penile length at birth. During mid-to-late gestation, penile growth is essentially linear due to the increase in testosterone levels reaching the mid-gestation peak (Figure 3). For infants born between 24 wk-36 wk of gestation, penile length in centimeters can be calculated using the following equation: Gestational age in week multiplied by 0.16, then subtract 2.27[15].

Table 2 Factors that affect the stretched penile length at birth

Factor	Description
Genetic factors	Genetic factors influence the tissue response to androgens and can contribute to variations in penile size and development
Ethnicity	There may be variations in penile length at birth among different ethnic groups. African and African-Caribbean ethnic backgrounds tend to have, on average, longer penile lengths compared to other ethnic groups. Individuals from Asian ethnic backgrounds tend to have slightly shorter penile lengths than African and African-Caribbean populations. Caucasian/European and Latino/Hispanic Ethnicities fall in the intermediate range in terms of penile length
Prenatal testosterone levels	Hormones, particularly testosterone, play a crucial role in developing the male reproductive system, including the penis. Testosterone influences the growth of the penis during fetal development
Gestational age	Premature babies may have a smaller penile length at birth than full-term infants. The duration of gestation can influence the development of the reproductive organs, including the penis
Birth length	This effect is observed in preterm infants
Birth weight	Babies who have a lower birth weight also tend to have shorter penises
Maternal Health and Nutrition	The health and nutrition of the mother during pregnancy can impact fetal development, including penile growth. Adequate nutrition and a healthy pregnancy can support normal development
Fetal growth and development	The fetus's growth and development rate during pregnancy can affect penile length at birth. Factors such as intrauterine growth restriction can potentially influence penile size
Certain medical conditions	Some medical conditions, such as Klinefelter syndrome and Down syndrome, can cause a decrease in SPL at birth
Health conditions during pregnancy	Certain medical conditions during pregnancy, such as hormonal imbalances or endocrine disorders in the mother, can affect fetal development, including penile length
Environmental factors	Environmental factors, including exposure to toxins, pollutants, or substances that can disrupt hormone levels, might impact penile development in utero
Maternal exposure to certain medications	Some medications, such as corticosteroids and certain anticonvulsants, have been linked to a decrease in SPL at birth

SPL: Stretched penile length.

The penis is typically small at birth, with an average stretched length of 3.5 cm in full-term newborn males. The penis may appear larger than expected due to increased adipose tissue, which is usually non-erect[16]. The foreskin is typically fused with the glans. A full-term baby's stretched penile length at birth varies based on gestational age, while for preterm babies, it depends on both gestational age and body length[17]. It's worth noting that it's normal for newborns to have variations in penile size and that the size will continue to develop throughout childhood. Table 2 shows the factors that affect the penile length at birth. The postnatal growth rate of the penis is influenced by hormonal, genetic, nutritional, and health-related factors. Penile growth in the first year of life is relatively slow, with an average stretched length of about 4.5 cm by age 12 months. This means that the penis grows approximately 1 cm in length during the first year of life; most of the growth occurs in the first six months due to the first post-natal testosterone surge known as mini puberty (Figure 2)[18].

During the early years of life, the growth of the penis is minimal until puberty. Between the ages of one and five years, there is a slow and steady growth in both the length and width of the penis, which is influenced by growth hormones (GHs) and other factors. The growth rate remains consistent during the pre-pubertal stage (6 years-11 years) but is not as pronounced as during puberty[19]. The child's overall growth influences penile growth and anticipates the rapid growth that occurs during puberty. With the surge in testosterone levels during puberty, the penis undergoes significant growth in both length and girth[20]. This pubertal growth spurt, coupled with hormonal changes, signifies the completion of penile development into its adult form. The average adult penile length is about 9.16 centimeters when flaccid and 13.12 cm when erect. After puberty, there is unlikely to be any further penile growth. However, some individuals may experience penis growth into their early 20s[21]. Figure 3 shows the rate of increase in penile length in both antennal, postnatal, and adulthood.

It's important to understand that there are normal variations in penile growth among individuals, and each person's growth trajectory may differ slightly due to genetic and environmental factors. Factors such as nutrition, general health, and physical activity can also play a role in the growth and development of the penis during childhood[22]. Table 3 shows the factors affecting penile length from birth to adulthood. It is crucial to understand the complex mechanisms and key milestones of penile development to identify and address any issues or abnormalities, such as microphallus and micropenis[23]. Further research into this process's molecular and genetic basis may lead to potential therapeutic interventions to optimize penile development and improve outcomes for people with penile anomalies.

Table 3 The factors affecting penile length from birth to adulthood

Factor	Description
Genetics	The most important factor affecting penile length is genetics. Studies have shown that up to 80% of the variation in penile length can be attributed to genetics. Therefore, penile length is a highly heritable trait that is passed down from parents to children. In addition, Certain genetic conditions can affect penile growth. For example, boys with Klinefelter syndrome tend to have smaller penises than boys without the disorder
Race and ethnicity	Studies suggest that there may be differences in penile size among different ethnic and racial groups, although these variations are generally within a normal range and not significant
Nutrition and Health	Proper nutrition and overall good health positively influence growth and development, including penile growth
Puberty	Significant penile growth during puberty due to hormonal surges, especially testosterone
Hormonal levels	Hormonal imbalances or medical conditions affecting hormone levels may influence penile growth. Testosterone, the primary male sex hormone, plays a critical role in the development and growth of the penis during puberty. Growth hormone also contributes to overall growth, including penile growth during adolescence
Environmental factors	Some environmental factors, such as exposure to certain chemicals or toxins, may also affect penile growth. For example, boys exposed to phthalates, a type of chemical found in some plastics, had smaller penises than boys not exposed to phthalates. However, more research is needed to confirm these findings
Medical conditions	Certain medical conditions or disorders, such as endocrine disorders and chronic illnesses, affecting endocrine function can influence penile length
Obesity	Excess body fat can make the visible portion of the penis appear smaller due to the fat pad in the pubic area. Maintaining a healthy weight and reducing excess fat can help perceive penile length
Circumcision	Surgical removal of the foreskin (circumcision) affects the appearance of the penis but not its actual length
Exercising and physical activity	Regular physical activity and exercises targeting the pelvic area may help maintain good blood circulation and penile health
Medical conditions and disorders	Some medical conditions or disorders can affect penile growth or cause anomalies in penile development. These can include hormonal disorders, congenital abnormalities, and certain genetic conditions. Disorders like Peyronie's disease, characterized by fibrous scar tissue in the penis, can cause curvature and shortening of the penis
Penis disorders and injuries	Trauma, surgical procedures, or diseases affecting the penis can cause changes in penile length. In some cases, surgical procedures might impact the size or appearance of the penis
Age and aging	Natural changes in penile length and appearance as men age may occur due to changes in blood flow, tissue elasticity, and overall health

EPIDEMIOLOGY

Microphallus can be identified at birth or during early childhood when the penis fails to develop to a normal size. However, it's important to note that in some cases, this condition may not become apparent until puberty, when the male experiences delayed or insufficient growth of the penis[24]. There is a slight variation in the average penile length between different races and ethnic groups. For instance, the average penile length for White newborns is 2.6 cm, 2.5 cm for East Indian newborns, and 2.3 cm for Chinese newborns. African and African-Caribbean ethnic backgrounds tend to have, on average, longer penile lengths compared to other ethnic groups. Individuals from Asian ethnic backgrounds tend to have slightly shorter penile lengths than African and African-Caribbean populations. Caucasian/European and Latino/Hispanic Ethnicities fall in the intermediate range in penile length. The prevalence of microphallus/micropenis showed a wide variability in different parts of the world. However, it is generally reported to be about 1/300 male births. These variations between the countries depend on the diagnostic criteria used and the studied population. For example, the incidence of micropenis in North America is approximately 1.5 per 10000 male newborns[25]. A study from Brazil showed an increased incidence of micropenis to reach 6.6/1000. This high rate may be due to environmental (increased exposure to endocrine-disrupting chemicals) rather than racial differences[26]. Another study from Bulgaria showed that the incidence of micropenis was 6.4/1000 in children between 1 year-5 years[27]. Therefore, there is a huge discrepancy in the actual overall incidence of micropenis with a wide range of incidence, between 0.015%-0.66% of male neonates, due to a lack of standardization of measurement and the differences in racial and environmental circumstances[25,26].

ETIOLOGY OF MICROPHALUS AND MICROPENIS

Microphallus and micropenis can be caused by various factors, including genetics, hormones, anatomy, and the environment[3]. Genetic mutations involved in male reproductive system development can result in microphallus[28]. AIS, a genetic disorder, can lead to underdeveloped or absent male genitalia, including a micropenis[29]. Hormonal imbalances during fetal development, caused by deficiencies in testosterone synthesis enzymes, can affect penile growth [30]. Conditions affecting the pituitary gland or chromosomal abnormalities like Klinefelter syndrome can be associated with microphallus[31]. Genetic syndromes such as Bardet-Biedl syndrome and Prader-Willi syndrome may include

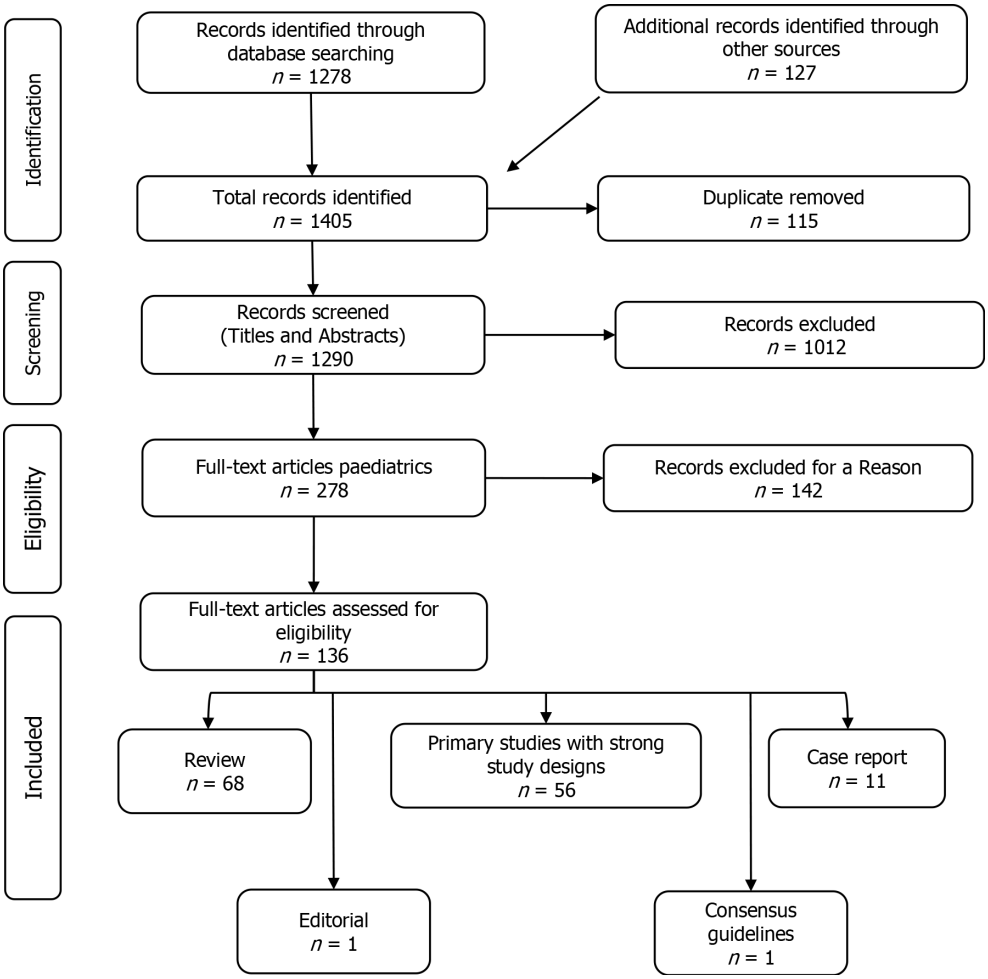

Figure 1 The flow chart of the included studies.

microphallus as a feature[32].

Hormones, especially testosterone, play a crucial role in the normal growth and development of the male reproductive system, including the penis. Primary Hypogonadism and testicular function insufficiency can decrease testosterone production[32]. AIS reduces or eliminates the response to androgens, including testosterone, leading to underdeveloped male genitalia[33]. Enzyme deficiencies affecting testosterone synthesis or conversion can disrupt penile growth[33]. Disruptions in hormonal balance during fetal development can result in microphallus or micropenis[34]. Disorders affecting hormone production, such as adrenal gland dysfunction or pituitary gland/hypothalamus dysfunction, can impact penile development[35,36]. GH deficiency or abnormalities in insulin-like growth factor-1 (IGF-1) production can affect overall growth, including penile growth[37]. Severe or prolonged hypothyroidism in fetuses or infants can potentially affect genital development, causing micropenis[38].

Anatomical and structural abnormalities in the penis, such as severe hypospadias, chordee, or underdevelopment of erectile tissues, can lead to micropenis[39,40]. Vascular abnormalities that affect blood flow to the penis, as in Peyronie's disease, can hinder proper penile growth[41]. Structural abnormalities in the ligaments or connective tissues supporting the penis can also contribute to a smaller penile size. Inadequate penile shaft length during fetal development can result in micropenis[42].

Environmental factors can disrupt hormone levels and impact penile development. Exposure to endocrine-disrupting chemicals like phthalates, BPA, and certain pesticides can interfere with hormone function during fetal growth[43]. Medications or hormones used during pregnancy[44], maternal substance abuse[45], radiation exposure[46], infections [47], inadequate nutrition[47], occupational exposure to chemicals, and improper use of antibiotics[48] or hormonal medications[49] can all potentially contribute to micropenis. Certain medical interventions and treatments for genital disorders can also result in a smaller penis[50]. In some cases, the cause of micropenis remains unknown, labeled as idiopathic micropenis[51]. Table 1 summarizes the common causes of micropenis.

MEASUREMENT AND MONITORING OF PENILE LENGTH AND SIZE

Accurate diagnosis of microphallus and micropenis is crucial due to the significant psychological impact, stress, and anxiety imposed on the patients and their families[52]. Unfortunately, the measurement and monitoring of penile length

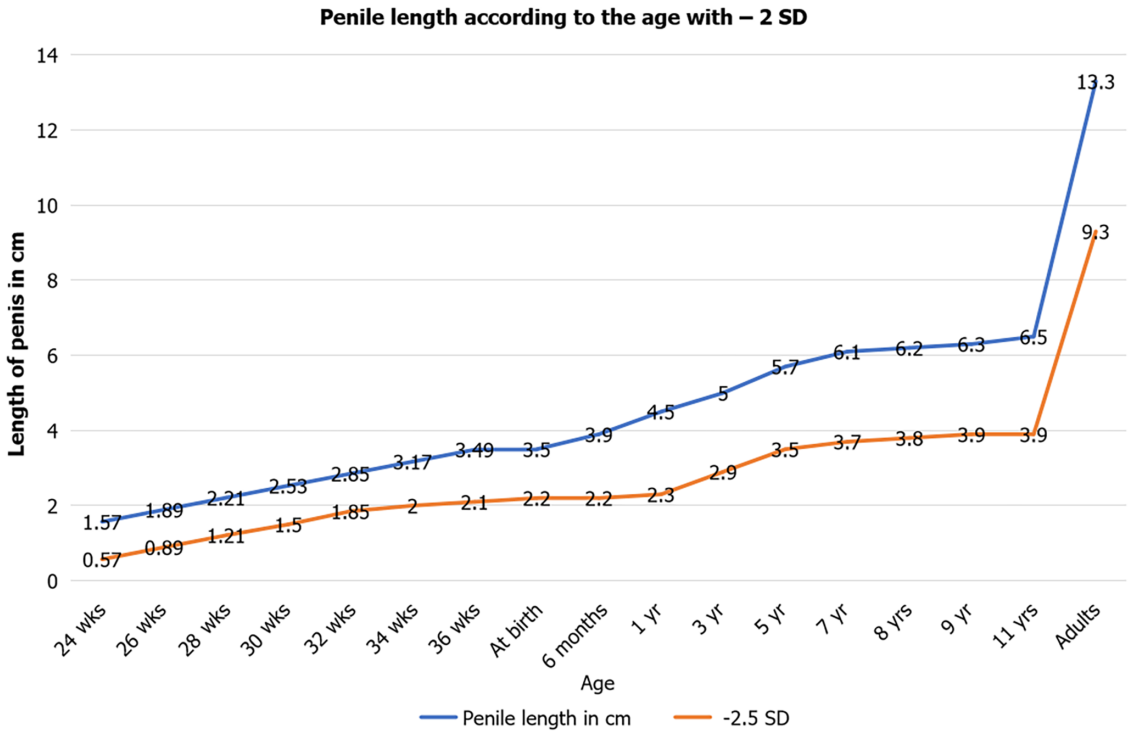

Figure 2 The rate of increase in penile length in both antenatal, postnatal, and adulthood. Micropenis is defined when the penile length is below -2 SD. The figure idea is based on data from Tsang[3]. SD: Standard deviation.

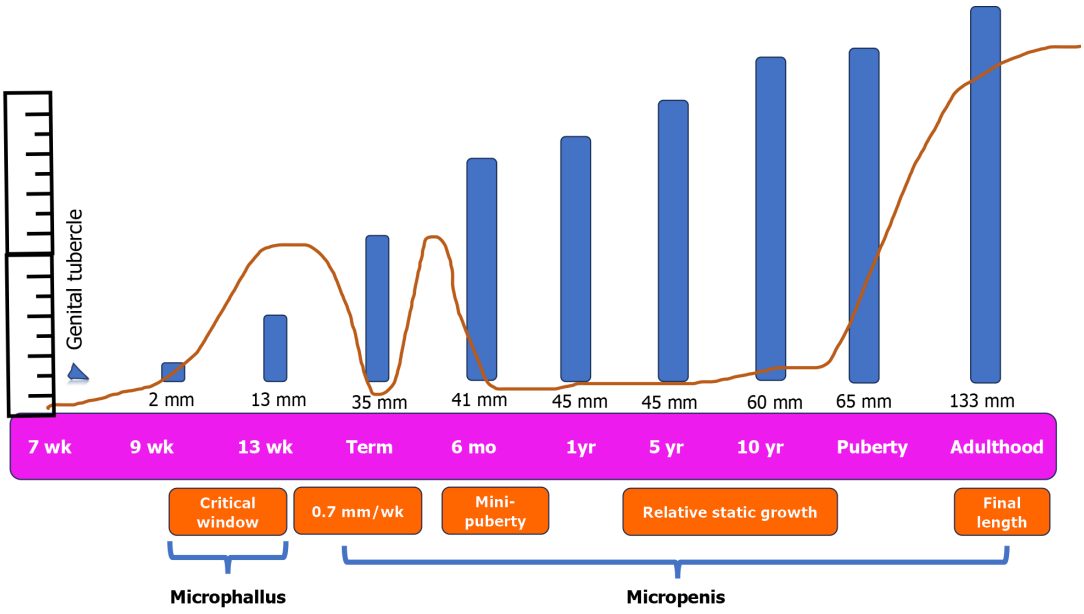

Figure 3 The prenatal and postnatal penile development and its relation to the testosterone surge. Note that any disruption of penile development during the critical window will cause microphallus, which is usually associated with congenital malformation such as hypospadias, while after that causes micropenis. Note the prenatal testosterone surge is associated with rapid penile growth of 0.7 mm/wk. The first postnatal testosterone surge occurs between the second and third month when the testosterone level reaches 260 ng/dL and is associated with rapid penile growth, known as mini puberty at six months of postnatal age. The second postnatal testosterone surge occurs at puberty and continues throughout adulthood. The figure idea and data were obtained from Chitayat and Glanc[10], Blaschko *et al*[11], Penington and Hutson[13], and Tuladhar *et al*[15].

and size in children during medical examinations is a controversial topic. Some experts believe that measuring and monitoring penile length and size is crucial to detect any abnormalities early on, while others consider it unnecessary and can be embarrassing for the child. However, measuring and monitoring penile length and size can be important for various reasons, including medical evaluation, research, and patient counseling[53]. There is no consensus on measuring penile length and size in children, and experts have different recommendations. Some suggest measuring the length of the erect penis from the base to the tip, and others suggest measuring the length of the flaccid penis from the base to the

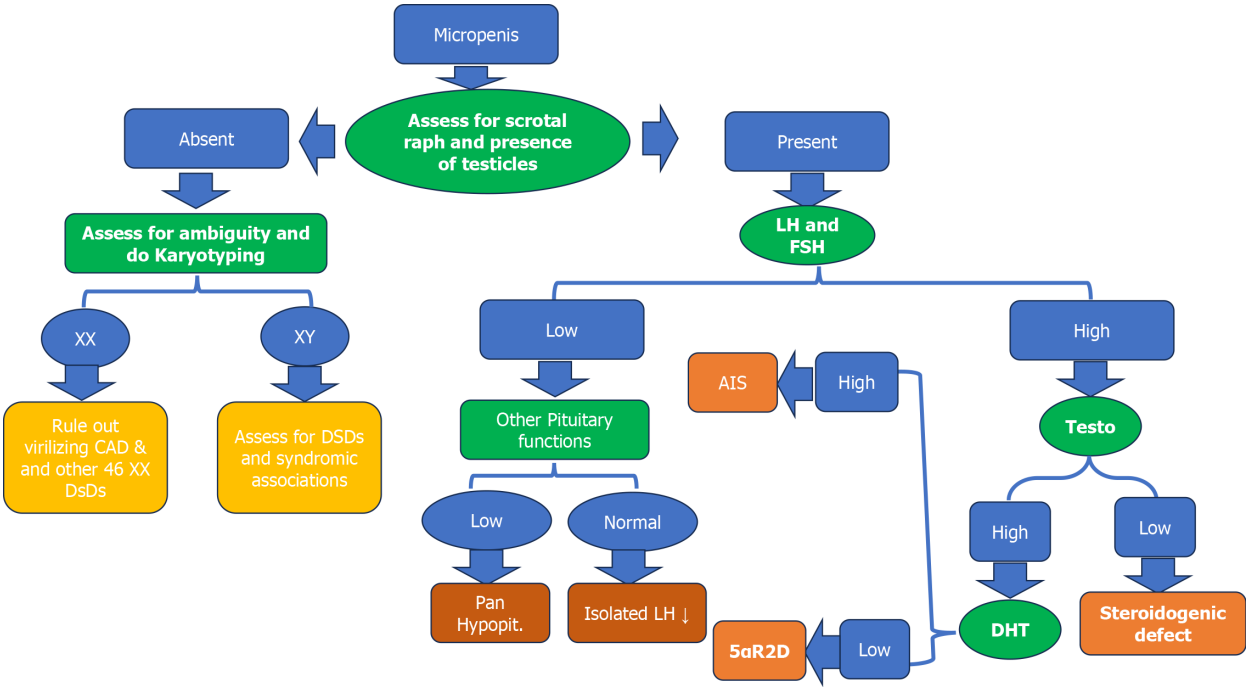

Figure 4 Flow chart for hormonal assessment of micropenis. Note that follicle-stimulating hormone, luteinizing hormone, testosterone, and dihydrotestosterone are measured in the basal state in min-puberty (at the age of 6 months) and a stimulated state after human chorionic gonadotropin injection after the age of 6 months. 5αR2D: 5α-Reductase-2 deficiency; AIS: Androgen insensitivity syndrome; CAD: Congenital adrenal hyperplasia; DHT: Dihydrotestosterone; DSDs: Disorders/differences of sex development; FSH: Follicle-stimulating hormone; Hopopit: Hypopituitarism; LH: Luteinizing hormone.

middle of the glans. Healthcare professionals may measure penile length and width during routine check-ups to monitor growth and development. Measurements are typically taken while the penis is stretched to obtain an accurate assessment. Stretched penile length is measured from the pubic ramus to the tip of the glans[54]. To measure the stretched penis, the flaccid penis is gently stretched horizontally, and the length is measured from the pubic bone to the tip of the glans while keeping the penis stretched as straight as possible[55]. The examiner should press the suprapubic fat pad inwards as much as he can and retract the foreskin, if present, during the measurement. Another way to stretch the penis is to use a modified syringe to make negative suctioning of the peins, stretching it while the suprapubic fat is pressed internally (Figure 5). A ten mL disposable syringe is cut off at the needle side, and the piston is re-inserted into the syringe on the cut side (Figure 6). The open side of the syringe is applied to the penis to exert a negative suction of the penis by pulling back the piston, causing the penis to be pulled inside the syringe[7].

For measuring a flaccid penis (completely relaxed, not erect) while the infant or child is lying on the back, a penile ruler or tape measure is used to measure the penis from the pubic bone to the tip of the glans along the dorsal (top) side[56]. To measure a fully erect penis, a ruler or tape measure is used to measure from the pubic bone to the tip of the glans along the dorsal (top) side. The penis should be fully extended and straight during the measurement to obtain the most accurate length. The measurement should be from the pubic bone, which is the base of the penis, to the tip of the glans without any bends or curves[57]. Multiple measurements should be taken to increase accuracy, and the average should be calculated. The measurements should be recorded in both centimeters (cm) and inches (in) for reference and comparison. While measuring the child's penile length, we should consider the wide natural variation in penile size and individual growth patterns. The physician should respect privacy and comfort during measurement, especially in clinical or medical settings[54].

Measuring penile length accurately is crucial in diagnosing true micropenis. A penile length measurement of 2.5 SD below the mean for age, in the presence of external and internal male genitalia compatible with a 46, XY male karyotype, is sufficient to diagnose micropenis[7]. Penile length can be measured using a penile ruler or measuring tape, or through specialized software and equipment that analyze images of the penis. This method involves taking standardized photographs and using computer software to measure penile length precisely; it is often used in research studies and clinical trials[58]. Penile plethysmography is a technique that measures changes in penile circumference or volume to assess sexual functioning and response, primarily in clinical and research settings[59]. Ultrasound imaging can provide detailed information about the anatomy of the penis, including measurements of penile length and diameter. It is often used in medical settings to evaluate penile abnormalities or during the assessment of erectile dysfunction[60].

Monitoring the length and size of the penis regularly can provide valuable insights into growth patterns and help address any concerns related to penile development. It is crucial to approach these measurements with sensitivity, especially when dealing with individuals who may have concerns about their penis size. To compare measurements with the average growth expected for a particular age group, we should use growth charts specific to penile length for age[61]. During puberty, we should periodically measure penile length (*e.g.*, every 6 months-12 months) to track changes in size as the individual progresses through adolescence[62]. Measuring and monitoring the penile length and size can be

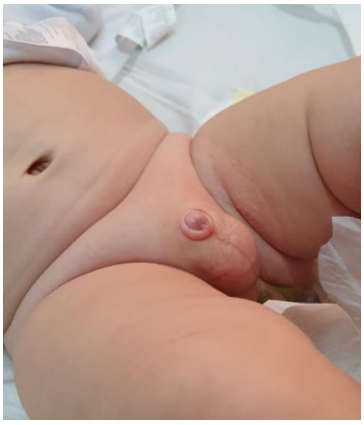

Figure 5 Pseudomicropenis due to a buried penis; the penis is partially or completely concealed beneath the scrotum or excess skin or fat in the pubic area.

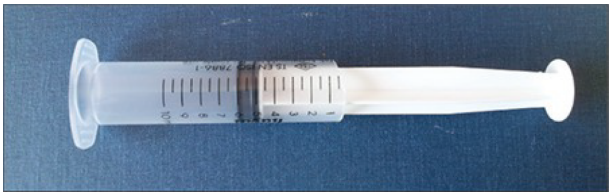

Figure 6 A suction 10-mL syringe exerts traction on the infant penis for stretched penile measurement. The idea is adopted from Hatipoğlu and Kurtoğlu[7].

important for various reasons, such as assessing growth and development, diagnosing conditions like micropenis, or evaluating the effects of treatments. When measuring or monitoring penile length, following these guidelines for accurate results is crucial[42]. Firstly, use the same method and conditions, such as the same level of arousal and stretch, for each measurement. This ensures consistency and accuracy in the results. Secondly, providing a comfortable and private measurement environment is vital to reduce any anxiety or tension that might affect the measurements. Thirdly, remember that penile size varies among individuals, and there is a wide range of normal variation. Therefore, it is important not to base self-esteem or self-worth solely on penile size. It is really crucial to differentiate between a true micropenis and a pseudo micropenis. In the latter condition, the penis seems small due to the prominence of surrounding tissue or due to the presence of a penile web that adheres the penis to the underlying skin. A thorough physical examination is essential to exclude pseudo-micropenis and avoid unnecessary invasive diagnostic procedures that can induce psychosocial stress for the patient and his family. Another surgical condition to be aware of is the chordee of the penis, which can cause an abnormally curved shaft and may falsely underestimate the penile length[63].

DIAGNOSTIC WORKUP FOR CASES WITH MICROPHALLUS

When clinical evaluations confirm microphallus or micropenis, further assessment is necessary to diagnose underlying conditions and plan appropriate treatment options. Search for signs of ambiguity or dysmorphism. General examination may give keys for diagnosis. For example, hearing impairment and anosmia may indicate the presence of Kallman syndrome, while cardiac defect may indicate Noonan syndrome. In some cases, genetic testing may be recommended to identify any genetic abnormalities that could be contributing to the condition[64,65]. Chromosomal analysis may reveal conditions such as Klinefelter syndrome (with an extra X chromosome), which can affect genital development. Hormonal evaluation is essential to determine if any hormonal imbalances could be causing the microphallus[16]. This may include measuring testosterone precursors, testosterone, DHT, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, estradiol, and other relevant hormones such as cortisol, thyroid stimulating hormone, GH, and IGF-1. Low LH levels may indicate hypogonadotropic hypogonadism, and a magnetic resonance imaging (MRI) of the brain and pituitary functions evaluation is needed[66]. If LH is high, then we test for serum testosterone. A steroidogenic defect is suspected if serum testosterone is low with high LH. If serum testosterone is high with high LH, we may need to test for DHT levels[67]. Low DHT with high LH and testosterone levels and a high testosterone/DHT ratio may indicate 5α-Reductase 2 deficiency (5αR2D). High LH, testosterone, and DHT levels may indicate AIS[68]. A low testosterone/DHT ratio may indicate partial AIS. Serum electrolytes are also needed for any neonate presented with microphalus, especially when associated with any degree of ambiguity, as the risk for congenital adrenal hyperplasia is high, and the child may go into shock if not discovered early (Figure 4)[69].

Hormonal stimulation tests may be needed, especially those that involve stimulating the release of hormones, particularly GH or testosterone. Serum testosterone levels should be measured before or after administering hCG. testosterone levels below 300 ng/dL after hCG stimulation suggest gonadal dysfunction[70]. Monitoring the body's response to these stimulants can help identify hormone deficiencies or abnormalities. Testicular insufficiency can be diagnosed if there is a rise in serum LH and FSH after hCG injection without a significant rise in serum testosterone[71]. Testosterone synthesis defects can be diagnosed by measuring 17 hydroxyprogesterone, androstenedione, and dehydroepiandrosterone levels before and after hCG injection. Mullerian-inhibiting hormone and inhibin B can be measured to indicate the presence of functional Sertoli cells and testicular tissue[72].

To assess the anatomy of the penis and surrounding structures, imaging studies such as ultrasound or MRI may be conducted depending on the clinical presentation[73]. Ultrasound imaging may be used to assess the anatomy of the genital region, including the penis and testicles[74]. It can help identify any structural abnormalities or congenital conditions affecting penile growth. MRI may be recommended to obtain detailed images of the penis and surrounding structures, particularly if ultrasound results are inconclusive. Brain MRI is useful for identifying midline structural defects, such as pituitary hypoplasia, dysplasia, or stalk dysplasia syndrome. If a person has a small posterior pituitary gland, a thinned or missing pituitary stalk, or posterior pituitary ectopia, these findings may suggest hypopituitarism [75]. Therefore, the cause of hypopituitarism can be determined through MRI imaging. Bone age assessment with an X-ray of the left hand and wrist can provide information about the individual's growth and development by assessing bone age[76].

A psychological evaluation may also be necessary to assess the psychological and emotional well-being of the individual, particularly in adolescents and adults, to address any concerns related to body image and self-esteem[77]. Every patient with microphallus should be assessed psychologically to evaluate their emotional well-being, mental health, body image, self-esteem, social interactions, academic performance, coping mechanisms, communication, family dynamics, decision-making, and long-term well-being[78]. It identifies mental health issues, evaluates the condition's impact on a child's perception of themselves and their body image, identifies social interaction difficulties, learning or concentration issues, and suggests appropriate interventions. The assessment also provides insights into the family dynamics and support system's influence on the child's mental health, evaluates their understanding and ability to make informed decisions, and helps design individualized psychological interventions[79]. The psychological assessment aims to assess a child's mental health and well-being, providing a comprehensive understanding of their psychological state, needs, and resilience factors, contributing to a better quality of life in the long term[80].

MANAGEMENT OF MICROPHALLUS/MICROPENIS

After adequate and proper evaluation of the microphallus by thorough clinical examination, hormonal assessment, and imaging studies, we need proper management through a multidisciplinary approach to address this condition's physical and psychological aspects. The main treatment goal in boys with micropenis is mainly based on increasing the length of the penis, assuming that it increases the child's self-esteem and body image, reassuring the parents, and alleviating their anxiety[81]. The management should be tailored to the individual's unique circumstances and needs, aiming to improve quality of life and address the condition's physical and psychological aspects. To ensure comprehensive and effective management, it's important to involve a team of healthcare professionals, including endocrinologists, urologists, psychologists, and counselors. Traditionally, the primary objective in treating boys with microphallus is centered on enhancing the penile length, believing that it positively impacts the boy's self-esteem and body image, simultaneously offering reassurance to the parents of the newborn[82].

HORMONAL THERAPY

Hormone replacement therapy, such as testosterone supplementation, may be considered if the microphallus is associated with hormonal deficiencies. After addressing the hormonal imbalances or deficiencies contributing to the micropenis development, hormonal therapy can help optimize the hormonal balance in the body, potentially promoting the growth and development of the penis. Therefore, the underlying defect should be confirmed before starting hormonal therapy, and the treatment will be tailored accordingly. Fortunately, sex hormone therapy helps in diagnosis and treatment as it helps assess androgen responsiveness. The timing of hormonal therapy should start early after the age of 6 months (after mini puberty)[83,84].

In idiopathic micropenis (80% of cases) and hypogonadotropic hypogonadism, testosterone is administered briefly to assess penile response through intramuscular injection, topical application, or suppository. Four doses of 25 mg (or 100 mg/m^2) of intramuscular testosterone cypionate, enanthate, or undecanoate are given at 3-wk intervals to complete three months[85]. It helps to assess androgen responsiveness. The course can be repeated with shorter intervals if there is no adequate response. The three different forms of intramuscular testosterone differ in their duration of action, with testosterone enanthate lasting for the shortest time, while undecanoate lasting the longest; therefore, it is given at 4-wk intervals[86]. Unfortunately, there is no consensus on dosage, administration method, or testosterone treatment duration for children with micropenis. Side effects are minimal, although temporary growth rate acceleration, advancement of bone age, premature growth spurt, and precocious puberty may occur. Other side effects may occur, such as pain at the injection site, headache, high blood pressure, and pronounced gynecomastia[87]. Intramuscular testosterone therapy is associated with a good response that typically involves a 100% increase in penile length during the initial treatment.

Additionally, testosterone therapy is used to treat scrotal hypoplasia in young children. Rectal testosterone is used in some cases with hypogonadotropic hypogonadism (panhypopituitarism and congenital hypogonadotropic hypogonadism) in a daily dose of 1 mg-5 mg and exhibited an increase in penile length and scrotum width. An increase in penile length of 3.5 cm is considered an adequate response. Adding aromatase inhibitors could increase the response to testosterone therapy[88]. Anastrozole is a nonsteroidal aromatase inhibitor that decreases the amount of estrogen in the body. Papadimitriou added oral anastrozole in a dose of 1 mg/d to intramuscular testosterone enanthate for three months to treat idiopathic-isolated-relative micropenis at the beginning of puberty. They showed a significant improvement in penile length by about 20%, while no effect on height velocity and bone maturation[89].

Topical testosterone also shows a good effect. Arisaka *et al*[90] showed increased penile lengths in 50 infants and children aged between five months and eight years by administering 5% testosterone cream for 30 d. Topical testosterone can stimulate GH secretion and promote bone growth, indicating the long-term effect of topical testosterone application on promoting both skeletal and penile growth[90]. Clinical studies have indicated positive effects of testosterone treatment on penile growth during infancy; however, whether this growth continues into adolescence and adulthood remains unclear. Topical dihydrotestosterone gel has been used to treat underutilized children with 5α-reductase deficiency or with partial AIS, an alternative to intramuscular testosterone, since 1990[91]. Table 4 compares between micropenis due to 5αR2D and AIS, including management.

When applied topically, DHT gel absorption can be unpredictable and inconsistent. There is also a risk of cross-contamination if the application instructions are not followed properly, which can affect people in close contact. The procedure for applying DHT transdermal gel to genital skin lacks standardization, with varying doses and durations reported in different studies[16]. Some studies show a significant increase in stretched penile length due to DHT treatment, while others report adverse effects such as changes in lipid profiles and mild skin reactions. The response to DHT treatment, as measured by stretched penile length, can vary depending on the underlying diagnosis and age at which therapy is started. DHT treatment has increased stretched penile length in pre and peripubertal patients with partial AIS and 5α-reductase deficiency but not in adults[92,93] Reduced androgen sensitivity in DHT-dependent tissues due to intracellular DHT deficiency may explain the limited effect of exogenous DHT in 5α-reductase deficiency[13]. It is important to determine the best age for therapy to maximize its effect. Micropenis is preferably treated in infancy (better to be after mini puberty and before 2 years of age) or at the onset of puberty[94]. Research suggests that this may be related to the time when androgen sensitivity is at its peak. The high expression of androgen receptor (AR) in early infancy suggests that androgens may be useful at this stage. However, it is unclear whether early use of androgens has any long-term benefits on penile length in adulthood[95,96]. Selective androgen receptor modulators (SARMs) are currently in the developmental phase and are pending approval. One anticipated benefit of SARMs is their proposed selectivity with fewer systemic side effects[97].

Gonadotropins are essential to the maturation and proper functioning of gonads. In 1993, Almaguer *et al*[98] documented the first use of hCG for micropenis treatment. They found that six neonates experienced significant penile growth following three daily intramuscular injections of 1500 IU of hCG[98]. Since then, recombinant gonadotropins have been suggested as an alternative treatment to testosterone for male infants and peripubertal boys with congenital hypogonadotropic hypogonadism. This treatment aims to replicate the physiological activation of the hypothalamic-pituitary-gonadal axis[99]. The first case of recombinant gonadotropin treatment for congenital hypogonadotropic hypogonadism was reported in 2002 by Main *et al*[100]. This treatment included recombinant LH (20 IU-40 IU) and FSH (21.3 IU) administered twice weekly for approximately seven months. The treatment successfully improved penile length and stimulated testicular growth and physiological mini-puberty[100]. Other studies have also noted the effectiveness of gonadotropin treatment in increasing stretched penile length in boys with congenital hypogonadotropic hypogonadism during their first year of life. Bougnères *et al*[101] described continuous gonadotropin infusion by an insulin pump to treat two newborns with micropenis and congenital hypogonadotropic hypogonadism. Patient 1 began subcutaneous infusion at eight weeks with rhLH 56 IU and rhFSH 67 IU daily until 25 wk of life. Patient 2 initiated subcutaneous infusion at 20 wk with rhLH 50 IU and rhFSH 125 IU daily until 48 wk of life. This treatment resulted in stretched penile length increasing from 8 mm to 30 mm in the first newborn and from 12 mm reaching 48 mm in the second newborn, accompanied by increased testicular volume and elevated serum testosterone, inhibin B, and anti-mullerian hormone levels in both neonates[101]. However, comprehensive reports on gonadotropin treatment during the neonatal and infant periods still need to be made available. Further research is necessary to compare the relative efficacy of hCG and LH, given differences in their half-life. Additionally, long-term studies are required to investigate outcomes like fertility.

GH therapy is a controversial but viable treatment option for micropenis, especially when it is linked to GH deficiency or short stature. GH promotes the growth of various tissues, including genitalia, and its application in micropenis cases aims to enhance overall growth and potentially improve penile size[102]. Oh *et al*[103] found synergetic effects of GH therapy on penile growth by enhancing the AR expression levels and reducing the testicular volume losses. However, the response to GH therapy may vary among individuals. The duration of treatment is determined based on an individual's specific growth needs but often needs an extended period. A multidisciplinary team of healthcare professionals, including pediatric endocrinologists and urologists, evaluates and monitors the effectiveness of the therapy. While GH therapy can be beneficial in certain cases, its impact on penile growth may not be significant for all individuals, and its use is tailored to the patient's unique condition and health requirements. The decision to use GH therapy is made within a comprehensive treatment plan considering the patient's overall health and underlying causes of micropenis. It may also be combined with other treatments for a multimodal approach[104].

Combined hormonal therapy for micropenis typically involves the administration of both testosterone and other hormones, such as gonadotropins, gonadotropin-releasing hormone analogues, aromatase inhibitors, topical DHT, GH, or other hormones that play crucial roles in male sexual development and function[23,99,100,103,105]. The decision to pursue combined hormonal therapy is based on a thorough evaluation of the underlying causes of micropenis, which

Table 4 Differences between 5α-reductase type 2 deficiency and androgen insensitivity syndrome

	5αR2D	AIS
Primary genetic defect	It is due to a deficiency in the 5α-reductase type 2 enzyme that converts testosterone to DHT	Genetic mutations in the androgen receptor gene result in the inability of cells to respond to androgens (*e.g.*, testosterone)
Inheritance	Autosomal recessive	X-linked recessive
Chromosome location	2p23	Xq11-q12
Genotype	Typically, 46, XY karyotype (male genotype)	Typically, 46, XY karyotype (male genotype)
Phenotype	Ambiguous genitalia in male infants. Varying degrees of under-virilization during puberty	Variable degrees of feminization and incomplete masculinization despite the presence of male internal and external genitalia
Hormonal profile	Reduced levels of DHT. Normal or elevated levels of testosterone, elevated LH	Elevated testosterone levels due to a lack of androgen receptor responsiveness, elevated LH, and normal MIF
Response to androgens	Reduced response to androgens due to inadequate conversion of testosterone to DHT. DHT plays a key role in the process of sexual differentiation in the external genitalia and prostate during the development of the male fetus	Lack of response to androgens despite normal or elevated testosterone levels
Internal reproductive organs	Typically have normal male internal reproductive organs (*e.g.*, testes, vas deferens, epididymis). Testes located in the inguinal canal or scrotum	Typically, they have normal male internal reproductive organs. Testes located in the abdomen or inguinal canal
External genitalia	Ambiguous or underdeveloped male external genitalia	External genitalia: Neonate: Female (complete type). But may appear as normal male external genitalia but with varying degrees of feminization (partial type)
Pubertal development	Affected males still develop typical masculine features at puberty (deep voice, facial hair, muscle bulk) since most aspects of pubertal virilization are driven by testosterone, not DHT	Minimal virilization, with absent or minimal facial hair, high-pitched voice, and breast development
Fertility	Reduced fertility due to underdeveloped reproductive organs	Infertility due to absent or underdeveloped reproductive organs
Psychological Impact	Gender dysphoria may occur due to ambiguous genitalia and delayed or incomplete virilization	Gender dysphoria may occur due to female-appearing genitalia and lack of virilization
Treatment	Hormone replacement therapy may be considered to supplement DHT. Testosterone therapy may be used to induce virilization	Hormone replacement therapy is not effective due to insensitivity to androgens. In complete type, the patient is treated as female, and estrogen therapy is indicated with orchidectomy. Orchidectomy aims to prevent possible malignant degeneration of the testes. Psychological support and surgical interventions may be considered

5αR2D: 5α-Reductase Type 2 Deficiency; AIS: Androgen Insensitivity Syndrome; DHT: Dihydrotestosterone; LH: Luteinizing hormone; MIF: Müllerian-inhibiting factor.

may include genetic factors, hormonal imbalances, or other medical conditions[7]. The primary goal of combined hormonal therapy is to correct hormonal imbalances that may contribute to micropenis. For example, combined treatment may address testosterone and gonadotropin deficiencies, which are essential for normal penile development[16]. The specific combination and dosage of hormones used in combined hormonal therapy are individualized based on the patient's medical history, hormonal profile, and specific needs. Combined hormonal replacement therapy is more likely to be associated with potential side effects, such as changes in blood lipid profiles, fluid retention, and mood swings[106]. Therefore, regular monitoring of hormone levels and physical and clinical assessments are crucial to assess the therapy's effectiveness and side effects and adjust the treatment plan as needed. Table 5 summarizes advantages and disadvantages of various hormonal therapies for microphallus.

SURGICAL MANAGEMENT

Surgical management of micropenis has come a long way since the early 1970s[107]. Guidelines for penile elongation were established in 1996 by Wessells *et al*[108], which recommended surgical intervention only for men with a penile length of less than 4 cm in the flaccid state or 7.5 cm in the stretched state. However, surgical management of micropenis in infants and children is a complex and highly specialized area of pediatric urology. The medical team usually waits until the child reaches an appropriate age, often in late adolescence or adulthood, due to the ongoing growth and development of the genitalia.

Table 5 Advantages and disadvantages of various hormonal therapies for microphallus

Hormonal therapy	Advantages	Disadvantages
Intramuscular testosterone	Promotes penile growth and development by addressing testosterone deficiencies. Enhances the development of secondary sexual characteristics. Improves muscle mass, bone density, and overall well-being	Potential side effects include acne, mood swings, increased aggression, and accelerated skeletal maturation. May suppress natural testosterone production if used long-term. Requires regular monitoring of hormone levels and administration through injections or topical applications
Topical	Non-invasive administration, more comfortable. Gradual absorption with more stable and consistent hormone levels. Less Pain and Discomfort. Easy Application and more convenient treatment option	Erratic absorption with hormonal fluctuations, limited efficacy, and uncertain outcomes. Risk of transfer to others, especially children and women, through close physical contact with the application site. Skin Sensitivity and allergic reactions to the topical product. Lack of Standardization
hCG	Stimulates the testes to produce testosterone, aiding in penile growth. Can be used in combination with other hormonal therapies for enhanced results. Administration *via* injections or subcutaneous pellets	Potential side effects may include fluid retention, breast tenderness, and mood swings. Requires careful monitoring and adjustment of dosages to avoid adverse effects
Aromatase inhibitors	Inhibit the conversion of androgens to estrogen, potentially increasing testosterone levels and aiding penile growth. May be considered for individuals with aromatase excess syndrome	Limited evidence on efficacy and safety for promoting penile growth. Possible side effects include joint pain, mood swings, and bone density issues
GH therapy	Stimulates growth and may indirectly impact penile growth. Beneficial for children with growth hormone deficiencies or short stature	Limited evidence regarding the direct impact on penile growth in microphallus cases. Potential side effects include fluid retention, joint pain, and increased risk of diabetes
GH therapy	Combines multiple hormones (*e.g.*, testosterone, hCG) to synergize and enhance penile growth potentially. May optimize hormonal treatment effectiveness for promoting penile growth	Increased complexity of treatment regimen with potential for elevated side effects due to multiple hormonal agents. Requires vigilant monitoring and management of potential adverse effects

hCG: Human chorionic gonadotropin; GH: Growth hormone.

Surgical interventions for micropenis are typically deferred until the child reaches an appropriate age, often in late adolescence or adulthood, due to the ongoing growth and development of the genitalia. It is often reserved for the most extreme cases. The medical team carefully considers the timing of surgery. Surgical procedures can vary depending on the specific needs of the child. Common surgical techniques may include penile lengthening, girth enhancement, visual appearance improvement, augmentation phalloplasty, or even replacement of the phallus. Penile lengthening procedures aim to increase penile length through various techniques, such as releasing the suspensory ligament or V-Y dorsal incisions[109,110]. On the other hand, girth enhancement procedures aim to increase penile girth, often involving fat grafting or the injection of hyaluronic acid[111]. A complete replacement of the penis with an augmentation phalloplasty is considered in augmentation phalloplasty. This technique is a more extensive surgical procedure. Other methods like sliding elongation and penile disassembly, have also been documented[112].

Surgical management of micropenis carries potential risks, including limited increase in penile length (1 cm-3 cm), scarring, changes in sensation, and effects on sexual function. The potential benefits include improved penile size and appearance[113,114]. Perceived penile length can be enhanced by eliminating suprapubic fat, achievable through weight reduction measures or surgical removal using liposuction or more extensive procedures[115]. However, despite the advancements in surgical techniques, they cannot fully replicate the normal anatomy and function of the penis. It is important to carefully consider these procedures' risks, benefits, and potential outcomes. Further research is essential to identify the optimal surgical procedure, focusing on long-term patient satisfaction and minimizing postoperative complications.

PSYCHOLOGICAL COUNSELING, EDUCATION, AND SUPPORT

Psychological support and counseling are essential for managing microphallus and micropenis. Mental health professionals can assist individuals in coping with the emotional and psychological difficulties associated with the condition, leading to improved self-esteem and overall well-being. Counseling and support are crucial for infants, children, and adolescents with micropenis to help them navigate their physical, emotional, and social challenges. Micropenis can profoundly impact a person's self-esteem, body image, and overall well-being[116].

Pediatricians can provide psychological counseling and support during the different stages of development. During infancy, pediatricians can offer emotional support and guidance to parents concerned about their child's condition[117]. Accurate information about micropenis and potential treatment options must be provided. They can also properly connect parents with pediatric endocrinologists and urologists who can assess the cause of the micropenis and recommend treatment, if necessary. During childhood, integration between pediatricians, families, and schools is crucial. Depending on the child's age and maturity, age-appropriate education can be provided, delivering information about

micropenis in a way that is appropriate for their age[118]. This education should explain that physical differences are normal and not a cause for shame. Positive self-esteem and body image can be encouraged by emphasizing a child's other qualities, talents, and achievements. Children should understand that their self-worth goes beyond physical appearance. Strategies should be taught to help them deal with teasing or bullying if it arises, and children should be encouraged to communicate with parents and educators[119].

Puberty is a critical stage for all individuals, especially those with micropenis. Adolescence can be incredibly challenging for those with micropenis, as it is a period of increased self-awareness and self-identity. Therefore, building self-esteem, self-acceptance, and self-compassion is crucial to providing accurate information about sexual health, relationships, and intimacy that is appropriate for their age. Adolescents should learn the importance of communication, consent, and safe sexual practices[120]. They should be encouraged to seek out supportive friends and groups focusing on self-acceptance and diversity. Joining support groups or engaging in therapy sessions with individuals facing similar challenges can provide emotional support and a sense of community. Sharing experiences and coping strategies can benefit those dealing with the condition. If adolescent struggles with body image issues, depression, or anxiety related to their condition, we should consider referring them to a therapist or counselor experienced in dealing with body image and self-esteem issues[121].

In all stages of development, open and non-judgmental communication between the child or adolescent, their parents, and healthcare providers is essential. Professionals should approach this topic with sensitivity and respect, focusing on the individual's emotional well-being and overall development. Additionally, interventions to address micropenis, such as hormone therapy or surgery, should be discussed and decided upon with the individual's and their parent's full consent, when applicable. Ultimately, the goal is to help individuals with micropenis develop a healthy self-image, self-confidence, and a positive outlook on their future, regardless of their physical condition[122].

LIFESTYLE MODIFICATIONS

Lifestyle modifications for children and adolescents with micropenis aim to create a supportive and healthy environment that fosters self-confidence and overall well-being. This modification includes promoting healthy body image and self-esteem, providing accurate information about their condition, teaching strategies to address bullying or teasing, and encouraging peer and social support[123]. Additionally, emphasizing a healthy lifestyle, including a balanced diet, adequate sleep, exercise, and hygiene, is essential and can positively impact overall well-being. Healthy lifestyle habits contribute to better physical health and may indirectly improve self-esteem and body image. Sex education should be part of the education, focusing on relationships, consent, and safe practices. Seeking professional help for psychological distress, considering medical consultation and treatment options, and maintaining a supportive family environment are also vital[124]. The approach for lifestyle modifications should be individualized, considering the child or adolescent's unique needs and circumstances. It is essential to provide a safe and supportive environment where the affected individuals feel accepted and loved regardless of their physical condition. Ultimately, the goal is to empower these individuals to thrive, set goals, and develop a positive self-image regardless of their physical condition[125].

CONSEQUENCES OF MICROPHALLUS/MICROPENIS

Throughout various historical epochs, the size of the male genitalia has been considered a symbol of masculinity, leading to extensive debate in societies with distinct social and cultural nuances. Apart from its role in sexual intercourse, the penis has been associated with male fertility and sexual performance, making its size a crucial aspect of male identity. The use of phallic size to support male dominance and superiority has been common in various cultures and historical periods and has continued to be reinforced by contemporary media, especially in the adult entertainment industry[126, 127]. This societal view that links the length of the penis to masculinity has placed a heavy burden on those who suffer or believe they suffer from microphallus.

A micropenis, or microphallus, can have significant physical, psychological, and social impacts on those affected. A smaller-than-average penis can lead to sexual difficulties, affecting sexual self-esteem and relationships due to challenges during sexual intercourse, including penetration and maintaining an erection[128]. This condition may also be linked to other reproductive system abnormalities, potentially impacting fertility and family planning. Dealing with a micropenis often brings about profound psychological distress, causing feelings of shame, embarrassment, anxiety, depression, and lowered self-esteem due to societal expectations about male genitalia[129]. These emotional struggles can have a lasting effect on mental well-being. Social interactions and relationships can be strained, as individuals with a micropenis often face stigma, discrimination, or teasing, impacting both romantic and platonic relationships. Daily activities related to personal hygiene, urination, and other functions may also pose challenges due to the size of the penis, affecting overall quality of life[130]. Seeking resolution, individuals with micropenis may explore medical interventions such as hormone therapy, penile lengthening procedures, or psychological counseling to address the physical and psychological aspects of the condition. However, these interventions can be invasive, costly, and may not always provide satisfactory results[131]. Dealing with a micropenis can challenge traditional notions of masculinity and male identity, given the societal association of penis size with masculinity and sexual prowess, adding to the psychological burden[132]. Individuals with micropenis may encounter difficulties in educational or professional settings due to the psychological stress or anxiety tied to their condition, potentially affecting their career prospects and advancement opportunities. Effectively addressing the consequences of a micropenis requires a holistic approach involving medical care, psychological support, education,

and societal awareness initiatives to promote understanding and acceptance[133].

MONITORING AND FOLLOW-UP

It is crucial to monitor and follow up with infants, children, and adolescents who have a micropenis. For infants, it is vital to have regular check-ups with a pediatrician and hormone assessments to rule out any underlying conditions[134]. During childhood, annual check-ups should continue, emphasizing psychological support and education to promote positive body image and address potential bullying. For adolescents, annual check-ups and psychological support are essential, along with sex education, peer support, and consultation with specialists for potential treatments[135]. Long-term follow-up into adolescence and beyond is necessary to provide continued mental health support, health check-ups, reassessment of treatment options, and maintaining a supportive family environment. The goal is to provide comprehensive care that addresses the physical and psychological aspects of micropenis and adapts to the individual's needs as they grow[136]. Table 6 summarizes follow-up care for various life stages of patients with micropenis.

LIMITATIONS OF THE STUDY

This study has some limitations. First and foremost, it relies heavily on the quality and reliability of data from existing literature, which may exhibit variations in accuracy and consistency across different sources. Moreover, the presence of publication bias is a significant concern, as it can skew the selection of studies towards those with notable findings, potentially affecting the overall conclusions. Additionally, data selection bias may be inherent in the process of choosing relevant literature, favoring studies with specific focuses, which might only encompass part of the subject matter. Notably, the absence of primary research data poses a substantial limitation, as the study primarily draws from secondary sources. The heterogeneity among the included studies regarding methodologies, populations, and diagnostic criteria can impede the synthesis of results and generalizability. Temporal bias is a consideration since the included literature might need to reflect current medical practices and criteria. The study might also exhibit language bias if confined to literature published in a specific language, potentially excluding relevant research in other languages. Moreover, the scope may only encompass micropenis cases and treatment options from some demographics and regions, affecting the generalizability of the findings. The influence of conflicts of interest, particularly in studies discussing treatment modalities, presents another potential bias. The psychological aspects discussed in the study can introduce complexities and subjectivity in interpreting results, as the psychological impact of micropenis varies among individuals. Identifying research gaps highlights the limitations in data sufficiency and the potential lack of effective data for certain treatments. Therefore, while this study provides valuable insights, these limitations should be considered when interpreting its findings and identifying areas for future research and methodology improvement.

TAKE-HOME MESSAGE

This comprehensive review of micropenis and its treatment options underscores the importance of multidisciplinary care, considering both medical and psychological aspects of this condition. The findings emphasize the need for early diagnosis and intervention to address affected individuals' physical and psychological well-being. While surgical interventions like penile lengthening procedures show promise, their long-term efficacy and safety require further investigation. Non-surgical approaches, such as hormonal therapy and psychological support, also play vital roles in the management of micropenis.

RECOMMENDATIONS

Neonatologists should measure stretched penile length in all neonates at birth. Neonatologists should consider micropenis with non-palpable gonads as an emergency. The presence of isolated micropenis suggests gonadotropin deficiency. Timely assessment and management of micropenis are crucial. Healthcare professionals should prioritize early diagnosis of micropenis and provide comprehensive counseling for individuals and their families. Early intervention can significantly alleviate the psychological distress associated with this condition. A multidisciplinary team, including urologists, endocrinologists, and mental health professionals, should collaborate in managing micropenis to address both the physical and psychological aspects. We also need to regularly assess the effectiveness of treatment approaches and update guidelines and best practices based on new evidence and evolving medical technologies.

CONCLUSION

Early management and comprehensive care for micropenis during infancy, childhood, and adolescence are crucial to ensuring the physical and emotional well-being of the affected individuals. This article highlights the significance of

Table 6 Follow-up care for various life stages of patients with micropenis

Life stages	Follow-up protocols
Infants (0-2 yr)	Regular Pediatric Check-ups: Schedule routine pediatric visits to monitor growth and development. Hormone Assessment: If micropenis is identified in infancy, consult a pediatric endocrinologist to evaluate hormone levels and rule out any underlying medical conditions. Parental Education: Inform parents about micropenis and any potential treatment options
Children (3-12 yr)	Annual Check-ups: Continue with regular pediatric check-ups, focusing on growth and development. Psychological Support: Provide psychological counseling for both child and parents and encourage open communication to address self-esteem or body image issues. Education and Awareness: Ensure the child has accurate information about their condition and promote a positive body image. Bullying Awareness: Discuss bullying prevention strategies and provide resources if needed
Adolescents (13-18 yr)	Annual Check-ups: Transition to annual check-ups focusing on overall health. Psychological Support: Continue to offer psychological support, especially given the increased self-awareness and potential body image concerns during adolescence. Sex Education: Provide age-appropriate sex education, discussing relationships, consent, and safe sexual practices. Peer and Social Support: Encourage the adolescent to seek out supportive friends and support groups. Consultation with Specialists: It is recommended to consult with a pediatric endocrinologist or urologist to evaluate the need for potential treatments like hormone therapy or surgery. The adolescent should be fully informed and involved in decision-making
Long-term follow-up (throughout adolescence and beyond)	Mental Health Support: It's important to provide mental health support to individuals who may be struggling with body image or self-esteem issues. Regular Health Check-ups: It's also important to encourage regular health check-ups to monitor overall health and well-being. Reassessment of Treatment Options: Periodically reassessing the need for medical interventions in consultation with healthcare specialists is also recommended. Supportive Family Environment: Maintaining a supportive and open family environment where individuals feel comfortable discussing their concerns and seeking help if needed is crucial

timely intervention and support. By providing accurate information, fostering self-esteem, addressing bullying, and seeking professional guidance when necessary, we can empower individuals to navigate their journey toward a fulfilling adulthood with positivity and confidence. It is vital for healthcare professionals, parents, and those affected to work together to create a supportive and understanding environment that recognizes that physical differences do not define one's worth. By doing so, we can help ensure that those with micropenis can lead healthy, happy, and fulfilling lives.

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REVIEW

Optimal timing for plastic surgical procedures for common congenital anomalies: A review

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Abstract

Apart from listening to the cry of a healthy newborn, it is the declaration by the attending paediatrician in the labour room that the child is normal which brings utmost joy to parents. The global incidence of children born with congenital anomalies has been reported to be 3%-6% with more than 90% of these occurring in low- and middle-income group countries. The exact percentages/total numbers of children requiring surgical treatment cannot be estimated for several reasons. These children are operated under several surgical disciplines, viz, paediatric-, plastic reconstructive, neuro-, cardiothoracic-, orthopaedic surgery *etc*. These conditions may be life-threatening, *e.g.*, trachea-oesophageal fistula, critical pulmonary stenosis, *etc*. and require immediate surgical intervention. Some, *e.g.*, hydrocephalus, may need intervention as soon as the patient is fit for surgery. Some, *e.g.*, patent ductus arteriosus need 'wait and watch' policy up to a certain age in the hope of spontaneous recovery. Another extremely important category is that of patients where the operative intervention is done based on their age. Almost all the congenital anomalies coming under care of a plastic surgeon are operated as elective surgery (many as multiple stages of correction) at appropriate ages. There are advantages and disadvantages of intervention at different ages. In this article, we present a review of optimal timings, along with reasoning, for surgery of many of the common congenital anomalies which are treated by plastic surgeons. Obstetricians, paediatricians and general practitioners/family physicians, who most often are the first ones to come across such children, must know to guide the parents appropriately and convincingly impress upon the them as to why their child should not be operated immediately and also the consequences of too soon or too late.

Key Words: Plastic surgery; Congenital anomalies; Pediatric plastic surgery; Facial cleft; Microtia; Vascular anomalies; Syndactyly; Hypospadias; Optimal timing; Pediatric surgery

Core Tip: This review comprehensively summarises the optimal timing for surgical intervention for various common congenital anomalies which are operated on by Plastic Surgeons. There are various considerations, both general and specific to a condition which dictate the most appropriate time to operate, and find a balance between several advantages and disadvantages of an earlier or more delayed surgery. Parents of children suffering from these anomalies must be guided appropriately by the obstetrician, paediatrician and the family physician, with convincing reasons as to why so much delay is required, before these children are referred to a plastic surgeon. This article is an attempt in that direction.

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INTRODUCTION

Apart from listening to the cry of a healthy newborn, it is the declaration by the attending paediatrician in the labour room that the child is normal, which brings utmost joy to parents. Congenital anomalies are being increasingly recognized as an important global cause of paediatric disease[1]. The global incidence of children born with severe congenital anomalies has been reported to be 3%-6% with more than 90% of them occurring in low- and middle-income group (LMIG) countries[2]. These countries also report 90% of deaths in these children. A significant number of survivors also suffer life-long disabilities, with birth defects accounting for a staggering 25.3 to 35.8 million disability-adjusted life years (DALYs) worldwide[1]. DALYs are a well-established metric for measuring the burden of disease in terms of both mortality and morbidity. One DALY is 1 healthy year of life lost due to disability or premature death[1]. World Health Organization's global burden of disease study reports that these anomalies rank 17th in causes of disease burden. Despite being impressive figures, these are definitely underestimates because of several reasons, viz, non-inclusion of all the anomalies in the study, absence of national congenital anomaly surveillance systems/registry in many LMIG countries and the difficulties in evaluating incidence, morbidity and mortality. The cultural stigma associated with congenital anomalies prevents many parents from going to and availing medical services or even leads to infanticide. There is also an inherent bias of hospital-based data. The last cause by its nature, excludes from estimations those infants with immediately life-threatening conditions who die prior to reaching treatment[3]. Deficient diagnostic capacity and poor awareness are contributing factors. Some anomalies may not be obvious at the time of birth, *e.g.*, submucous cleft palate (Figure 1), congenital muscular torticollis (sternomastoid tumour) *etc*. A great number of children in LMIG countries are born in homes or in places where no records are maintained. Sarkar *et al* reporting data from eastern India found the prevalence of congenital malformations in the newborns as 2.22% which was comparable with data of 2.72% and 1.9% from earlier studies from India[4].

The children with congenital anomalies are operated under several surgical disciplines, *e.g.*, paediatric-, plastic reconstructive-, neuro-, cardiothoracic-, orthopaedic surgery *etc*. These conditions may be categorised as follows: (1) Life-threatening: *e.g.*, oesophageal atresia with or without trachea-oesophageal fistula, critical pulmonary stenosis, *etc*. and require immediate surgical intervention. Even in these conditions, the patient should be optimally stabilised hemodynamically and reasonably well investigated in the shortest possible time after transfer to an appropriate centre with available technical expertise and then taken for surgery; (2) need very early intervention: *e.g.*, hydrocephalus, may need intervention as soon as the patient is fit for surgery; (3) need the so-called 'wait and watch' policy: Natural history of the disease may be such that it is advantageous to follow this policy, also sometimes known as 'masterly inactivity'. However, it is really not so. Rather, one has to be more careful that intervention is not unduly delayed and the disease does not worsen or lead to some secondary problems. For example, while one waits for oculomotor ptosis surgery, it should not cause deleterious effects on vision. A patent ductus arteriosus is kept under observation and followed upto a certain age in the hope of spontaneous recovery. The natural history of haemangiomas is one of spontaneous resolution with better outcome than if surgical intervention would have been rushed, usually on the demand of parents to do something at the earliest. Additionally, infantile haemangiomas are now being treated with drugs (*e.g.*, propranolol) which cause resolution of the lesion earlier. Umbilical hernias and many children with sternomastoid tumour may resolve of their own, the latter with some non-operative measures; and (4) need elective surgery based on several 'common to all' and some 'specific to deformity' related considerations: A category of patients exists where the operative intervention is done based on several considerations which serves the child best by delivering an optimal outcome, *e.g.*, the age and weight of the child, size of the part to be operated *etc*. There are advantages and disadvantages of intervention at different ages which may need to be balanced. The structures may become more robust with growth of child. The greater risks of anaesthesia to smaller children is always a cause of concern although it has become less important than in the past.

Most of the emergency surgical interventions done by paediatric surgeons at the time of birth (or within a few days) are life-threatening and their successful treatment leads to normal survival. However, almost all the congenital anomalies

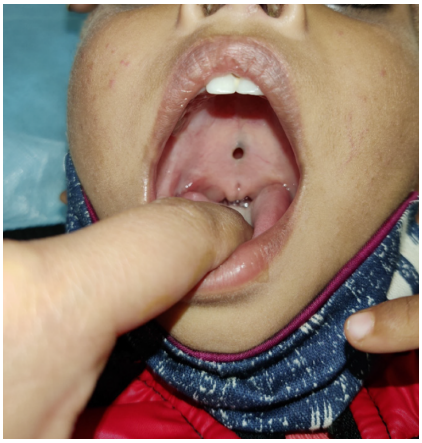

Figure 1 A submucous cleft palate is often missed until the child is much older and has poor speech development. Note the zona pellucida and bifid uvula in this 3-year-old child.

or birth defects coming under care of a plastic surgeon are operated as elective surgery, with several as multiple stages of correction, at appropriate ages. It is well known that children are anatomically and functionally different from adults and are not to be considered as mini-adults. Some plastic surgical procedures just cannot be done as soon as the child is fit for anaesthesia/surgery. For example, pinna reconstruction requires harvesting of sufficient autologous costal cartilage which is not available till 6-7 years of age or so. Moreover, the reconstructed pinna does not grow to adult size with age. Similarly, micropenis cannot be treated till late adolescence period. Breast reconstruction in a female child afflicted with Poland syndrome can be considered only in late teens.

In this article, we present a review of optimal timings, along with basis/reasoning behind them for surgery of many of the common congenital anomalies treated by plastic surgeons. Obstetricians, paediatricians and general practitioners/family physicians, who most often are the first ones to come across such children, must be able to guide and convince the parents appropriately. It must also be emphasized that although parents must consult a plastic surgeon as soon as possible, they should not expect that the child will be operated immediately. It is a common observation that many patients reach late to a plastic surgeon because they never consulted anyone. There is another category who were not guided appropriately by the medical personnel as to when they should consult a plastic surgeon. All the parents wish that they take a healthy newborn baby from labour room to home with no aesthetic defects because that is what is visible to relatives and friends visiting to see and bless the new born as is the custom in many societies. It will be ideal if every parent is directed to consult a plastic surgeon at the earliest who tells them about the surgical procedure and its timing and keeps the child under his/her follow up so that no undue delay occurs. On follow up visits, the plastic surgeon not only replies to the queries of the parents arisen since last visit and reassure them that everything best possible is being done for their child but also checks if the child is thriving normally. Another advantage of these visits is that the child's parents get an opportunity to meet parents of other children, waiting in the out-patient department, who are in different stages of their post-operative follow up. This reassures them and also provides them with a real picture of what to expect.

Several of the most common congenital birth defects can be treated by a plastic surgeon operating as an individual (*e.g.*, hypospadias, torticollis *etc*.) while others need a multi-disciplinary team (*e.g.*, cleft lip and palate, craniosynostosis *etc*.). Great strides have been made in the treatment of numerous congenital conditions because of the intensified efforts toward achieving better functional and aesthetic results. Advances in microsurgery, craniofacial surgery, tissue expansion and anaesthesia *etc*. have significantly impacted the outcome of these unfortunate children. It goes without saying that all children with congenital anomalies requiring surgical intervention must remain under the constant follow up of a paediatrician as well.

The most common congenital defects requiring surgical correction by a plastic surgeon can be divided into three groups: (1) Aesthetic defect alone, *e.g.*, cleft lip alone, unilateral microtia/anotia have mainly cosmetic concerns with far less of functional issues; (2) functional defect alone, *e.g.*, submucous cleft palate alone; and (3) combined functional and aesthetic defect, *e.g.*, bilateral microtia with atresia of external auditory canals, congenital muscular torticollis, congenital blepharoptosis, congenital constriction ring syndrome, syndactyly/polydactyly/absent thumb, haemangiomas and vascular malformations, craniosynostosis, pectus excavatum, *etc*.

GENERAL CONSIDERATIONS FOR TIMING OF SURGICAL INTERVENTION

Paediatric plastic surgery is a surgical subspecialty focused on the reconstructive and aesthetic improvement of a child's appearance with the goal of restoring functionality and improving quality of life for those with anomalies, be they congenital or acquired from an illness or traumatic event.

The question being discussed in this article is, what is the optimal time a child should be operated for a particular congenital condition and why he/she should be operated at that age, why not earlier or later. There are a number of general considerations, common to all congenital conditions which call for a delay in operation and some specific consid-

erations for each condition which determine the actual timing for that condition. The specific considerations are discussed separately for each condition later.

The general considerations 'common to all' are: (1) Age and weight are very important. It allows the surgeon to work with larger tissues and give more symmetry to reconstructed structures like in cleft lip, pinna (microtia) *etc*. A number of anomalies require accurate measurements. Thus, in cleft lip or ptosis surgery, even 0.5 to 1.0 mm over/under measurement may ultimately spoil the result as the child grows and need revision later in adult age. 'If one makes a small mistake in a neonate, it becomes a "big" mistake in an adult! It also avoids inadvertent injuries to very small structures (like fingers, thumb *etc*.) and gives more working space to surgeon, *e.g.*, during palatoplasty. Amount of blood loss may also be important (*e.g.*, craniosynostosis); (2) in all aesthetic defects, the timing is usually also dependent on whether the reconstruction requires tissues like skin grafts, skin flaps, cartilage, bone *etc*. and their availability in sufficient amount, extent of operation, *etc*; (3) condition of patient. As a rule, every patient who undergoes an elective surgical intervention, is expected to be hemodynamically stable. It is very important to evaluate every child, especially one who is syndromic or has multiple, associated congenital anomalies, especially of cardio-respiratory system. The child should be healthy, thriving well and be able to withstand anaesthesia and surgery. Over a period of last few decades, with advances in paediatric anaesthetic gadgets and techniques, availability of trained paediatric anaesthesiologists, greatly improved pre and post operative care in neonatal and paediatric Intensive Care Units, at least in bigger centres of metropolitan cities, postponement of operation for this reason at least has diminished. Aesthetic and elective surgical procedures demand safety with nil mortality. Most minimal morbidity/complications are the prime objectives; (4) other associated, obvious or hidden, congenital conditions may delay the surgery, if life-threatening. For example, isolated cleft lip repair may have to be delayed because of ventricular septal defect which needs an earlier management; (5) long term psychological disturbances are also very important for most congenital defects requiring surgical management. An improved understanding of psychological implications of genital surgery have changed the timing of surgery in hypospadias, epispadias *etc*. over a period of last several decades. An undue delay in reconstruction of cleft lip or pinna may force a child to avoid school to safeguard himself/herself from the taunts of his/her classmates. While a few children may be impervious to ridicule and may get along without correction, others may need a change of school even after correction for full psychological benefit. Thus, the personality of the child also matters a lot. As a rule, all congenital anomalies especially with aesthetic defects not only affect the psychology of the child but also of the parents of the affected child; (6) technique of operation also matters. Thus, one-staged or multi-staged operations cannot have same time guidelines, *e.g.*, in cleft lip and palate cases. Similarly, different techniques of pinna reconstruction demand different age group of the child; (7) a very important factor, which has not been given due importance by the novice surgeons, is that all ideal timings are advocated by highly experienced surgeons working in high volume centres with teams of experienced anaesthetists, nursing and other paramedical staff and resident doctors for usual postoperative care and any untoward rare problem. Thus, cleft lip/palate and craniosynostosis management requires a team of several specialities and paramedical personnel available in developed countries and advanced centres in metropolitan cities of LMIG countries. If these are not available, it may not be wise to mechanically follow the time guidelines. In these countries, many children have lower body weight and body frame and one may need to delay the operation for some more time. For example, unless the body size is big enough to harvest the required size and volume of costochondral cartilage, microtia reconstruction should not be undertaken; and (8) in LMIG countries, every child is not fortunate enough to get the best of institutional treatment at optimal timings. Many reach very late to the appropriate clinician and centre and one may have to accept less than best outcome. Thus, 'late comers' in congenital muscular torticollis may not get perfect facial symmetry but do improve tremendously and are offered treatment even in adulthood. The number of trained plastic surgeons falls severely short of required to deal the massive numbers of these children.

SPECIFIC CONSIDERATIONS FOR TIMING OF SURGICAL INTERVENTION

Cleft lip and palate

Children affected with cleft lip and/or palate (CLP) form a large spectrum of deformities from unilateral microform cleft lip alone on one end and bilateral cleft lip and palate with severely protruding premaxilla on the other end (Figure 2A and B). In addition, the nose is also deformed to varying extent and needs correction[5]. These clefts are quite common and may be unilateral or bilateral. About 1 in 700 children are born with a cleft lip and/or cleft palate. They are also sometimes associated with other congenital anomalies, especially of the heart. They may be syndromic or non-syndromic. Almost 500 syndromes have been associated with them[6]. The importance of presence of other congenital anomalies and being part of a syndrome is that very often this delays the usual timings of operation as they may have to be given priority[7]. Apart from obvious problems of feeding and aesthetics at the time of birth, a CLP has significant effect on the child's hearing and speech with psychosocial difficulties at school due to bullying and teasing by peers because of appearance of their face and consequently attaining lower educational standards. Frequent visits to doctors and operative interventions are stressful in themselves[8]. The objectives of cleft lip and palate surgery are to achieve symmetry of lip, nose, maxillary arches and their normal growth upto adulthood when the facial growth stops and no further alterations are expected. The separation of nasal and oral cavities should allow development of normal speech (without any velopharyngeal incompetence) and normal hearing.

A newborn with cleft lip and palate does not constitute a surgical emergency. Many paediatricians and obstetricians who see the child in labour room have a mistaken notion that this child needs immediate closure of the defect lest the child starves to death or else needs a nasogastric tube feeding (Figure 3). Thus, cleft lip and palate constitutes one condition where a plastic surgeon needs to see the child immediately after birth for appropriate feeding and other advice.

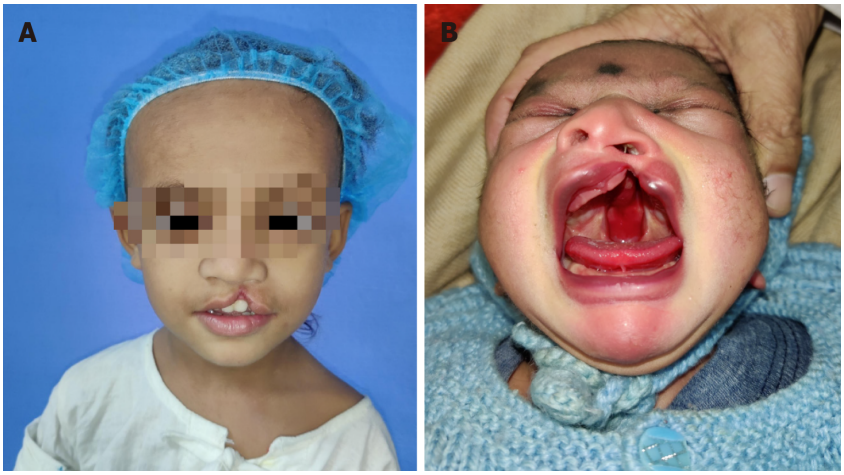

Figure 2 Cleft lip and palate. A: 2-years-old boy with left unilateral incomplete cleft lip; B: Neonate with complete left cleft lip, alveolus and palate with Simonart band.

Figure 3 Neonate with a nasogastric tube inserted by the paediatrician for feeding.

The problem of feeding is for initial few months only when the mother cannot breast feed the child directly because cleft in the palate does not allow the child to develop intraoral negative pressure required. The mother is instructed to express the milk manually from her breast in a bowl and then feed the child using a small spoon with child's head being elevated at 45 degrees and milk dropped over the posterior part of the tongue. The child gulps the milk down with each breath/cry. The child swallows a lot of air along with milk, regurgitates milk into nose and may stop more intake of feed. The child is made to burp by holding vertically against the chest of mother and patting on the back of child repeatedly so that more milk is accepted. A successful feeding by the mother is judged by the satisfactory gain in weight measured every week initially. Some workers also advocate feeding with bottle using a bigger opening in the nipple so that the milk drops of its own without the child sucking it. A number of feeding devices made by dental colleagues to feed the child are probably not required as the mother and child soon adjust to bowl-spoon routine.

Many children are given presurgical infant orthopaedic dentofacial treatment before the cleft lip and palate are operated. It is started as soon as possible after birth. Here, the cleft maxillary and soft tissue segments are moved closer while waiting for surgical reconstruction. Although it can be used in any patient, it is typically reserved for cases with a very wide cleft. The objective is to reposition the alar base and restoring the skeletal and soft tissue anatomy. Repositioning of the maxillary alveolar segments provides symmetric maxilla and nasal floor and a narrower alveolar cleft. In unilateral cases of complete cleft lip and palate, segments of the maxillary arch are brought in an anatomically neutral position without collapse or constriction (Nasoalveolar moulding)[9-12].

Millard gave his 'rule of 10' for cleft lip repair. The child should be over 10 wk of age, over 10 pounds of weight with a hemoglobin of at least 10 gm/dL and a leucocyte count of less than 10000/mm^3. The bottom line is that the child should be thriving well and without any other congenital anomalies which can increase the risk of anaesthesia[13]. Because of free communication of oral and nasal passages in cases of cleft lip and palate, these children are prone to upper respiratory infections which may need postponement of operation and appropriate management. The timing is also

dictated to some extent by the technique. Thus, some surgeons do correction of nasal deformity (primary rhinoplasty) with cleft lip repair and thus, it may be better to operate at a slightly higher age than if only lip repair is done. Similarly, in patients with complete cleft of lip, alveolus and palate, most surgeons perform lip and anterior repair in first stage and palatal (hard and soft palate) repair in second stage 3-4 months later. However, some like to repair all in one-stage operation and obviously, child should be older and weigh more for this technique in view of prolonged surgery. One-stage cleft lip and palatal repair before the age of 6 months is rarely applied in clinical practice due to the technical difficulty of the surgical procedure and potential risks such as increased blood loss, airway obstruction and anaesthetic problems. However, there are surgeons who routinely do it and find no such shortcomings[14]. One of the very important considerations in these patients is that the surgeon and anaesthesiologist share the upper airway and have to take care throughout the operation that one does not encroach upon each other's interests.

The main concern in lip repair is of scar. It is known that scarless healing occurs in fetus and hence, experimental attempts have been made to diagnose and perform surgery in utero. This is still not done clinically. Surgery on the lip can be performed even at the time of birth sparing the psychological trauma to parents who wish to take a normal child to their home. However, this is not followed as it is very difficult to measure and accurately suture the cleft edges in a newly born (with oedematous facial tissues) and any minor discrepancy gets enlarged with facial growth, spoiling all the result with need to revise on non-virgin tissues later. Moreover, as the parents have not spent even a few hours with the neonate, even the best result may not be acceptable to them. Spending a few months allows greater development of the bond between child and parents and sudden tremendous improvement in the child's look after operation makes them far happier. Thus, there is absolutely no need to take the child from labour room to operation theatre. A big advantage of repeated visits to the plastic surgeon during the course of treatment is that it gives the parents an opportunity to meet and interact with parents of other affected children to gain insight into the course of management.

While cleft lip repair is almost totally aesthetic, the palatal repair has lot of functional consequences, the most important being speech. The goals of palatoplasty operation are to have normal speech, hearing and maxillofacial growth The palate forms the floor of the nose and the roof of the oral cavity. Thus, palatal cleft leads to a free communication between these two cavities. The palatoplasty has to be done before the child starts speaking. Over a period of last several decades the age at which the child starts speaking has been decreased by the paediatricians again and again as they have included babbling and cooing of infants as an important prelude to normal speech development[15]. Consequently, the plastic surgeons who used to perform palatoplasty at 18-24 months of age are now advocating 6-9 months or even earlier [16].

Apart from speech, the timing of cleft palatal repair has to consider several other issues concurrently. Prevention of otitis media in the first year of life are of prime importance. Otitis media with effusion in middle ear is almost universal in unoperated cleft palate patients and this leads to diminished hearing, affecting the speech development in return. Eustachian tube dysfunction is a primary cause of middle ear disease. The Eustachian tube in a child is not fully developed and is shorter than that of adults. Also, it is positioned more horizontally and its opening into the nasopharynx is narrower. An episode of upper respiratory tract infection leads to inflammatory swelling of mucosa with narrowing/blockade of the Eustachian tube meatus resulting in a negative pressure in the middle ear. Microbes in the nasopharynx enter the middle ear resulting in effusion and infection. Abnormal tensor veli palatini and levator veli palatini muscles also cause maladjustment in the regular opening of the Eustachian tube. It needs placement of tube (ventilating grommet) in the tympanic membrane and may be done as part of palatoplasty surgery or independently, if required earlier[17,18].

In unoperated CLP patients, the midfacial growth is similar to non-cleft children without apparent restriction of growth (Figure 4A)[19,20]. However, along with varying degrees of inherent maxillary deficiency[15], surgical intervention further retards the maxillary growth due to periosteal stripping and scarring following raw areas left on either side of mucoperiosteal flaps raised to close the palatal cleft[21]. These raw areas heal by secondary intention (contraction and epithelialization from the margins). Thus, timing in palatoplasty is a compromise where speech demands as soon as possible after birth and facial growth demands only after adulthood, i.e., after complete facial growth. Midfacial growth restriction after the palatoplasty operation becomes apparent much later in adolescence and may require corrective jaw surgery. Although this revision surgery is taken to be part of an overall treatment plan, it can definitely not be viewed as inconsequential. It is quite complex and burdensome and can further affect facial and palatal growth due to scar and wound contraction. In situations where limited interdisciplinary facilities are available, the child may be left with excellent speech but severe mid face hypoplasia. After the repair of lip, alveolus and palate, the child needs to be sent to a speech therapist who assesses the speech and gives therapy for its normal development[22]. The speech abnormalities are multifactorial. There may be hypoplasia and hypomobility of the levator and tensor veli palatini muscles and their abnormal course and insertion into the palate. Unless properly repositioned, it may not allow for perfect speech development. Assessment for any velopharyngeal incompetence is done and, in some children, pharyngoplasty is required at around 5 years of age. Velopharyngeal incompetence is the inability to completely close the velopharyngeal sphincter which is required for the normal production of all but the nasal consonants. It results in nasal air escape and hypernasality leading to decreased intelligibility of speech[23].

As the child grows, one finds that the dentition is disturbed with malocclusion. Some teeth are absent while others are mal-positioned or mal-aligned or even supernumerary or smaller than normal (Figure 4B). Thus, a pedodontist and an orthodontist need to see the child as the teeth erupt. These specialists have to work with primary, mixed and permanent dentitions[8]. Orthodontic treatment in children with CLP consists of maxillary arch expansion, correction of upper incisor misalignments, gross rotations of incisors, crossbites and correction of class III skeletal growth pattern. Some patients require secondary alveolar bone grafting. When the bone grafting is done before or along with palatoplasty, it is called primary while secondary bone grafting implies that the procedure is performed after the repair of cleft palate[24-26]. Primary bone grafting prevents collapse of the maxillary arch and stabilizes it creating more uniform growth of maxilla and improvement of articulation. The disadvantage is that it can provoke attenuation of the maxillary growth

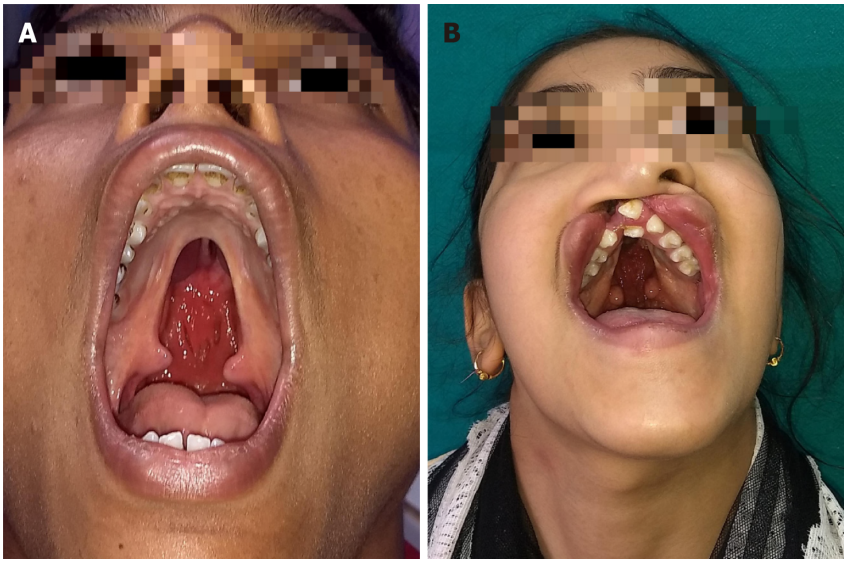

Figure 4 Cleft lip and palate in adults. A: Normal maxillary growth in an adult un-operated cleft palate patient; B: Adult un-operated patient of cleft lip and palate with multiple dental abnormalities and nasal deformity.

especially in the vertical dimension. The optimal timing of secondary alveolar bone grafting balances the ability to provide bone for eruption and periodontal support of the teeth adjacent to cleft, establish continuity of dental arch in the alveolar cleft, allowing orthodontic space closure and future placement of an implant or bridgework, achieve closure of any oronasal fistulae, establish nasal skeletal base and improve speech on one side and the minimization of inhibition of maxillofacial growth on the other side[24]. Secondary bone grafting is usually performed just before permanent canine eruption, seen in radiograph to be about half or more of its normal size, using autogenous cancellous bone from iliac crest (8-11 years of age.) It minimizes the growth disturbances of the maxillary arch, giving it integrity with periodontal support for the teeth proximal to the cleft. It is now widely used and considered to be a standard procedure for alveolar repair[27]. Some children may need orthognathic surgery (osteotomies, distraction osteogenesis and bone grafting). These interventions are usually done only in late teenage, a little earlier in females (14-16 years) as compared to males (16-18 years). Some patients may require dental implants and prosthetics in adult age. Correction of the underlying skeletal base by alveolar bone grafting, Le Forte I advancement or prosthetic reconstruction of the anterior maxilla in bilateral clefts is done prior to undertaking secondary rhinoplasty.

The repair of cleft in the lip and palate and other measures by the plastic and dental surgeons restores the nasal floor maxillary symmetry but several problems remain which are due to misplaced nasal cartilages. Their correction is done either as part of lip repair (primary rhinoplasty) or later (secondary rhinoplasty)[28]. Primary rhinoplasty can be limited or extensive. By giving reasonable shape to nose, primary rhinoplasty allows most children to defer definitive rhinoplasty. A secondary or definitive rhinoplasty operation is carried out only after the facial growth is complete and maxillary arch is symmetrical and hypoplasia taken care of. Definitive rhinoplasty is usually undertaken at 14 to 16 years of age in girls and 16 to 18 years of age in boys[6]. During definitive rhinoplasty, aggressive osteotomies, septal correction and nasal cartilage repositioning/carving and fixations are done as there is neither a risk of their alterations by growth nor the growth itself will be altered by them. The goal is to create a permanent symmetry, desired nasal shape, dorsum as well as base and relief from various deformities, not only congenital but also due to effects of previous surgical interventions[6,29]. In addition to restoration of nasal shape, secondary rhinoplasty also requires to correct functional component as majority of these patients have functional airway obstruction which is due to external nasal deformity, septal deviation, vomerine spurs, inferior turbinate hypertrophy or maxillary hypoplasia. Secondary cleft rhinoplasty has been considered one of the more challenging subcategories of rhinoplasty because each of previous procedures add to scarred nasal skin, cartilage and mucosa and absent virgin tissue planes and often needs autogenous costal cartilage graft as well[30].

Some surgical interventions may also be required for secondary deformities which need correction only after the facial growth is over and the patient himself/herself puts forth the demands to the surgeon (*e.g.*, improper direction of hair or alopecia in the area of moustache region). The fact is that the treatment of these children by a plastic surgeon is longitudinal in nature starting right at the time of birth and stopping only after adulthood. Throughout this period, the child is under care of not only a plastic surgeon but also of paediatrician, otorhinolaryngologist, speech therapist, dental surgeons (pedodontist, orthodontist *etc.*).

In several LMIG countries, a large percentage of the CLP children reach a plastic surgeon far later than the above optimal timelines because of shortage of trained medical personnel as well as remote areas and financial constraints. These children are still offered operative intervention and satisfactory, if not perfect results obtained. In these patients palatoplasty may be done first so that speech problems are minimised. Aesthetic outcome of isolated clefts of lip are usually dependent on the skills of the surgeon and even late middle-aged have also been offered treatment with excellent results! Palatoplasty is generally not offered to adults because of poor speech outcome.

Microtia and other deformities

Congenital absence of pinna or external ear is a very common anomaly (Figure 5). The deformity can range from mild malformation to complete absence of the pinna (anotia). Congenital aural atresia, a common association with microtia, is failure of development of the external auditory canal. Microtia may be part of a syndrome (*e.g.*, hemifacial microsomia). Microtia is of great aesthetic concern to parents of newborn and to the child once he/she starts going to school and wishes to avoid the torment meted out by the classmates. Pinna is also required to wear glasses, may not be now but later on when an individual needs correction of refractory errors or at least in old age for presbyopia. It is also needed for wearing sunglasses and hence needs restoration. So, theoretically, as for several other conditions with aesthetic concerns, it should be reconstructed before the child starts going to school (around 3 years). However, there are a number of important considerations as to why this is just not possible. The two most important one's are: (1) The reconstructed pinna does not grow. The normal pinna continues to grow after birth and attains 85% of adult size by the age of 5 years or so[31]; and (2) pinna reconstruction for microtia is done using autologous costochondral cartilage[32]. The amount of cartilage required depends upon the technique of reconstruction while the amount available depends on the age and body build of the child. While Brent[33] operated on children more than 6 years of age (preferred 7-8 years of age) with costal cartilage from opposite side, Nagata advocated use of ipsilateral cartilage at 10 years of age with a chest circumference of 60 cm at the level of xiphisternum[31,34-36]. Many other pioneers of pinna reconstruction like Bauer, Firmin, Park also prefer 10 years of age because of insufficient amount of costal cartilage at lesser ages and better results at 10 years[37]. In general, 6th, 7th, 8th and 9th costal cartilages are harvested for pinna framework fabrication. Pinna reconstruction is a multi-staged operation. A very early operation may make the pinna very small (as compared to the opposite normal side) and the amount of cartilage may be highly deficient in length and volume. The reconstructed pinna may also prove to be rotated and abnormally positioned once the adult size of normal pinna is reached. There may be a chest deformity also. An undue delay may affect the psychology of the child while still greater delay may even land the surgeon in harvesting a cartilage which is ossified and difficult to carve. Thus, the best time for reconstruction has to be balanced and comes to around 10 years for most children.

The time to attend the school and the development of self-image of a child are much before the time-lines of a good pinna reconstruction. They do have an impact on the emotional state of the child and the parents which needs to be looked after by the plastic surgeon and the paediatrician till the pinna is reconstructed. It is important to note that although the operation is to be done at a particular age, the parents and child must visit the surgeon regularly, may be yearly. As the child grows and understands, he/she is taken into confidence for the reasons of delay and may be shown pictures of pinna reconstructed by the surgeon on every visit. All must understand that once the cartilage is harvested, there is no way to harvest more for a second operation, if it proved inadequate earlier. The ear being reconstructed is for rest of life. First attempt is the best attempt, be it pinna reconstruction or academic exams!

In addition to aesthetic reasons, ability to hear normally is another big concern. Thus, the other consideration of concern is whether microtia is unilateral or bilateral and whether the external auditory canal is absent. All children need to be seen by the otologist at 3-4 months of age and kept under follow up for assessment of hearing capabilities. Even in unilateral cases, one must know that the other side is normal, the protection of which is very essential for development of speech. Identification of extent of hearing loss allows appropriate corrective measures to be instituted. In patients with unilateral microtia, absent canal is usually not reconstructed as the child adjusts to monoaural hearing. Patients with bilateral microtia with bilateral absence of canal are usually functionally deaf bilaterally. Autologous ear reconstruction takes care of cosmetic outcomes only. It does not address the functional hearing issues in patients of microtia with associated atresia of external auditory canal. It precedes canal restoration as the plastic surgeon needs virgin tissues for cartilaginous pinna framework placement at the appropriate site while delay in canal restoration in bilateral cases is bound to affect development of speech. To achieve best results from auricular reconstruction for cosmetic purpose and canal restoration for hearing rehabilitation, the plastic surgeon and the otologist colleague must look at all aspects of the procedure and plan in advance. Thus, the site of surgical incision, position of bone conducting hearing implant *etc*. must be discussed. Treatment of functional deficits must not compromise aesthetic reconstructive options and vice versa. These children need non-operative management for hearing in the form of bone conducting hearing device on soft band or osseointegrated implants like bone anchored hearing aid (BAHA) during this period[31,36]. Bilateral cases have to be staggered and one side should have settled before undertaking the opposite side lest the manipulations of child's head spoil the reconstructed pinna. Canal restoration has to be done at least after 3 months of framework placement and lobule transposition and is generally combined with the last stage of pinna reconstruction. Even after canal restoration the child may need BAHA.

Similar to many other congenital conditions, microtia may also be associated with other congenital malformations like facial clefts and cardiac defects. The latter may have a bearing on the timing of pinna reconstruction.

Other deformities of pinna such as prominent ear or lop ear (Figure 6) also have aesthetic concerns and affect the psychology of the child because of bullying by children at school and calling them names like 'bat ear' or 'Mickey Mouse ear'. Hence, child should be operated before admission to school. Some severe cases of lop ear need reconstruction as for microtia and hence they follow similar timelines for operation.

Craniosynostosis

Craniosynostosis is defined as premature fusion of one or more cranial sutures in the newborn. It leads to restrictions in the growth of calvarium, predictable compensatory deformations and also affects the brain morphology with resultant neurological sequelae[38]. The growth of the skull is typically restricted perpendicular to the fused suture and expanded in a plane parallel to it (Virchow's law). This fusion can be either isolated (or non-syndromic, in 80%; *e.g.*, sagittal synostosis) or part of a syndrome (or syndromic, in 20%; *e.g.*, Apert syndrome, Crouzon syndrome *etc.*)[39]. The incidence

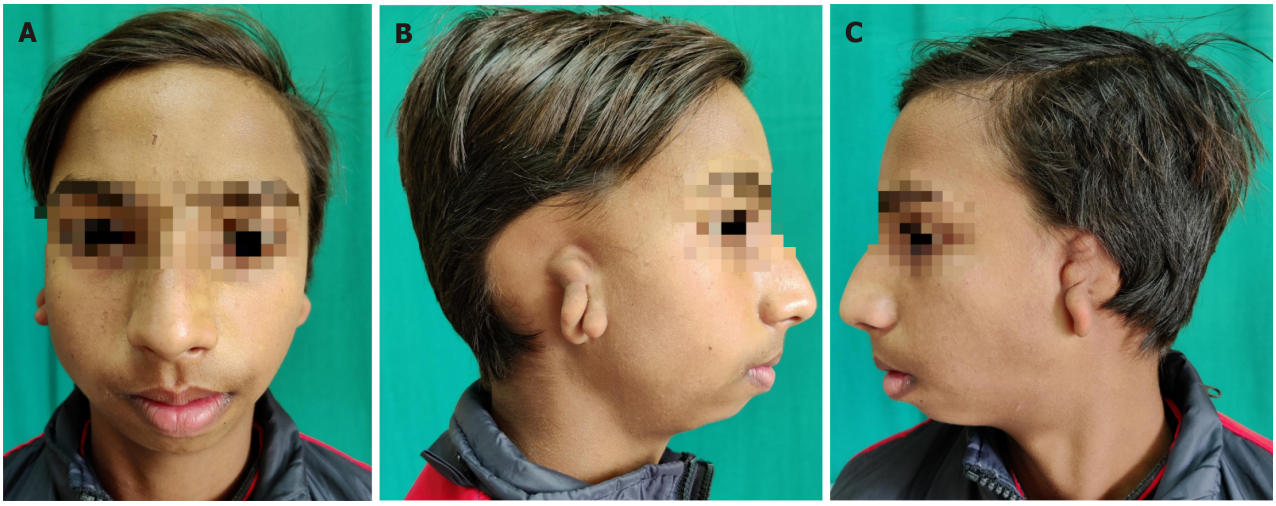

Figure 5 Bilateral microtia in a 12-year old boy. A: Front view; B: Right ear; C: Left ear.

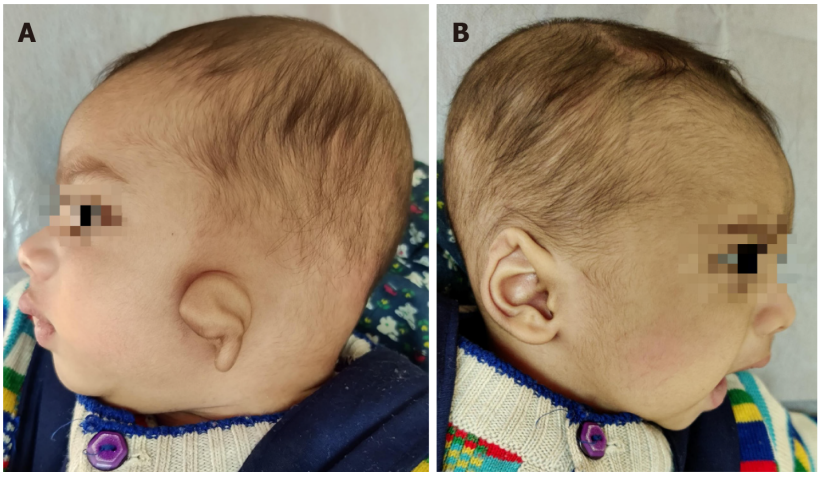

Figure 6 Neonate with unilateral lop ear. A: Left affected ear; B: Right unaffected ear.

is approximately 1 in 2500 births[40]**.** While surgical treatment is the mainstay of treatment, its timing is controversial[41-44]. The considerations for timing of operative intervention are aesthetic and functional. By restoring the normal shape of the cranium as soon as possible, cosmetic goals are achieved. Just like other aesthetic deficiencies, craniosynostosis also leads to psychosocial stress for the parents and the child. The functional goals of craniosynostosis surgery are to restore adequate intracranial volume enough for unimpeded cerebral growth and expansion, reducing the risk of raised intracranial pressure (ICP) and to minimize cognitive sequelae. An uncorrected deformity does not tend to worsen over a period of time. At the same time, it does not improve of its own as well. The calvarium of a newborn undergoes significant changes until one year of age. Maldevelopment of skull and brain go side by side. The weight of brain is 400 gm at birth, double at 6 months and triple at 2.5 years of age, reaching almost adult size of 1400-1600 gm by 5 years. Greater the number of fused sutures, greater is the potential for raised ICP[40]**.** Thus, operative intervention is important not only for cosmetic purposes but also to safeguard against brain dysfunction.

Being a highly complicated problem, even all plastic surgeons do not perform this surgery. It requires major centres with a team of plastic surgeon, paediatric neurosurgeon, paediatrician, ophthalmologist, anaesthetist and trained nursing staff *etc.* to operate on such patients[45]. Different units have their own protocols for operative intervention[41-44] and hence, the child should be under care of the team as soon after birth as possible[46]. Some unfortunate children reach the clinician late because the parents consider their abnormal shape to be due to child's preferred posture while lying. Indications for emergency surgery include an immediate threat to the airway or eyes or the presence of raised ICP[39]. Surgery before the age of 1 year (most surgeons start at 3-6 months) reduces potential impacts on crucial brain development, risk of raised ICP and deformities of skull base[47]. Advantages also include increased malleability of the softer and younger bone amenable to bending and reshaping. The ongoing growth of brain encourages continued growth of the cranial vault and ossification of craniectomy defects due to surgery occurs satisfactorily. No bone grafting is required for these defects as the osteogenic potential of the dura enables spontaneous bone regeneration. However, softness and increased malleability of bone also means that its fixation by plates and screws is unsatisfactory. There are greater risks of anaesthesia and excessive blood loss in smaller children[39,48] (plus complications of massive blood

transfusions) and more chances of re-do surgery required during youth because of ongoing craniocerebral disproportion resulting in craniostenosis again. After 1 year of age, the bones become more and more mineralised, thick and brittle and pose difficulty in remodelling. The deformity also increases and becomes too severe for improvement. The small defects left in calvarium do not ossify of their own and need bone grafting[39]. Despite surgery, some children do develop raised ICP which needs to be looked after to prevent complications. Thus, the timing of surgery needs a balance between prevention of undesirable effects of craniosynostosis and child's ability to tolerate a prolonged and complicated major surgery.

Congenital blepharoptosis

Congenital blepharoptosis is characterized by an abnormal drooping of the upper eyelid resulting in narrowing of the palpebral fissure and may be: (1) Simple, seen as an isolated anomaly; or (2) complicated, which is associated with maldevelopment of the surrounding structures, ocular motor anomalies, blepharophimosis syndrome and Marcus Gunn ptosis[49]. Examination of the infant is difficult and the presence of congenital ptosis is generally confirmed from photographs taken during childhood by the parents. It may be uni- or bilateral and is mostly due to defective development of the muscles and is nonprogressive. Although the primary reason for correction of ptosis surgery is functional (vision), production of symmetry in lid height, contour and eyelid crease lead to better cosmesis taking care of any psychological consequences.

A number of operative techniques are available to correct the ptosis. The timing of surgical correction depends on the severity of the ptosis and the strength of the levator muscle. The eyelids are primarily elevated by the contraction of the levator palpebrae superioris muscle which is innervated by the superior branch of the third cranial nerve[49]. Usually, it is done at 4-5 years as it allows accurate measurements preoperatively which need reliable cooperation of the child during physical examination, improving the surgical outcome in return. Also, the risk of postoperative exposure keratopathy is less as these children cooperate better with forced closure exercises and the status of cornea can be better monitored[50]. An earlier surgical correction is indicated when the droopy upper eyelid interferes with the visual axis causing stimulus deprivation amblyopia[51,52] or associated strabismus induced amblyopia. Sometimes, a temporary operation may be needed to tide over the period in first year of life itself. Greater the delay in treatment of amblyopia, more difficult it becomes to achieve good final visual acuity and after 7-10 years, it may not be possible to reverse amblyopia. Thus, timely treatment of ptosis is very important. Refractive error is another criterion, especially anisometropia or increasing amblyogenic astigmatism. An earlier intervention is required in cases with torticollis of ocular origin as this may delay mobility in toddlers because of the balance problems from extreme chin-up head posture.

Congenital muscular torticollis

Congenital muscular torticollis is the third most common congenital musculoskeletal anomaly, after clubfoot and congenital hip dysplasia. There is shortening of the sternomastoid muscle in the neck and it presents as a unilateral deformity leading to inclination of the head towards the affected side (Figure 7). Torticollis is not a simple static, fixed deformity. Rather, it has far-reaching effects on craniofacial growth, development of spine and possibly ocular and vestibular development. In an attempt to compensate for torticollis, the child elevates the affected shoulder and laterally shifts the head. This involuntary compensation may lead to the development of craniofacial asymmetry, cervical scoliosis and compensatory lumbar scoliosis. It may result in rotation, lateral flexion of head, deviation of the face, limitation in neck movements and diplopia as well[53]. Thus, congenital muscular torticollis has aesthetic as well as functional consequences. It must be remembered that that torticollis is a sign also and hence, other causes must be ruled out.

Because of short neck in children, many cases especially the mild ones may be missed even by clinicians. Affected children present with a painless swelling in the neck, called 'sternomastoid tumour' which resolves spontaneously, usually within a year or so. In patients where the tumour persists, it is replaced by fibrosis and contracture in the muscle which, if not treated, leads to progressive facial asymmetry. The cranial asymmetry is already determined by 6 months of age. Non-surgical treatment in the form of cervical collars, massage and physiotherapy with active and passive stretching exercises of sternomastoid muscle is started as soon as diagnosed. However, this non-operative treatment is not successful after 1 year of age[54]. The patient's age at operation, duration of the disease and severity of the deformity before the operation are the major determinants affecting both cosmetic as well as functional outcomes. The surgical intervention is required to correct the abnormality for aesthetic and functional reasons. Although optimal age has not yet been established but intervention from 1-4 years is said to be optimal, if diagnosed by that time[55-57]. Earlier the better. After that age, as the delay occurs, it may not be possible to achieve perfect symmetry. However, marked improvement in symmetry, aesthetics and psychology has been reported after operation in teenagers and young adults, the so called 'late comers' by several workers including authors[58-60].

Pectus excavatum

Pectus excavatum is the most common congenital chest wall deformity (constituting 95% of patients of all chest wall deformities) with males being affected several times more. The sternum is sunken and gives an appearance of a dent in the anterior chest. The sternum and lower costal cartilages are pushed posteriorly with severity ranging from mild depression to ones where the sternum almost abuts the vertebral bodies. In 90% of patients, the deformity can be detected in first year of life. It increases in severity gradually till adolescence when growth spurt makes it rapid until full skeletal growth is achieved. After adulthood the deformity remains constant for most patients throughout life[61]. Scoliosis may be present in 15% of cases[62]. This can be very disturbing to young teenagers who develop poor self-esteem and body image perception leading to psychological disturbances. In general, any deformity of the anterior chest is not acceptable to adolescent males as well as females leading to significant restrictions in their social activities. This is seen so commonly

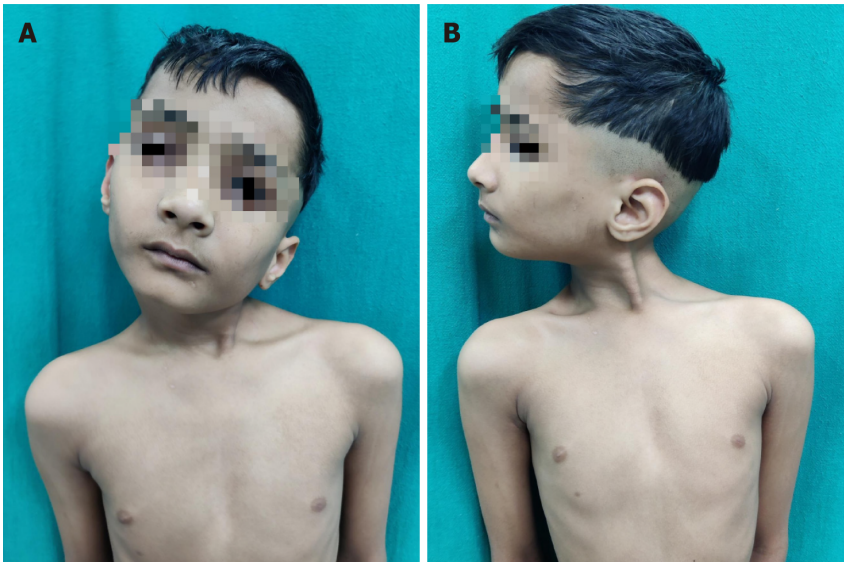

Figure 7 Eight-year-old child with left congenital muscular torticollis. A: Note the shortened left sternocleidomastoid muscle as a fibrotic band, the facial asymmetry, restricted mobility of the neck; B: Lateral view of the same.

with the increasing number of teenaged males with gynecomastia and females with hypoplastic breasts thronging the aesthetic surgery clinics all over the world. These children try to camouflage the chest contours and avoid sports and swimming activities *etc*. Corrective surgery tremendously improves the quality of life[63].

Functional effects are on heart and lungs. In milder cases, the cardiorespiratory functions may be within normal limits. More severe cases may require surgical intervention for cardiac and/or respiratory impairment. Older children, teenagers and young adults may find exercise intolerance and endurance issues or even exercise-induced asthma[64]. Thus, there are aesthetic as well as functional considerations for deciding the optimal age. Earlier, mild to moderate cases of pectus excavatum were advised operation from 2-5 years even if they were asymptomatic because the surgery could be done more easily as compared to in bigger children. However, surgical techniques which involve resection of large amounts of rib cartilages performed too early may risk chest wall growth and result in pulmonary dysfunction[65,66]. Thus, most surgeons wish to wait till 8 years or later when there is adolescent growth spurt[61]. Symptomatic cases might need intervention earlier. The optimal age for repair appears to be in the range of 12 to 16 years as the rib cage is more malleable allowing for rapid recovery with a lower recurrence rate. Adults with persistent deformities extending into 4th-5 th decades have achieved excellent results after corrective surgery[61]. Fonkalsrud in his retrospective study of 375 patients found the age at surgery ranged from 2.5 to 53 years with 47% from 2-11 years[67]. Horch *et al*[68] advocate that in children with no reduction of the respiratory volume and no compression of mediastinal structures, a sternal elevation procedure or rib transection may not be required. The aesthetic defect, without altering the rib cage pathology, can be treated using customized silicone implants or autologous tissues such as cartilage, fat *etc*. in teenagers and adults[69,70]. However, very few patients are suitable for augmentation procedures and maximum require sternal elevation only[68]. While obviously the former do not address the functional issues, their migration may produce additional deformity.

Poland's syndrome

Poland's syndrome is a rare congenital anomaly characterized by unilateral chest wall hypoplasia and ipsilateral hand abnormalities. In a classical case, there is unilateral symbrachydactyly with ipsilateral hypoplastic anterior chest and anterior axillary fold, diminished bulk of infraclavicular subcutaneous tissue, absence of the costosternal portion of the pectoralis major muscle, lack of the pectoralis minor muscle, aplasia or deformity of the costal cartilages/ribs, hypoplasia of nipple-areola complex and finally hypoplasia of the breast at the time of thelarche[71]. The extent of these deformities in a given case are variable. Extremely rarely, the syndrome can involve structures bilaterally. Apart from these, an extremely large number of other abnormalities have also been described in these patients[72].

The major anomalies requiring operative intervention are those of the hand and the anterior chest (including breast) [73,74]. The child is usually brought for hand deformities as chest wall deformity may not be obvious in an infant or toddler (Figures 8 and 9). In the latter cases, the surgeon brings to the notice of parents about the hypoplastic chest wall and the surgical interventions which may be required after puberty, especially in girls. There is a wide range of spectrum of hand deficiencies and surgery may have to be started from 6 month onwards in patients who require multiple stages or need to separate first web space while those requiring a single stage may be taken up at 18 months or so[73,75]. Reconstruction of breast needs to be done only after the contralateral breast has developed, usually in late teens (Figure 10). Teenaged and adult male patients may ignore chest wall hypoplasia, if not very severe and may not opt for any surgical treatment.

Syndactyly/polydactyly/thumb hypoplasia/cleft hand

Syndactyly and polydactyly are the most common of congenital malformations affecting limbs. While it is obvious that

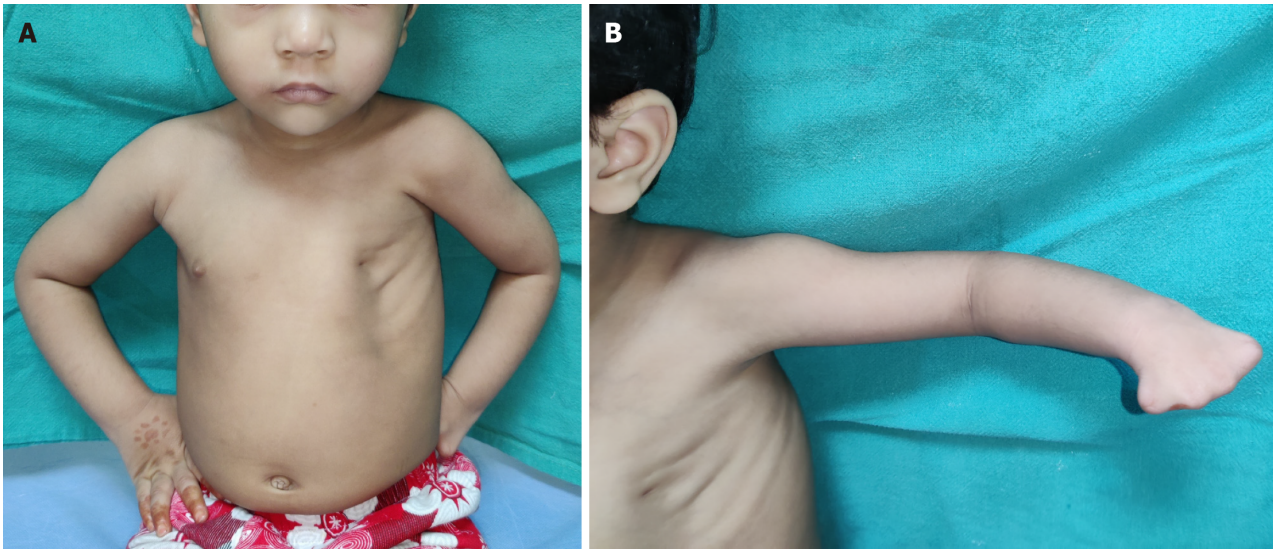

Figure 8 Poland syndrome in a 3-year-old girl child. A: The chest can be reconstructed only after complete development of the opposite normal breast; B: Symbrachydactyly in the left hand.

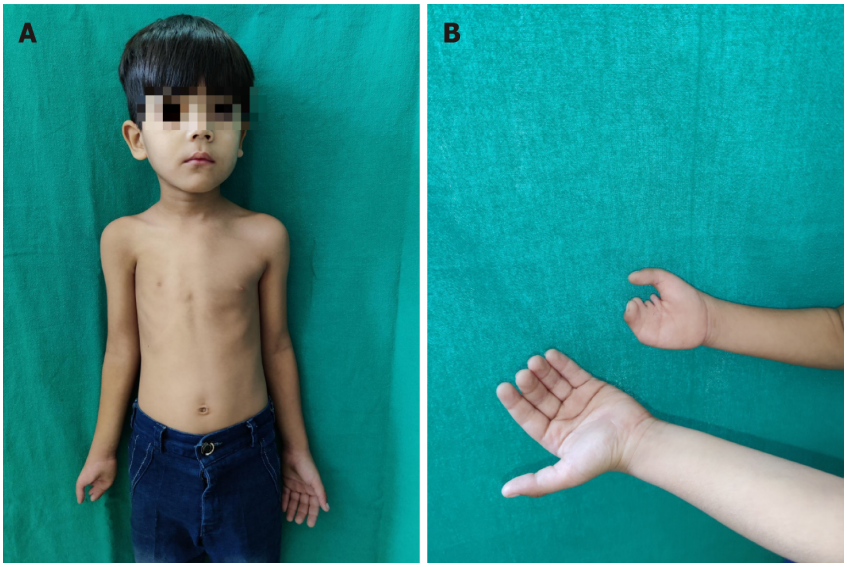

Figure 9 Poland syndrome in a 6-year-old male child brought to the out-patient department for right hand deformity. A: The parents did not notice the mild hypoplastic right chest and nipple areola complex. He may not need or desire a chest reconstruction at all later in life; B: Right hand deformity.

many parents with minor problems may not opt for treatment, it is also true that many cases of syndactyly or polydactyly may have no functional/psychological problems to the child and hence no treatment desired. Many parents/grown-up children even consider supernumerary digit as bringing good luck. Hence, actual prevalence may not be known. They may be isolated or part of syndromes[76]. Both show a great spectrum of deformities and may be uni- or bilateral and can involve hands and/or feet. Thus, there may be partial fusion of skin between two adjacent fingers or all the fingers and thumb may be united upto whole length to give rise to a mitten hand (Figure 11). There may be bony fusion, extra or missing phalangeal bones or placed erratically. When fusion is upto finger tips, the nails are fused to each other despite the different length of digits and this causes deformities of fingers in a growing hand, prevention of which is an important consideration for an early operation. Additionally, when more than two adjoining fingers are joined, all of them cannot be released in one operation because of risk of damage to neurovascular bundles. A waiting period of at least 3 months should be given which also allows time for massage, exercises and some maturation of scar. Similarly, polydactyly may be as insignificant as a nubbin to as severe as a duplication of hand (mirror hand). There may be polysyndactyly, pre- or post-axial polydactyly and other severe deformities.

It is thus, clear that the surgical release/amputation has to be based more on functional considerations (before the development of fine motor skills) apart from aesthetic considerations. Patterns of prehensile function are established by the age of 2 years. Timing of operation on fingers is also decided by their size as there is a risk of damaging minute neurovascular bundles, difficulty in multiple dressings required post operatively, care of the skin graft used almost as a routine for provision of skin cover after release of syndactyly and physio-occupational therapy. Administration of

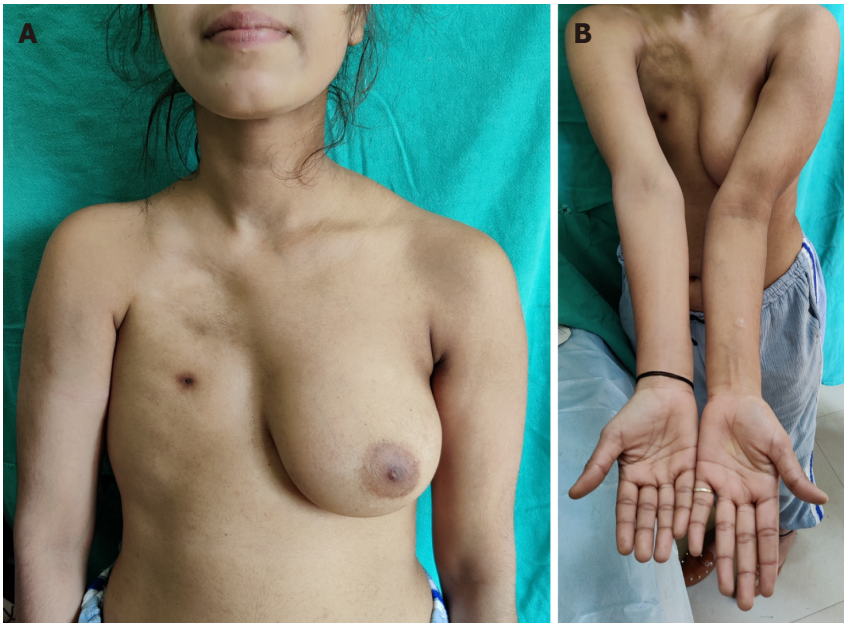

Figure 10 Poland syndrome in a girl in her late teens. A: Right chest hypoplasia, absent breast seen. Reconstruction of right breast can be done with the left as a template; B: Short humerus on the affected side.

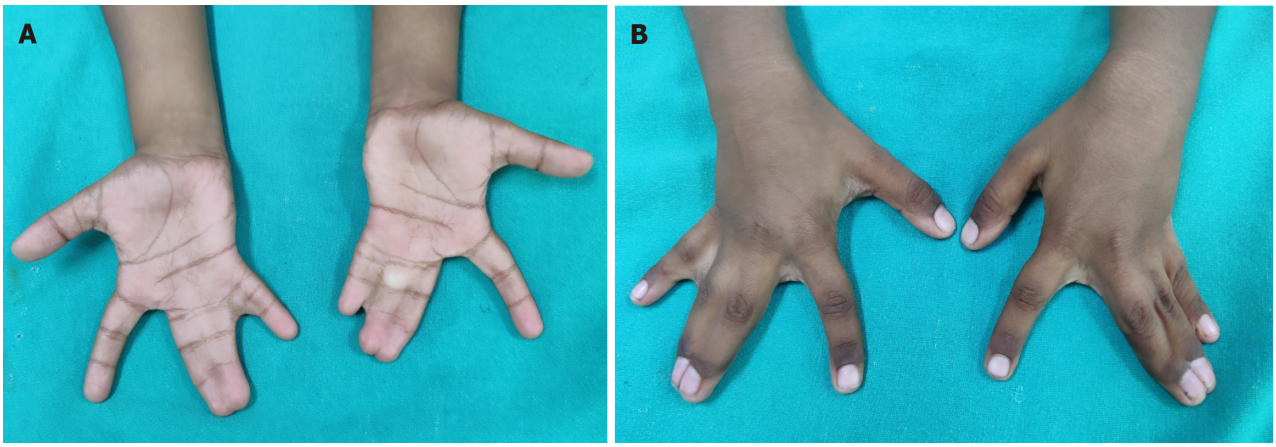

Figure 11 Syndactyly. A: Complete syndactyly of bilateral 3rd web spaces and incomplete syndactyly 4th webspace (palmar view); B: Dorsal view.

anaesthesia to infants is another consideration. Surgery can usually be done in most cases by 12-18 months or so. However, in cases requiring multiple operations, interventions may have to be started earlier.

In patients of syndactyly where the fused digits have significant difference in length or there are abnormal bones or bony fusions, intervention should be started by 6-9 months lest functional deformity results due to asymmetrical growth (Figure 12). This is especially true for syndactyly between the ring and little finger and between the thumb and index finger. In bilateral cases, surgery can be done simultaneously on both hands by two teams as the child is dependent on parents for all his daily needs[76]. Operative blood loss is not a consideration as these operations are always done under tourniquet control. It is ideal that the child goes to school with normal anatomy and function which is so important for development of their fine motor skills and psychosocial development. In mitten hand (*e.g.*, Apert syndrome), the first and third web spaces may be separated first and the second and fourth web spaces may be done in second stage. First web is most important so that the child starts using the thumb in a normal way. First surgery must be started by 6 months of age. Last but not the least, the congenital anomalies of the hand are sometimes part of various syndromes and cardiovascular anomalies which may be life-threatening and demand prior correction. Consequently, usual timelines may have to be delayed. Children with partial simple syndactyly can be delayed without any consequences.

In patients of polydactyly, planning is required to decide the digits to be kept and the ones to be amputated. Simple ablative surgery alone, especially in duplication of thumb, can result in instability and malalignment of the remaining thumb[77]. The main goal of operative intervention in these cases is restoration and maintenance of function and pinch activity along with improved appearance. While a nubbin can even be amputated under local analgesia at 1-2 months of age, a more developed supernumerary digit needs removal under general anaesthesia in a preschool child. Even polydactyly of the feet should be treated prior to school so that the child can wear normal shoes (Figure 13).

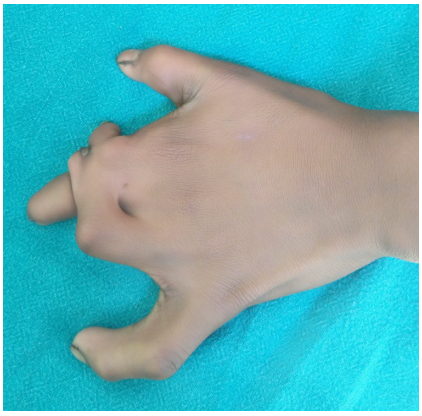

Figure 12 Complex complicated syndactyly with aberrant transverse bones and bony fusions.

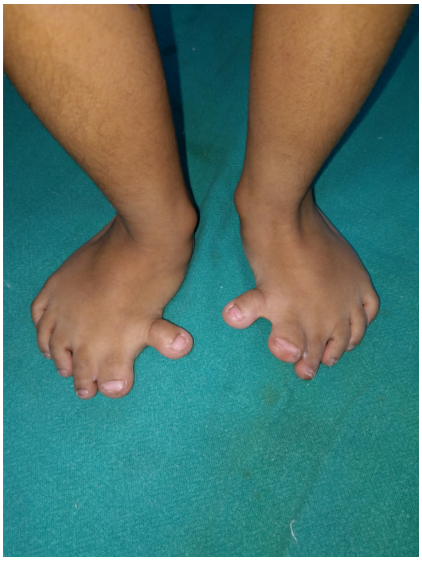

Figure 13 Bilateral feet pre-axial polydactyly and syndactyly in a teenager.

Cleft hand has been described as 'a functional triumph, but a social disaster' as many function well without correction (Figure 14)[78]. However, those with a progressive deformity (*e.g.*, transverse bones, syndactyly, *etc*.) or absence of first web space or thumb need early intervention in early infancy. Other cases may be delayed till 1-2 years of age.

Thumb hypoplasia of lesser severity may not warrant any intervention but the most severe needs pollicization (Figure 15). Cortical plasticity and motor relearning are said to be of critical importance in functional development following pollicization[79]**.** There is a large region of the sensorimotor cortex dedicated to hand with significant chunk devoted to thumb. The cortical plasticity unveils formerly quiescent connections with sprouting of active afferents from cortical regions nearby. The changes within the sensorimotor cortex are amplified with practice by the child. Advantages of an early surgery are lessened by the very small size of the nerves, blood vessels and musculotendinous tissues. Pollicization from 1-2 years of age gives bigger child to the anaesthetist, bigger hand to work on by the surgeon and yet takes advantage of plasticity of brain of the infant/toddler. Older children and teenagers presenting late are, however, not denied the reconstructive surgery. Similar considerations apply in duplication of hand (mirror hand) where surgical intervention is needed to move one digit in place of thumb.

Constriction ring syndrome

Congenital constriction ring syndrome[80], also called amniotic band syndrome, is a non-genetic condition resulting from entrapment of different fetal parts, most commonly limbs including digits (lower more commonly than upper limbs) in a fibrous band while the fetus is still in utero. There are skin indentations or grooves varying in depth and circumference (Figure 16A). A wide variety of rare presentations can be seen with involvement of trunk, viscera and face[81]. There may be associated cleft lip and palate, craniofacial anomalies, club foot, polydactyly, neural tube defects *etc*. The most common problems which concern a plastic surgeon may range from a cutaneous constriction ring which is not very tight with no distal effects to the one with very tight circumferential involvement leading to distal deformities including lymphedema, acrosyndactyly/syndactyly and even intrauterine amputation (Figure 16B). The limb proximal to constriction ring is normal. There are aesthetic considerations as well. The considerations for timing for surgery on consequences of amniotic

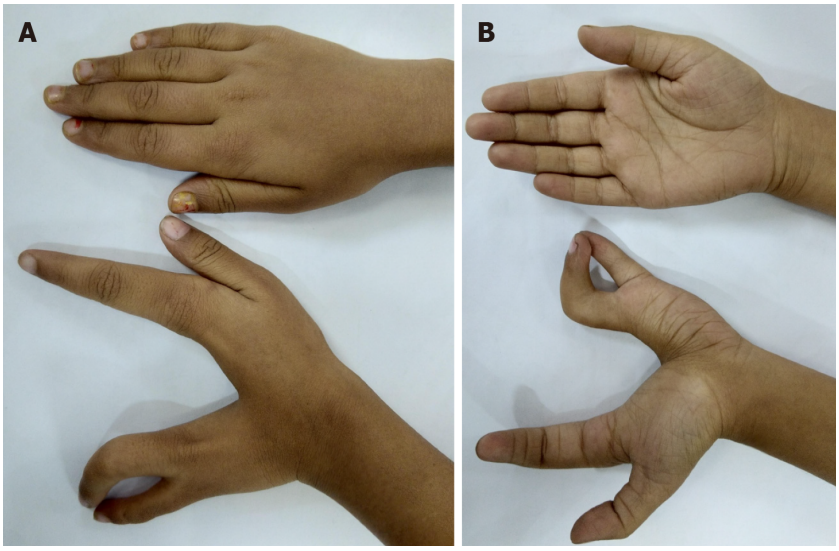

Figure 14 Left cleft hand with satisfactory function but poor aesthetic appearance. A: Palmar view; B: Dorsal view.

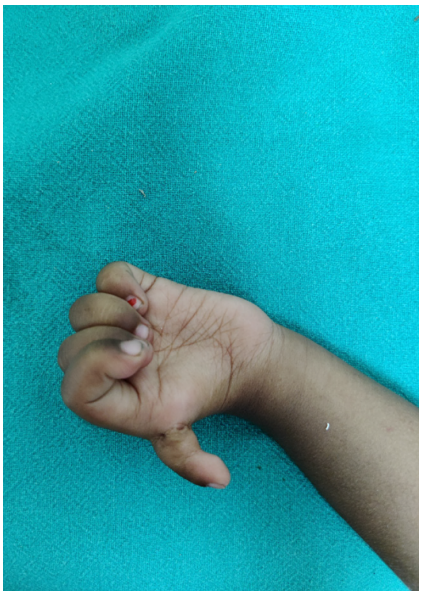

Figure 15 'Pouce flottant' thumb dysplasia should be operated with a correct balance between cortical plasticity and adequate size of tissues. In many cases, if the thumb of the opposite side is normal, the child does not use the reconstructed thumb even after pollecization.

bands on extremities include extent of compromise of blood supply to the part distal to constriction as severe hypoperfusion can lead to gangrene with risk of sepsis and threat to life of the child. This calls for an immediate surgical intervention. In other cases, general considerations of risks of surgery and anaesthesia-related complications and other associated life-threatening congenital anomalies requiring earlier management come into play. Thus, such children may have surgery done after 6 months of age. To prevent distal circulatory compromise, traditionally two stage surgery (Z-plasty of half the constriction ring in first stage followed by remaining half after 3 months) has been advocated. One-stage surgery with the obvious advantage of avoiding one more operation, is being reported with comparable results in the long-term follow-up[82]. Patients with superficial bands not affecting circulation and no/minimal lymphedema have cosmetic indications only and may be delayed. Presence of severe distal lymphedema demands an earlier intervention. It improves easily as there is no induration by scar tissue. Craniofacial and body wall anomalies should be approached by surgery based on clinical practice guidelines individually for each case. The routine use of ultrasound of the fetus in the prenatal period has allowed some observations on the prenatal natural course[83-85]. The outcomes have ranged from spontaneous resolution without any long-term consequences to progressive limb strangulation with subsequent amputation. There are reports of successful fetoscopic surgery for tackling amniotic bands in utero in animals as well as human[83-85].

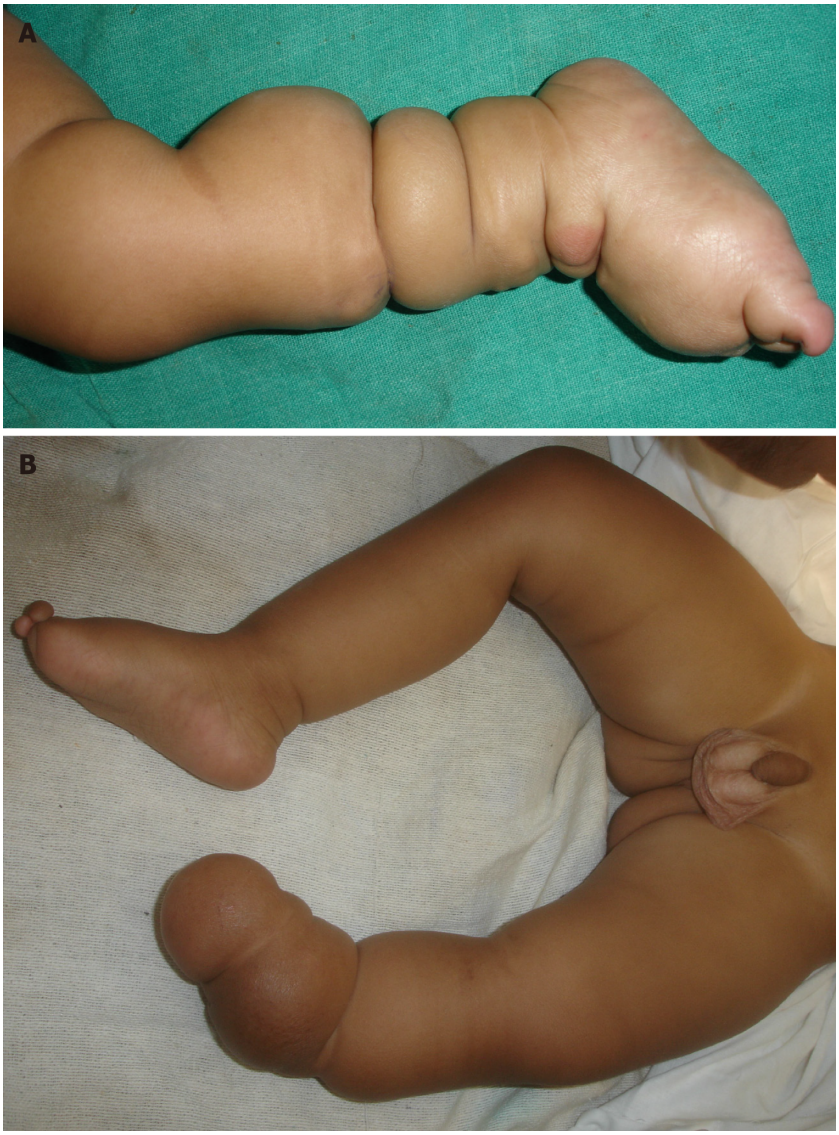

Figure 16 Constriction Ring Syndrome. A: Multiple constriction rings in the lower limbs with lymphedema foot; B: Intrauterine amputation at level of distal left leg with proximal constriction rings seen.

Hypospadias

Hypospadias is a very common congenital anomaly affecting male children. The external urethral meatus, instead of being situated at the penile tip, terminates proximal to normal site on the under surface anywhere upto perineum. In addition, there is ventral bending or curvature of the penis, known as chordee (Figure 17). More proximal the opening, greater is the difficulty in passing urine in standing position without spoiling clothes. Similarly, greater the chordee, greater the ventral curvature of penis, especially during penile erection and thus, affecting sexual function in adults. Similar to other congenital anomalies, hypospadias may be an isolated problem or else may be associated with other congenital surgical anomalies like inguinal hernia, undescended testis or even intersex states[86]. The timing for operative intervention is controversial[87,88]. The considerations for operative timing arise from the functional reasons. Child becomes a subject of ridicule by the peers once he starts going to school and has to squat for urination. This has a lot of influence on psychosocial development of the child[89]. Gender identity, *i.e.*, concept of being male or female, is well established by the age of 3-4 years with some awareness as early as 18 months[90]. Hence, corrective surgery must be over by 3 years of age. Early surgery also reduces the psychological stress of parents. The parents must be also be told that they should not get circumcision done because the preputial skin is required for urethroplasty. In adulthood, aesthetic problems may arise due to tell-tale evidence of surgical intervention or its deficiencies. Over a period of last several decades, there have been several improvements in surgical techniques. Psychological effects of operating on male genitalia after the age of memory recall (6 months) have also been studied greatly. Thus, the aim is an operation by single stage technique by 6-12 months of age[91,92]. In addition to above issues, another issue of great importance is that of surgeon being comfortable in performing surgery on very small tissues of genitalia, so-called technical considerations. Use of magnifying loupes, availability of finer micro instruments and sutures by some expert surgeons have made this technically feasible[93]. Many surgeons still prefer to wait till the age of 18-24 months (or even more) for performing urethroplasty so that larger tissues are available. The penis grows only moderately during the preschool period. Any

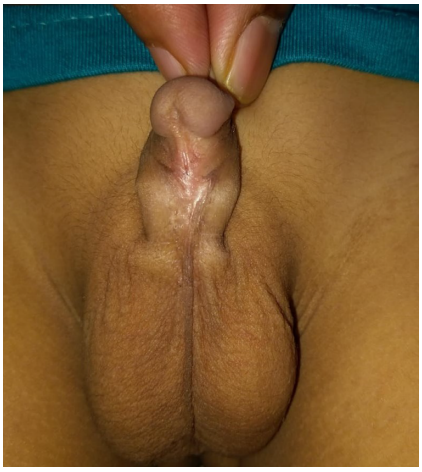

Figure 17 Mid penile hypospadias with chordee in an infant.

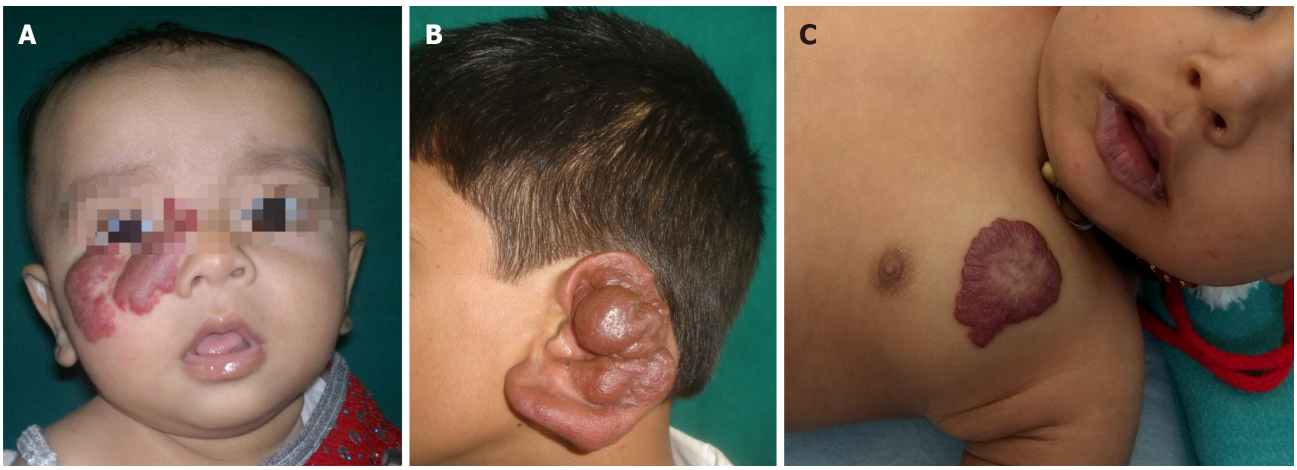

Figure 18 Vascular anomalies. A: A hemangioma on the face of an infant. Proliferation of the lesion can cause obstruction to vision in the right eye, may need propranolol therapy to expedite involution; B: A pulsatile arterial malformation of the ear in a teenager, increasing in size- needs embolization and surgical intervention; C: An innocuous hemangioma on the chest- can follow the wait and watch policy for such a lesion.

delay beyond 3 years has no benefits to the surgeon during operation[94]. A few children may have associated meatal stenosis and may need meatotomy very early (may be at 3 months of age), if they are symptomatic (straining during micturition) and surgeon's assessment finds meatal size to be the cause of problem.

Even after successful management, these patients must be followed till the adult age because the now grown-up patient may find some problems like scarred or tight skin, abnormal shape of glans, psychological issues *etc*. many of which may be treatable at this stage and improve the quality of life of the patient[95,96].

Vascular anomalies

The definitions, classification and treatment of vascular anomalies (vascular tumours and vascular malformations) has tremendously evolved over the last three decades[97]. They can occur in all parts of the body and are most common in head and neck region (Figure 18A and B). There is a very vast list of diseases which come under the category of vascular anomalies and obviously their treatment and need for surgical intervention cannot be same. Moreover, there is an extremely vast spectrum of severity with some just innocuous small lesions (Figure 18C) requiring no treatment to ones which have great morbidity and even risk to life. Hence, timing of surgical intervention has to be assessed by the surgeon taking all the factors into consideration. They are found in the internal organs as well. Thus, specialists of multiple disciplines like general surgery, plastic surgery, dermatology, neurosurgery and otorhinolaryngology *etc*. treat them and need coordination in their management[98]. The child needs treatment not only to minimize the physical side effects of vascular anomalies but also for aesthetic reasons.

Classically, infantile haemangiomas have been seen to proliferate initially over 9-12 months of age and then regress or involute over a period of time in almost 70% of children by 7 years of age. In those resolved completely of their own, some evidence is left in the form of atrophic scar *etc*. Thus, traditionally they have been managed with 'wait and watch policy' or the so-called 'masterly inactivity'[99]. However, over a period of last few decades, several effective medical therapies have come up which are being used by surgeons to expedite this maturation and involution process[98,100]. During this waiting period stretching over several years, the parents of these children also need to be counselled and

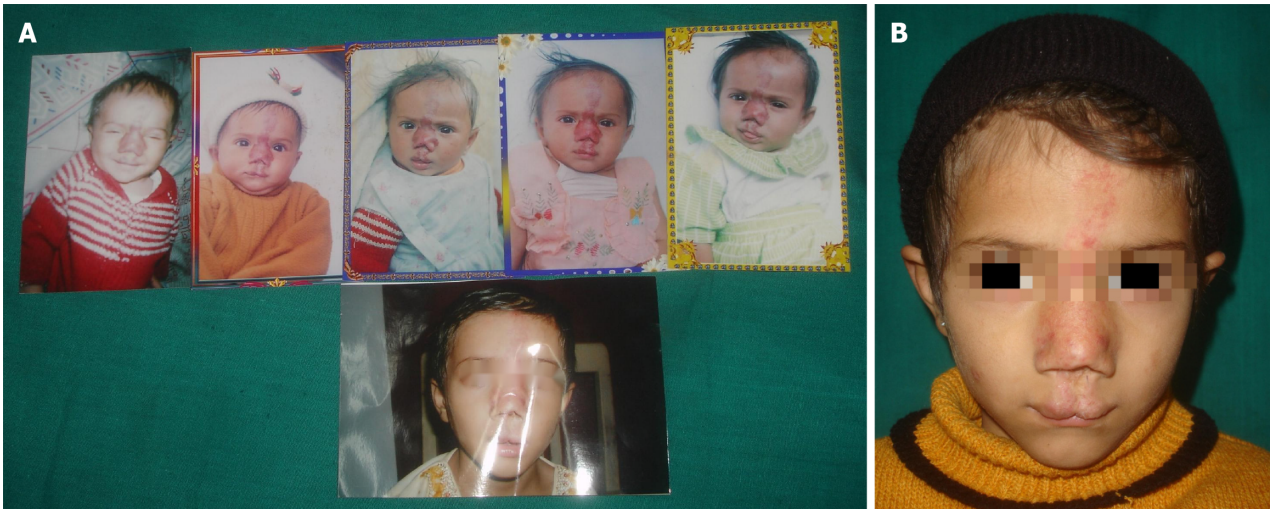

Figure 19 Congenital infantile hemagioma. A: Serial photographic record of a child with vascular lesion on the face; B: Final photograph at 10 years of age with residual scarring.

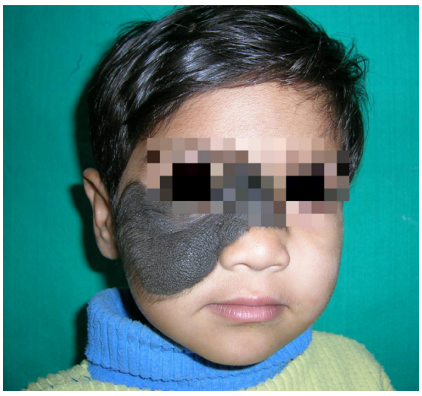

Figure 20 Congenital hairy nevus in a preschool toddler with a bear-like appearance- a huge stigma.

photographic records of the lesion taken at regular intervals be kept by the clinician as well as parents to see the progress (Figure 19). At the same time, the surgeon must show the pictures of children under his/her care showing spontaneous resolution to the parents to gain their confidence. Thus, at present, 'masterly inactivity' only refers to non-surgical management. Some children do need surgical intervention earlier because of functional reasons or recurrent ulceration/bleeding episodes. Those in the oral cavity and respiratory tract may obstruct airways and be life-threatening. Those present over the periorbital region may obstruct vision and lead to deprivation amblyopia. Nostrils may also get blocked. Laser therapy is effective in the management of many cases. Surgical intervention is best done for residual deformities and scars left after spontaneous resolution or laser treatment. Surgical excision is also sometimes done for very small lesions or those which may be better or equally well treated instead of expectant policy[101,102], especially with respect to resulting scar/residual lesion.

There is a very wide spectrum of vascular anomalies from those with purely cosmetic considerations (*e.g.*, Port-wine stain) to massive arteriovenous malformations with functional consequences and risk of massive haemorrhage. A very large spectrum of treatment modalities, *e.g.*, sclerosant therapy and lasers *etc.* are available. Interventional radiology has been used to block the feeder vessels by embolization followed by surgical excision.

Giant hairy/non-hairy Nevi

Giant congenital hairy/non-hairy nevi are melanocytic nevi which are rare but very important not only because of their association with risks of malignant transformation (malignant melanoma) and central nervous system involvement but also because of their major psychosocial impact on the child and his/her parents due to their ghastly appearance (Figure 20). These children become a subject of ridicule by their peers in school who often call them 'bear' because of their skin colour. Although skin of any part of body can be involved, these are frequently seen in trunk, followed by extremities and head and neck. More than one body region may be affected. In addition, there may be multiple satellite lesions of varying sizes all over the body (Figure 21). Some peculiar locations have led to various terms like 'bathing trunk nevus' (Figure 22), 'coat sleeve nevus' *etc.* Various definitions of their size have been given by different workers [103]. Thus, giant nevi present aesthetic as well as risk to malignant change as considerations for timing of surgical intervention.

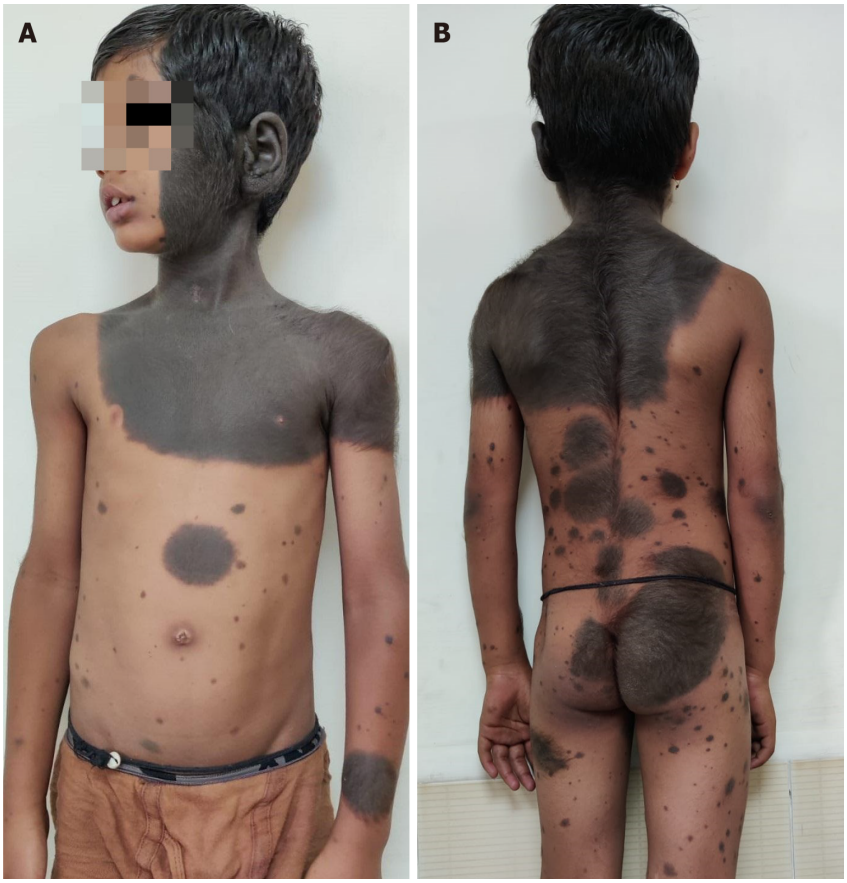

Figure 21 Giant hairy nevus. A: A child with extensive involvement of the head and neck, trunk with multiple small and large satellite hairy nevi; B: Back, buttocks and limbs also have extensive involvement.

The exposed parts of body constitute an absolute indication for their excision to restore psychological well-being. Development of nodularity, repeated excoriation, ulceration and infection are also indications for excisional therapy. Complete armamentarium of a plastic surgeon is required in their management depending on their size, site and depth of involvement *etc*[104,105]. Some of the treatment modalities, *e.g.*, dermabrasion, skin curettage, shave excision or lasers improve the appearance but since the complete burden of nevus cells is not taken care of, the risk of malignant change persists[106-108]. Hence, these patients must be under constant observation of clinician and must be taught to look for abnormal features and report them as soon as possible for confirmation or otherwise.

The risks of malignant change are different in different population groups. It has been seen to be very high in Caucasians while it is practically nil in Indian subcontinent. This is a very important consideration for surgical intervention. The risk of malignancy, which some workers have found to be 50% within first 3 years of life and 70% by 13 years in Caucasians, is the most contentious issue in management and demands complete excision of all the lesion as soon as possible[109]. However, the massive size of the lesion and the paucity of donor skin areas may not allow complete excision. In low/minimal risk population, these lesions need excision for their aesthetic considerations alone. In such a scenario, one would definitely like to make the child lesion free before going to school. However, for several massive lesions it may not be practically possible as they require multiple stages. Partial excisions in non-exposed areas may be acceptable as residual lesion is not likely to turn malignant. The first operation may be done at 6 months. It must be remembered that some of the best techniques of resurfacing after excision may not be possible in very small children. Thus, the lesion may have to be excised completely preventing risk of malignancy and removing the title of 'bear' to the child and cover provided with split skin graft before the child goes to school. The definitive resurfacing with tissue expansion or appropriate free flap can be provided later in teenage/adulthood. Once malignant melanoma is diagnosed, appropriate anti-cancer therapy needs to be instituted.

CONCLUSION

Congenital anomalies amenable to surgical treatment are very common. They are treated by surgeons of various surgical disciplines apart from a paediatric surgeon. They may need immediate surgical intervention to save the life of the patient. Almost all those anomalies which are treated by a plastic surgeon are done as elective procedures and there are optimum timings for surgical intervention ranging from, say 3 months for a cleft lip alone to adulthood for breast reconstruction in a Poland syndrome. Parents of these children are to be guided appropriately by the obstetrician, paediatrician and the

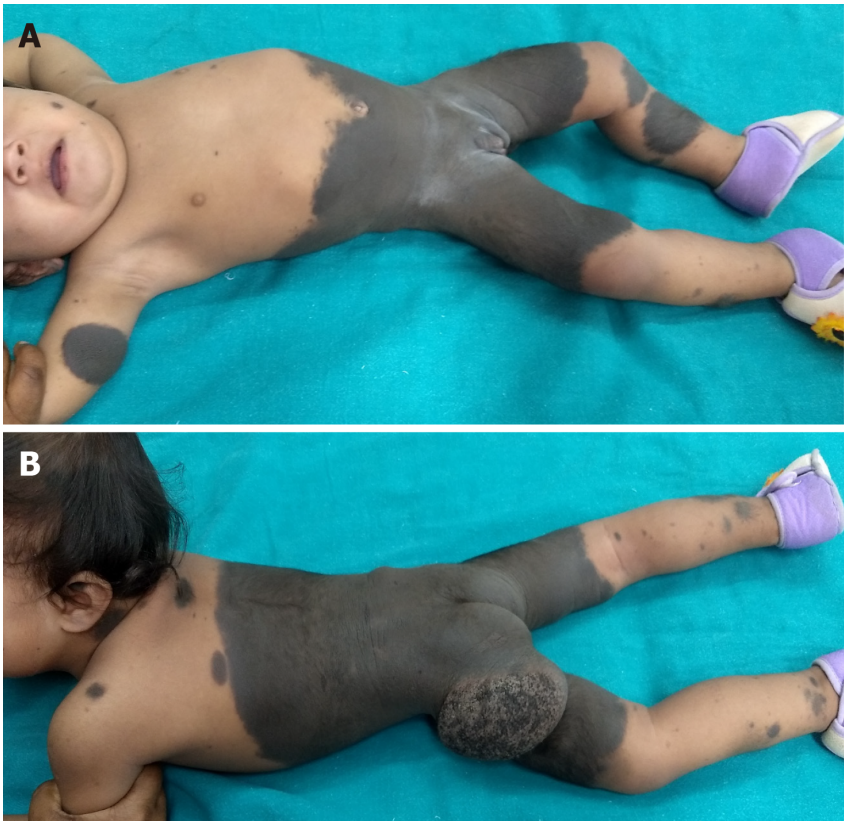

Figure 22 Bathing trunk nevus with satellite lesions. There is not enough donor area for complete excision and resurfacing. A: Supine view; B: Prone view.

family physician, with convincing reasons as to why so much delay is required, before these children are referred to and seen by a plastic surgeon. This article is an attempt in that direction.

FOOTNOTES

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REVIEW

Assessing the impact of concurrent high-fructose and high-saturated fat diets on pediatric metabolic syndrome: A review

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Abstract

High-saturated fat (HF) or high-fructose (HFr) consumption in children predispose them to metabolic syndrome (MetS). In rodent models of MetS, diets containing individually HF or HFr lead to a variable degree of MetS. Nevertheless, simultaneous intake of HF plus HFr have synergistic effects, worsening MetS outcomes. In children, the effects of HF or HFr intake usually have been addressed individually. Therefore, we have reviewed the outcomes of HF or HFr diets in children, and we compare them with the effects reported in rodents. In humans, HFr intake causes increased lipogenesis, hypertriglyceridemia, obesity and insulin resistance. On the other hand, HF diets promote low grade-inflammation, obesity, insulin resistance. Despite the deleterious effects of simultaneous HF plus HFr intake on MetS development in rodents, there is little information about the combined effects of HF plus HFr intake in children. The aim of this review is to warn about this issue, as individually addressing the effects produced by HF or HFr may underestimate the severity of the outcomes of Western diet intake in the pediatric population. We consider that this is an alarming issue that needs to be assessed, as the simultaneous intake of HF plus HFr is common on fast food menus.

Key Words: Fructose; Saturated fat; Metabolic syndrome; Insulin resistance; Type 2 diabetes; Ultra processed foods; Children; Obesity; Dyslipidemia; Non-alcoholic fatty liver disease

Core Tip: High-fructose (HFr) or high-fat (HF) intake favors metabolic syndrome (MetS) development by different mechanisms. When combined, HFr exacerbates the effects of HF, leading to a faster and more severe MetS development. Combined HF + HFr is usually present in ultra-processed foods. However, there is a lack of studies in the pediatric population evaluating the impact of restricting the combined intake of carbohydrates and fat in MetS. We reviewed the mechanisms by which HF + HFr produces more severe MetS to support the need for studies targeting the combined intake of HF + HFr in pediatric population to improve the outcomes of different interventions against MetS.

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INTRODUCTION

Industrialization of occidental society has led to profound changes in several aspects of human life, including the introduction of foods that are accessible to the majority of the population, have a long shelf life, and are highly palatable. This has opened the world market for ultra-processed (UPC) foods, a term that refers to industrial modification of food such that the final product is different from the original and generally contains little or no whole food[1]. High amounts of saturated fat or carbohydrates are added to these foods to achieve high palatability and making them energy-dense, the chronic consumption of which is believed to be the main driver of the current obesity pandemic.

In contrast to the current panorama, in both the Middle Ages and Renaissance, obesity was considered a status symbol of affluence and health, triggering the onset of metabolic disorders in adults. Childhood obesity gained attention approximately 40 years ago when the stable prevalence curve of obesity in the pediatric population was found to have become parabolic[2]. Over the last three decades, the global prevalence of obesity has increased by 27.5% in adults and 47.1% in children[3], highlighting the urgent need to understand the main causes of this phenomenon.

Undoubtedly, the increasing accessibility of high-calorie UPC foods in developed and developing countries has played an important role in the early onset of obesity[4,5], which is a key risk factor for the development of metabolic syndrome (MetS)[6]. In adults, MetS is defined as a set of diagnostic symptoms including central obesity with a waist circumference ≥ 102 cm in men or ≥ 88 cm in women, blood pressure (BP) ≥ 130/85 mmHg, triglycerides (TG) ≥ 150 mg/dL, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL (men) or < 50 mg/dL (women) and fasting glucose ≥ 100 mg/dL[7,8]. In contrast with the well-established criteria for defining MetS in adults, the diagnosis of MetS in children lacks universally accepted criteria[9].

In addition to the excessive consumption of UPC foods, other factors that may contribute to early onset MetS are maternal obesity and the consequent development of gestational diabetes and type 2 diabetes mellitus (T2DM). This metabolic environment enveloping the fetus is related to an alteration in the degree of fetal adiposity and triggers early-onset obesity[10]. An association between the visceral adiposity index, daily energy intake, and MetS has been reported in children and adolescents with obesity aged 8–15 years[11]. Thus, fetal metabolic alterations and early simultaneous consumption of high-fructose (HFr) and high-saturated fat (HF) diets may enhance the pathophysiological mechanisms that contribute to the development of MetS.

Fructose, in HFr, is a glucose isomer widely used as a glucose substitute owing to its high sweetening power. It is a carbohydrate with the highest lipogenic power because its glycolytic metabolism is not subjected to negative feedback regulation. Thus, the hepatic metabolism of fructose yields large amounts of substrate for lipogenesis[12-14]. Moreover, HFr consumption increases hepatic uric acid production, which directly contributes to insulin resistance[15]. Accordingly, a consumption of > 100 g/d HFr may have serious health repercussions[16].

Trans-saturated fats, in HF, are added to UPC products whose fats have been hydrogenated to increase their shelf life [17] and are considered a risk factor for the development of coronary heart diseases, insulin resistance, and obesity accompanied by systemic inflammation[18,19].

Common pathological consequences such as insulin resistance, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), hyperglycemia, and increased BP have been observed when HF or HFr diets were administered separately in animal models of MetS[20], probably because both HF and HFr diets share lipotoxicity as a common injury mechanism [20,21]. Nevertheless, at the molecular and cellular levels, HF and HFr exhibit different injury features. Moreover, when combined, HF and HFr reinforce their deleterious effects and produce more severe MetS phenotypes than when supplemented separately[22-24].

Therefore, this narrative review aimed to highlight the need for increased attention to the consequences of simultaneous intake of HF and HFr on the metabolic health of the pediatric population; this is because many UPC foods in fast food establishments consist of sugar-sweetened beverages and HF-containing foods, frequently targeting the pediatric population[25]. To address this issue, we briefly reviewed the mechanisms underlying the effects and evaluated studies on the outcomes of HF, HFr, and HF + HFr diet-mediated MetS development and epidemiology of pediatric MetS. Finally, we reviewed some studies to measure the beneficial impact of reducing fat and fructose-related calories in the diets of children. This raises the urgency of measuring the impact of the simultaneous intake of HF and HFr diets rather

than measuring only their individual outcomes in the pediatric population.

EPIDEMIOLOGY

MetS definition and prevalence

The International Diabetes Federation and the American Diabetes Association suggest that only children older than 10 years should be examined for MetS, whereas for children younger than 10 years, only high waist circumference measurements should be used for screening[26]. According to the International Diabetes Federation, children aged 10-16 years with central adiposity (≥ 90th) and presenting with two of the following criteria: TG ≥ 150 mg/dL; HDL-C < 40 mg/dL; systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg; and fasting plasma glucose ≥ 100 mg/dL[27] may be diagnosed with MetS. At the beginning of 21st century, only a few studies investigated MetS frequency in childhood and adolescence because of the various criteria used to define MetS[28]. Nevertheless, a systematic review including 169 studies was conducted in 2020 with 550405 children and adolescents from 44 countries across 13 regions worldwide[29]. The results showed that approximately 3% of the children (6-12 years) and 5% of adolescents (13-18 years) had MetS, with some variation across countries and regions. Although these numbers may appear modest, they are the result of a noticeable increase in the past four decades in the global prevalence of pediatric obesity, T2DM, and overall MetS[30].

MetS causes and risk factors

In the pediatric population, the onset of MetS is driven by an array of risk factors. A prominent factor is the presence of a family history of MetS, particularly when one or both parents have MetS or related disorders[31]. Moreover, lifestyle choices exert a prominent influence, with suboptimal dietary patterns and lack of physical activity significantly contributing to increased risk[32]. In this context, childhood obesity and excessive adiposity particularly concentrated around the abdominal region, have emerged as critical factors that increase the risk of MetS in children and adolescents [33]. This phenomenon is closely linked to a noticeable increase in the availability of high-fat foods and sugar-sweetened drinks, the consumption of which has markedly increased during the past decades, coinciding with an exponential increase in MetS incidence[34-39]. Furthermore, a high-fat diet exacerbates vascular oxidative stress and endothelial dysfunction before the onset of insulin resistance and systemic oxidative stress, thereby contributing to the onset of MetS [40].

Comorbidities and complications of MetS

Children and adolescents with obesity frequently display endocrine comorbidities including T2DM, dyslipidemia, polycystic ovary syndrome, central precocious puberty, and MetS[41]. MetS is not an isolated condition, but rather a precursor to a multitude of comorbidities and potential complications.

Among the most common comorbidities associated with MetS in children and adolescents are abdominal obesity, high BP, dyslipidemia, and T2DM[42-44]. In addition, MetS may lead to complications affecting various organ systems, including the liver and kidney[45]. Furthermore, long-term complications of MetS increase the risk of developing cardiovascular diseases (CVDs), such as atherosclerosis and coronary artery disease (Figure 1)[46,47].

DIFFERENTIAL EFFECTS OF HF, HFr, AND HF + HFR DIETS ON LIPID METABOLISM AND MetS

The intermediate metabolism of carbohydrates and lipids converges during acetyl-CoA formation for ATP generation or carbon molecule buildup to cope with the biosynthetic needs of the cells. The fate of these fuels is determined by an intricate network of signals involving the endocrine system, and cellular energy needs manifest as variations in the ATP/ADP and nicotinamide adenine dinucleotide [NAD(P)]/NAD(P)H ratios, which control metabolic fluxes *via* allosteric and post-translational modifications of enzymes. Here, we briefly summarize the fundamental aspects of fructose and fatty acid metabolism and their influence on the development of insulin resistance, emphasizing the disturbances in lipid metabolism. The effects of excessive consumption of carbohydrates and lipids on the overall alterations that influence MetS have been described elsewhere[20,24].

Biochemical mechanisms underlying HFr diet effects in MetS

Fructose consumption has not been associated with health issues until recently. Indeed, in the middle of the 20th century, fructose was used as a sweetener by individuals with diabetes because its metabolism is not insulin-dependent. Moreover, it increases acetone synthesis, restores nitrogen balance, and decreases water loss[48]. The following paragraphs summarize the biochemical basis of this notion has changed completely.

Fructose in sugar-supplemented beverages is usually added as HFr corn syrup-55, which is composed of a mixture of 55% fructose and 45% glucose in the form of monosaccharides[49], allowing rapid fructose absorption. Fructose does not undergo enzymatic hydrolysis in the intestine. Fructose absorption by the brush border and basolateral membranes of the small intestine is carried out by the carbohydrate transporter GLUT-5, although the GLUT-8 transporter may also be involved in this process[50-52].

Once in portal blood, fructose is transported into hepatic cells by GLUT2[53]. Fructose is metabolized in hepatocytes *via* fructolysis, which involves three enzymatic steps that yield triose-phosphate molecules that are available for lipogenesis. Fructose is first phosphorylated by fructokinase to fructose-1-phosphate (F1P), which is cleaved by aldolase B

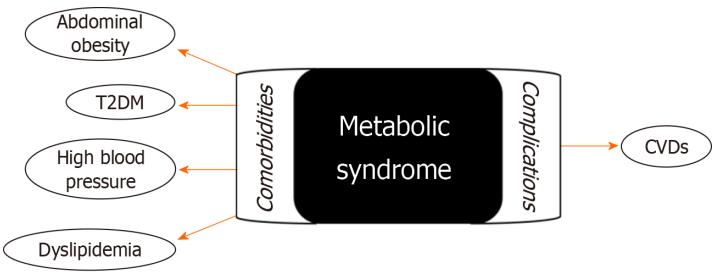

Figure 1 Comorbidities related to metabolic syndrome and their complications. T2DM: Type 2 diabetes; CVD: Cardiovascular disease.

into dihydroxyacetone phosphate (DHAP) and glyceraldehyde; glyceraldehyde is subsequently phosphorylated to glyceraldehyde-3-phosphate (G3P) by triokinases[54]. The unregulated and rapid phosphorylation of fructose by fructokinase and the bypass of fructose metabolism from the major regulatory steps of glycolysis explain the negligible concentration of fructose in the systemic circulation and increased production of triose phosphate available for glycolytic and gluconeogenic pathways[20].

The metabolic fates of G3P and DHAP are dependent on nutritional status, as triose phosphates are involved in gluconeogenesis and *de novo* lipogenesis (DNL)[55] in starved and fed animals, respectively. In fed animals, triose phosphates are converted to pyruvate, causing enhanced flux into the tricarboxylic acid cycle and increased citrate production, which is exported to the cytosol and transformed by ATP citrate lyase into cytosolic acetyl-CoA, the starting point for DNL[56]. DNL is stimulated by at least two factors derived from fructokinase-mediated fructose phosphorylation, namely, elevated uric acid synthesis supported by phosphate depletion[57], and F1P as a signaling molecule[58]. Moreover, excessive uric acid impairs insulin signaling, thus contributing to insulin resistance[59].

DNL is mainly involved in the development of certain features of MetS, including elevated blood levels of total cholesterol, TG, and free fatty acids (FFA)[60]. At the intracellular level, DNL increases the concentrations of diacylglycerols and ceramides in the liver and skeletal muscle, driving local and systemic insulin resistance[61,62]. Oxidative stress and chronic inflammation are also involved in insulin resistance and may be enhanced by fructose-induced DNL[63].

Biochemical mechanisms underlying HF diet effects in MetS

The hepatic accumulation of fatty acids, a characteristic of MetS, can be due to excessive fat ingestion. TG in HF diets are emulsified by bile acids within the intestinal lumen and then hydrolyzed primarily by pancreatic lipase to sn-2-monoacylglycerols and FFA[64]. In enterocytes, FFA are re-esterified, and the resultant TG are incorporated into chylomicrons for entry into circulation. Chylomicrons are taken up by muscle and adipose tissue owing to the activity of lipoprotein lipase, whereas chylomicron remnants are taken up by parenchymal liver cells[65,66]. An HF diet increases the blood levels of chylomicrons and their remnants. Abnormal chylomicron metabolism in diabetes is associated with atherosclerosis development[67].

Conversely, although the role of passive diffusion in fatty acid transport[68] is small, the active uptake of circulating fatty acids by the liver is dependent on fatty acid transporter proteins (FATP), the expression of which is regulated by peroxisome proliferator-activated receptor gamma. The FATP2 and FATP5 isoforms are found primarily in the liver. In hepatocytes, FFA may be metabolized in the mitochondria *via* fatty acid β-oxidation to produce ATP and ketone bodies. FFA can also be esterified to G3P and cholesterol for the synthesis of TG and cholesteryl esters, respectively, which accumulate in hepatocytes as small droplets or are secreted into the bloodstream as very low-density lipoprotein particles [69]. An imbalance between fatty acid utilization for energy generation, very low-density lipoprotein secretion, and TG storage causes lipotoxicity in hepatocytes, leading to the development of NAFLD and impaired insulin signaling.

Mitochondrial fatty acid β-oxidation produces large amounts of flavin adenine dinucleotide ($FADH_2$), NADH, and acetyl-CoA. NADH and $FADH_2$ are reoxidized to NAD^+ and FAD, respectively, by the mitochondrial electron transport chain (ETC). Excessive TG accumulation in hepatocytes causes mitochondrial dysfunction, inefficient electron transport through the ETC, electron leakage from the redox sites of ETC complexes, and generation of reactive oxygen species (ROS)[70]. Therefore, the overconsumption of fat impairs mitochondrial fatty acid β-oxidation by impairing NADH and $FADH_2$ oxidation. This produces reductive stress in the form of high NADH/NAD^+ ratios, which together with excess acetyl-CoA lead to the reversible acetylation and inhibition of several enzymes participating in catabolic pathways, including acyl-CoA dehydrogenases involved in β-fatty acid oxidation and ETC complexes, among others[71]. Overall, in addition to causing the accumulation of lipids such as ceramides and diacylglycerol, which interfere with the insulin receptor[61], enzyme inhibition enhances the impairment of ETC function and causes overproduction of ROS. In turn, ROS oxidize unsaturated lipids in fat deposits, causing lipid peroxidation. Moreover, HF diet-induced ROS triggers proinflammatory signaling, inducing nuclear factor kappa B -dependent production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, TNF- β, interleukin-6, interleukin-1β, and inducible nitric oxide synthase. Overall, this enhances insulin resistance and contributes to dyslipidemia[72,73], whereas, at the systemic level, dyslipidemia, ROS, and inflammation favor the development of atherosclerosis and CVD.

Biochemical mechanisms underlying combined HF + HFr diet effects in MetS

As mentioned above, there is a growing interest in exploring the combined effects of HF and HFr diets to mimic the composition of Western diets comprising high-fat foods accompanied by sugar-sweetened beverages typically served in

fast food establishments. At least three recent studies in rodents have shown that fructose addition to an HF diet worsens the deleterious effects of HF diet by increasing DNL and enhancing damaging effects of HF on mitochondrial function, oxidative stress, hepatic lipid metabolism, and insulin resistance[22,23,62]. Rats fed the HF + HFr diet displayed higher levels of blood TG, FFA, and insulin than those fed an HF diet alone. The increase in both FFA and TG was believed to be the result of DNL stimulated by HFr consumption, as an HF diet inhibits DNL in the liver and whole body, whereas the opposite occurs with an HF + HFr diet[74]. Hepatic insulin resistance was also higher with the HF + HFr diet than that with the HF diet, which was in concordance with higher ceramides levels, TG levels, mitochondrial dysfunction, and steatosis in rats fed an HF + HFr diet than that in rats fed only an HF diet[62].

The worsening of mitochondrial dysfunction, *i.e.*, impaired mitochondrial fatty acid oxidation, by the HF + HFr diet may be central to the more deleterious effects of this diet than of the HF or HFr diet alone[62], as impaired oxidative phosphorylation and increased severity of steatosis was observed in rats consuming HF + HFr than those consuming only an HF diet or HFr diet[23]. Moreover, mitochondrial dysfunction coincided with higher ROS production and oxidative stress with this diet than that with an HF diet or an HFr diet alone[23,62].

Fructose addition to an HF diet increased the acetylation and inhibition of carnitine-palmitoyl transferase 1a (CPT1a), which mediates fatty acid translocation of fatty acids into the mitochondrial matrix and is the rate-limiting step in fatty acid β-oxidation, and long-chain acyl-CoA dehydrogenase (ACADL), thus contributing to increased liver steatosis[22].

Thus, compared to an HF or HFr diet alone, fructose addition not only worsens but also accelerates the effects of an HF diet by impairing ETC function to a higher degree, probably eliciting higher NADH/NAD^+ ratios that induces a greater inhibition of fatty acid β-oxidation *via* increased acetylation of ACADL and CPT1a. This, in turn, may increase the concentrations of incomplete products of fatty acid oxidation, such as ceramides and TG, causing impaired insulin signaling, which may be reinforced by the exacerbated ROS production induced by HF + HFr diets in the liver mitochondria. Therefore, this raises the question of whether, in childhood, the frequent consumption of UPC fast foods containing HF + HFr triggers early onset of MetS than the consumption of foods containing only HF or HFr, and whether this worsens the outcomes of MetS, namely diabetes and its complications, including early onset of CVD.

STUDIES IN THE PEDIATRIC POPULATION

Pediatric clinical trials and research studies

A review of the methodology of clinical trials and their limitations, as well as cohort, cross-sectional, and other observational and associative studies related to both HF and HFr consumption patterns in children of different ages, ethnicities, and metabolic conditions, will help to understand the role of HF and HFr diets in metabolic disorders and MetS development. The prevalence of disorders related to lipid metabolism, such as obesity, diabetes, hyperlipidemia, dyslipidemia, glucose and insulin intolerance, hepatic steatosis, and heart risk, increases with age; however, these metabolic risk factors in childhood could predispose to the development of chronic degenerative diseases in adulthood[75-77]. Therefore, it is important to explore how HF and HFr individually, or the simultaneous intake of HF + HFr differentially alters metabolic parameters from childhood, becoming imminent risk factors in the subsequent development of metabolic diseases.

Types and effects of high-fat intake

The Dietary Guidelines for Americans 2020-2025 and the joint World Health Organization (WHO)/Food and Agriculture Organization expert consultation recommend limiting saturated fat intake to < 10% of the total energy intake[78]. This is mainly because high saturated fat intake is associated with an increase in metabolic risks, such as high body mass index (BMI), hepatic steatosis, insulin resistance, and high levels of TG, total cholesterol, low-density lipoprotein cholesterol (LDL-C)[79-81]. A high-fat diet and insulin resistance also promote FFA accumulation in the liver, leading to a loss of regulation of normal lipid liver metabolism and the development of NAFLD, which frequently occurs in children and has been reported in cases of severe hepatitis and hepatic fibrosis in children aged 10 years[82].

The focus on trans fatty acids (tFA) as a causal factor of MetS has increased in recent years. Although saturated fatty acids can be derived from natural sources, tFA have an industrial origin in the production of UPC foods. Industrial tFA are produced by the partial hydrogenation of unsaturated vegetable oils, which occurs when vegetable oils are heated. The main sources of industrial tFA are margarine, commercially baked products, deep-fried fast foods, packaged snack foods, and other UPC foods[76,83]. tFA have been identified as an important cause of CVDs and their resulting clinical endpoints, such as stroke and heart attack. Evidence indicates that a high tFA intake is associated with an increased probability of coronary heart disease. Although CVDs and coronary heart diseases mainly present in adults, atherosclerotic lesions in the aorta and coronary arteries can begin to appear in childhood and are positively associated with dyslipidemia and other CVD risk factors, such as elevated total and LDL-C levels. The WHO has prioritized elimination of industrial tFA in processed foods and recommends limiting tFA intake to < 1 energy percentage. Moreover, the United States Food and Drug Administration (FDA) has determined that partially hydrogenated oils are no longer safe for use in human food. However, evidence regarding an association between HF intake and either MetS development or lipid and glucose profile modifications is lacking[84].

Types and effects of high fructose intake

Fructose is one of the sweetest naturally occurring carbohydrates, either in the form of sucrose or high-fructose corn syrup (HFCS). It is an important lipogenic substrate that stimulates DNL. Fructose consumption has gradually increased worldwide over the last four decades as the UPC food industry has dramatically expanded, as has sugar-sweetened

beverage consumption in children[85].

The main food types with a considerable fructose contribution consumed by children are industrialized and homemade sweetened milk-based drinks and other beverages; whole fruits contribute little to fructose intake[86]. Even moderate consumption of fruits (0.8-1.5 servings per day, 6-7 d a week) can improve the lipid profile by decreasing the total cholesterol, serum TG, and LDL-C levels[87]. Although the WHO discourages feeding fruit juices or other sweetened beverages to children aged under 2 years, babies from 6 months of age drink fructose beverages or derivatives, thus modifying their water consumption patterns and increasing health risks[88]. Additionally, the WHO recommends limiting the consumption of sugar-sweetened foods (including fructose, sucrose, and HFCS) to 10% of the total energy intake and suggests that a reduction of 5% has beneficial health effects[89]. Fructose restriction from 28% to 10% in children aged 3–18 years improved glucose metabolism and lipid profile and decreased levels of blood aspartate aminotransferase, a liver and heart injury marker[90]. In the United States, 13% of the population consumes more than 25% of their total energy intake from added sugars. Alarmingly, the prevalence of added sugar is estimated to be higher in Mexico, ranging from 58% in school-aged boys to 85% in adolescent girls; total sugar intake in the Mexican diet was 365 kcal/d, of which 238 kcal/d was from added sugars and only 127 kcal/d from intrinsic sugars[91,92]. Huerta-Ávila *et al*[86] showed an association between fructose intake and high adiposity and cardiometabolic risk factors (*i.e.*, high levels of blood glucose, insulin, TG, total cholesterol, LDL-C, and HDL-C) in children from Mexico City (Table 1).

Health risks associated with HFr consumption in pediatric populations include dental decay, overweight, obesity, insulin resistance, diabetes, NAFLD, hypertension, and cardiovascular risks[93-95]. Studies have shown that those who consume a greater amount of sugar-sweetened beverages are more likely to have elevated fasting serum insulin levels, insulin resistance, waist circumference, and serum uric acid levels[96].

Deleterious effects of high fat plus high fructose intake

UPC foods significantly contribute to HF and HFr intake in children. Approximately 41.8% of preschoolers and 47.8% of school-age children have high intakes of these products[97]. The consumption of UPC products has undergone a huge increase worldwide, with high consumption among children up to 12 months of age, with an increase observed in 16-month-old children[98]. Thus, consumption of UPC products early in life in children aged 3-8 years plays a role in altering lipoprotein profiles and could be a key determinant of CVDs. Moreover, high consumption of UPC foods in 12-18-year-old adolescents was associated with the prevalence of MetS and its associated features. Likewise, epidemiological studies (Table 1) have correlated the consumption of UPC foods with metabolic alterations and chronic diseases, such as obesity, T2DM, NAFLD, and CVDs[99-105].

PREVENTION AND MANAGEMENT OF METABOLIC DISORDERS

Children and teenagers who consume HFr/HF diets usually develop metabolic disorders such as obesity, NAFLD, insulin resistance, T2DM, and other MetS components[106]. Therefore, it is important to educate the public regarding the risk of excessive consumption of these products and unhealthy lifestyles among children who develop several MetS components. Nevertheless, accomplishing this goal has been challenging, as initiatives aimed at decreasing the consumption of products containing HFr/HF in children and interventions designed for the treatment of associated diseases have not succeeded[107], as evidenced by the current childhood obesity pandemic[108]. However, many research groups, institutions, and organizations worldwide continue to develop strategies to reduce the consumption of products containing HF/HFr and treat disorders caused by excessive intake. An essential aspect of these prevention and treatment programs is the need for multidisciplinary teams to address the problems and improve the probability of success. These programs should prioritize dietary guidance while emphasizing physical exercise and, if needed, pharmacological, psychological, and surgical interventions[109].

Dietary interventions to prevent pediatric MetS

Owing to the limited efficacy of therapeutic interventions for the treatment of MetS associated with HFr/HF diets, there has been growing interest in preventive strategies. This may be feasible because many risk factors, excluding genetic influences, can be modified to serve as targets for preventive initiatives[110]. Interventions for both the prevention and treatment of the negative consequences of HF/HFr diets in children focus on reducing the intake of products containing HF or HFr, high-glycemic, and calorie-dense foods[111,112]. Moreover, exclusive breastfeeding should be promoted in infants up to 6 months of age[113]. In addition, children should be encouraged to consume vegetables, fruits, low-fat dairy products, moderate quantities of whole grains, fish, poultry, and nuts, and advised against excessive sodium intake [114].

Currently, there is no effective general dietary intervention that can be applied in all cases or all pediatric populations; thus, a personalized approach is recommended. However, for the management of children who are at risk or have already developed metabolic alterations owing to the consumption of HFr/HF diets, several dietary alternatives have been proposed. One key approach is to guide children on appropriate food portions. A 12-month trial revealed that nutrient-balanced portion-controlled diets using meal plans (55%-60% carbohydrates, 10%-15% protein, and 30% fat) were effective in decreasing BMI among children with obesity. Furthermore, these diets resulted in reduced hunger compared to low-carbohydrate and reduced glycemic load diets[115]. The traffic light diet is another, simple alternative that reduces energy intake and encourages overweight and obese children to consume healthy diets[116]. It involves restricting foods based on their caloric content, and categorizing them by color: Green foods – can be eaten without restriction; yellow foods – should be consumed cautiously; red foods - stop and think before eating these foods[117].

Table 1 Results of some clinical studies in pediatric populations with fructose consumption or restriction, consumption of foods with saturated fat or ultra-processed foods

MetS inductors and interventions	Population (age, *n*)	Measured parameters	Outcomes	Type of study	Ref.
Saturated fatty acids	6-16 (*n* = 108); OB children	lipid profiles	High saturated fat intake was associated with higher BMI, NAFLD positivity. TG, total cholesterol, LDL-C and HOMA-IR were significantly higher in SFA consumption group	Cross-sectional study	Maffeis *et al*[81]
Fructose restriction	8-18 (*n* = 20) Children with obesity and MetS	Lactate in serum (related with liver fat fraction and visceral adipose tissue)	Fructose restriction produced a 50% decrease in lactate, which was related with decreased *de novo* lipogenesis and insulin sensitivity	Clinical trial	Erkin-Cakmak *et al*[85]
Sugar sweetened beverages (Fructose)	6-12 (*n* = 1,087); OB children	Adiposity (BMI, WC), cardiovascular risk markers (glucose, insulin, HOMA-IR, TG, LDL-C, HDL)	TG, insulin, TC and LDL concentrations and HOMA-IR were significantly higher in OB children	Association study	Huerta-Ávila *et al*[86]
Moderate fruit consumption	5-19 (*n* = 14, 755)	Lipid Profile (TC, TG), fasting serum insulin (HOMA-IR)	Moderate fruit consumption (1.5 serving per day 6-7 d a week) was associated with lower odds of lipid disorders, improving the childhood lipid profiles	Cluster-controlled trial	Liu *et al*[87]
Fructose restriction	3-18 (Latino children *n* = 2; African-American children *n* = 16)	Anthropometric parameters (BW, WC, BMI), BP, biochemical measurements (serum lactate, TG, cholesterol and others), glucose and HOMA-IR	Fructose restriction (reduction from 28% to 10%) in children showed reductions in diastolic BP, serum lactate, TG, LDL-C. Glucose tolerance and hyperinsulinemia	Clinical trial	Lustig *et al*[90]
Fructose	12-16 (*n* = 1454)	HOMA-IR, uric acid, anthropometric parameters (BW, WC, BMI, body fat percentage)	High consumption of sugar sweetened beverages (> 350 mL/d) were more likely to have elevated fasting serum insulin and HOMA-IR, WC and serum uric acid compared to those not having fructose consumption	Cross-sectional study	Lin *et al*[96]
UPC foods (high fructose-high fat)	4-8 (*n* = 307)	Anthropometric parameters (BW, WC, BMI, WHR) and glucose profile	An increase in WC was observed in children with a higher UPC consumption. Not a direct association with altered glucose metabolism was observed	Longitudinal study	Costa *et al*[97]
UPC foods (high fructose-high fat)	3-4 (*n* = 346); 7-8 (*n* = 307)	Lipid profile (TC, TG, HDL, LDL-C)	The higher consumption of UPC, the higher increase in total cholesterol and LDL-C and altered lipoprotein profiles in children	Longitudinal study	Rauber *et al*[99]
UPC foods (high fructose-high fat)	12-19 (*n* = 210) Children from Brazilian family program	Urinary fructose excretion Anthropometric parameters and biochemical parameters	Significant association between MetS and the consumption of UPC foods	Cross- sectional study	Tavares *et al*[100]
Fructose	7-12 (*n* = 27); 13-15 (*n* = 25); 16-16 (*n* = 32); OB children	Anthropometric parameters (BW, Hgt, WC, BMI and WHR), serum lipid profiles	Higher fructose intake from beverages correlate positively with the percentage of body fat, WC, WHR, TC, TG and increased atherogenic indices	Observational study	Czerwonogrodzka-Senczyna *et al*[101]
Fructose	9-16 (*n* = 246)	Urinary fructose excretion	Metabolic dysfunctions and urinary excretion only at very high fructose intake levels (> 25% of total energy intake) or hypercaloric diets	Cohort study (DONALD)	Perrar *et al*[102]
Trans fatty acids	6-13 (*n* = 54); OB children	Anthropometric parameters, glucose and insulin. HOMA-IR, total lipids, postprandial levels of trans fatty acids	Obese children showed hyperinsulinemia and increased insulin resistance compared with controls. No differences for fasting plasma tFA or dietary tFA intake were observed	Clinical trial	Larqu *et al*[103]
High dairy fat products (total and saturated fat intake)	4-13 (*n* = 174) Intervention with low dairy fat products	Anthropometric parameters Pentadecanoic acid and lipid profile	Total fat and saturated fat intakes from dairy foods were lower in the intervention group (low fat dairy products consumption) but did not alter energy intakes or measures of adiposity	Randomized controlled trial	Hendrie and Golley[104]

OB: Obese; BW: Body weight (kg); Hgt: Height (cm); WC: Waist circumference (cm); BMI: Body mass index; WHR: Waist-to-height ratio; TC: Total cholesterol; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; LDL-C: Low-density lipoprotein cholesterol; HDL: High density lipoproteins; ALT: Alanine aminotransferase; AST: Aspartate amino transferase; NAFLD: Non-alcoholic fatty liver disease; SFA: Saturated fatty acid; tFA: Trans fatty acids; UPC: Ultra-processed; DONALD: The Dortmund Nutritional and Anthropometric Longitudinally Designed ongoing study; MetS: Metabolic syndrome.

Furthermore, nutritional labels, including nutritional facts tables, guide daily amounts, and front-of-package labels, have been implemented in numerous countries worldwide. These labels are particularly relevant to foods with high unhealthy nutrient content, such as HF/HFr products, which require restricted consumption. Front-of-package labels are the most comprehensible to the general public, particularly to children, resulting in the highest rates of self-reported awareness[118].

In addition, the short-term efficacy of low-carbohydrate and low-fat diets has been demonstrated. Within 6 months of implementation, these regimens resulted in a significant reduction in the BMI of children with obesity and considerably ameliorated fatty liver disease[119]. The fructose and glycemic index/glycemic load diet (FRAGILE) dietary intervention showed that a reduction in fructose intake, particularly HFCS, resulted in a significant reduction in metabolic risk factors and hepatic injury in children with NAFLD[120].

Established diet plans, such as the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) diet, have been applied in patients with metabolic disorders, such as obesity, hypertension, cardiovascular risk, and MetS, which are usually the result of unhealthy dietary patterns, including HF/HFr diets. These diets contain a substantial portion of plant-based foods, including fruits and vegetables, whole grain products, legumes, nuts, and seeds; moderate daily consumption of dairy products, and weekly consumption of poultry and fish[114]. Six weeks of adherence to the DASH diet improved MetS markers and high BP in a group of adolescent women, according to the findings of a randomized controlled trial designed to examine the effect of the DASH diet on metabolic outcomes[121]. In their evaluation of the effectiveness of a Mediterranean-style diet in children and adolescents with obesity, Velázquez-López *et al*[122] found that a 16-week intervention decreased BMI, glucose levels, and lipid profiles.

Other therapeutic options, such as probiotics or prebiotics, have been investigated to modulate the intestinal microbiota composition and improve alterations caused by the HFr/HF diet, such as obesity, MetS, and NAFLD. Various hypotheses have been proposed to explain the potential therapeutic benefits of probiotics and prebiotics in patients on HFr/HF diets[123]. Prebiotic supplementation inhibited fatty acid synthesis in animal models, which possibly reduced fructose-induced hepatic TG accumulation. This may be due to decreased expression of fatty acid synthase and acetyl CoA carboxylase, which are enzymes that regulate hepatic lipogenesis[124]. Probiotics improved inflammatory liver damage through the modulation of TNF-α and insulin resistance[125]. It has been demonstrated that the consumption of an HF/HFr diet over 8 wk resulted in both gut dysbiosis and a change in the permeability of the gut barrier. As an effective probiotic, *Limosilactobacillus reuteri* DSM 17938 may be used to prevent the detrimental effects of an HF/HFr diet on the gut[126].

Physical activity to prevent pediatric MetS

Compelling evidence indicates that modern lifestyle, including a diet composed primarily of HF/HFr-containing UPC foods and a sedentary lifestyle, has socially affected early childhood, as evidenced by an increase in the prevalence of childhood obesity[127]. Consequently, interventions promoting physical activity are frequently employed to counteract the negative effects of HF/HFr consumption in children. A Mayo Clinic review on childhood obesity outlined the following characteristics of physical activity: Age, individual preferences regarding the type of physical activity, and exercise tolerance, which should be considered when establishing physical activity objectives for children. Children aged ≥ 6 years are recommended to participate in at least 60 min of daily physical activity. Toddlers should have a designated

time of 60–90 min in an 8-h day for moderate to vigorous physical activity. Preschoolers, however, are recommended to have a longer duration (90–120 min) of physical activity[109]. Parents and schools should encourage children and adolescents to participate in outdoor play and recess activities and walk or bike to school. Positive factors affecting physical activity levels include family, peer, and communal encouragement; proactive and athletic self-perception; desire to prevent weight gain; and personal achievement[128].

Children who are obese face challenges in initiating and sustaining physical activity programs. These issues arise from limited motor abilities resulting from excessive body weight and an inclination toward a sedentary lifestyle. Consequently, such children often develop unpleasant emotions associated with physical activity, leading to a higher possibility of ceding physical activity and establishing a vicious cycle wherein sedentary habits become more prevalent [129]. Stabelini Neto *et al*[130] suggested that to promote a healthy metabolic profile, adolescents should perform at least 88 min/d of moderate-to-vigorous physical activity. These activities should include games, sports, recreation, planned exercises, and transportation, both in schools and communities. In children, low levels of physical activity are associated with an increased risk of developing MetS[131]. Lifestyle intervention in elementary school students involving a physical activity promotion program to raise awareness among parents and students about MetS-associated risks associated with MetS significantly reduced some major MetS components, including insulin resistance, high BP, and obesity[132].

Physical activity can also improve the composition and functionality of the gut microbiota, representing another benefit that lifestyle changes can bring to children affected by HF/HFr diets who have developed complications such as obesity, NAFLD, or MetS[133].

Pharmacological therapy to prevent pediatric MetS

Pharmacological treatment may be initiated when children fail to lose weight within 6–12 months of lifestyle modification or when they exhibit significant cardiometabolic risks associated with the consumption of HFr/HF diets, although few approved drugs are available for use in children[132]. The FDA has approved the following five drugs for chronic weight management in pediatric patients aged ≥ 12 years who are obese, with a BMI of 30 kg/m^2 and weight > 60 kg[134]: Orlistat, phentermine/topiramate (PHEN/TPM), liraglutide, semaglutide, and setmelanotide; these drugs employ various mechanisms of action to combat childhood obesity. Orlistat is a widely recognized medication for weight management in overweight children and adolescents and functions as a selective inhibitor of gastrointestinal lipases, thus inhibiting dietary fat absorption[135]. Orlistat, in conjunction with lifestyle modifications, has been shown in clinical studies to substantially decrease BMI and improve metabolic parameters in children with obesity[136]. As orlistat inhibits the absorption of fat-soluble vitamins, multivitamin supplementation is recommended. Adverse effects of orlistat include diarrhea, abdominal pain, flatulence, and greasy feces[109].

PHEN/TPM is frequently prescribed for short-term weight loss interventions owing to its appetite-suppressing properties. A reduction in caloric intake is induced *via* appetite suppression when topiramate, which acts on GABA A receptors, is combined with the noradrenergic agonist, phentermine[58]. The use of PHEN/TPM and lifestyle interventions in a 56-wk trial including adolescents with obesity showed a statistically significant reduction in BMI and favorably affected TG and HDL-C levels. Common adverse events include paresthesia, dizziness, dysgeusia, insomnia, constipation, palpitations, tachycardia, and dry mouth[137].

Liraglutide and semaglutide are agonists of the glucagon-like peptide-1 (GLP-1) receptor and affect glucose metabolism and appetite regulation by binding to GLP-1 receptors located in both the brain and peripheral tissues[138, 139]. Kelly *et al*[140] showed that in obese adolescents, the use of liraglutide plus lifestyle therapy led to a significantly greater reduction in BMI. Common side effects of GLP-1 receptor agonists include gastrointestinal symptoms such as nausea, vomiting, diarrhea, and constipation, which typically diminish over time[141].

Setmelanotide, a synthetic analog of melanocyte-stimulating hormone, is a promising treatment for some types of pediatric obesity. It functions as an effective melanocortin-4-receptor (MC4R) agonist in children and adolescents with obesity resulting from genetic mutations that impact the MC4R pathway[142].

Although the FDA has not yet approved metformin for the treatment of obesity in children, there is evidence of its use in studies on pediatric patients with other metabolic disorders, such as insulin resistance, polycystic ovary syndrome, MetS, and childhood T2DM, where metformin has been associated with weight loss, improved insulin sensitivity and glucose levels, and reduced inflammation[131]. Because of the limited pharmacological options for treating metabolic disorders related to HFr/HF diets, such as obesity, MetS, and NAFLD, there is an extensive field of research wherein new therapeutic alternatives are being analyzed to address these issues that affect both pediatric and adult populations. Table 2 shows a summary of recent studies focused on the treatment of these metabolic disorders, which are not limited to the pediatric population. These studies include compounds present in olive oil such as oleanolic acid alone[143] or in combination with metformin[144], as well as some polyphenols[145] and triterpenic acids present in this oil[146]. Other compounds of natural origin under study are γ-oryzanol with ferulic acid[147], quercetin[148], ursolic acid[149], and the non-protein amino acid citrulline[150]. Microencapsulation of herbal extracts with beneficial properties against metabolic syndrome has been studied to improve their pharmacokinetic properties[151]. On the other hand, the use of agents that improve intestinal microbiota and glucose and lipid levels by influencing host metabolism has been explored[152,153]. Likewise, the beneficial effect on lipid metabolism of such divergent drugs as the antihypertensive drugs amlodipine and valsartan[154], GLP-1 receptor agonists[155] and acetyl CoA carboxylase inhibitors of natural or synthetic origin[156] have been explored.

Controversial role of fructose in the induction of MetS in humans

Despite the substantial amount of evidence about the negative effect on metabolic health of HF + HFr diets, there is still controversy about whether fructose consumption is harmful to health in humans. One of the main arguments against a specific effect of fructose on the increase of intrahepatic fat, serum triglyceride levels and insulin tolerance has been that

Table 2 Recent studies focus on new therapeutic alternatives for obesity, metabolic syndrome, and Non-alcoholic fatty liver disease

Model/population	Treatment	Outcomes	Ref.
Fructose-induced NAFLD; Neonatal rats	Oleanolic acid	Attenuates the subsequent development of HFr diet-induced NAFLD	Nyakudya *et al* [143]
HFr induced metabolic dysfunction; Rats (*Rattus norvegicus*)	Oleanolic acid (60 mg/kg); metformin (500 mg/kg)	Both treatments increased mono- and polyunsaturated FFAs, associated with increased *glut-4*, *glut-5* and *nrf-1* and decreased *acc-1* and *fas*	Molepo *et al*[144]
MetS induced by HFr diet; female mice	Tyrosol, hydroxytyrosol and salidroside	Improved glucose metabolism and lipid metabolism, including reduced levels of total cholesterol insulin, uric acid, LDL-C, and aspartate aminotransferase	Zhan *et al*[145]
Obesity and related diseases in humans	Olive oil triterpenic acids	Improves glucose and insulin homeostasis, lipid metabolism, adiposity and cardiovascular dysfunction in obesity	Claro-Cala *et al* [146]
HF and HFr diets induced metabolic dysfunction; Sprague dawley rat	Wheat flour, enriched with γ-oryzanol, phytosterol, and ferulic acid	Alleviates hepatic lipid accumulation and insulin resistance through their elevation in the phosphorylation of AMPK and Akt	Guo *et al*[147]
HF diet induced NAFLD; C57BL/6J mice	Quercetin	Decreased insulin resistance and NAFLD activity score, by reducing the intrahepatic lipid accumulation through its ability to modulate lipid metabolism gene expression, cytochrome P450 2E1 (CYP2E1)-dependent lipoperoxidation and related lipotoxicity	Porras *et al*[148]
HFr induced NAFLD; Sprague dawley rats	Ursolic acid	Ursolic acid administration against dietary fructose-induced NAFLD in newborn rats by reducing fructose-induced hepatic lipid accumulation	Mukonowenzou *et al*[149]
Mouse 3T3-L1 fructose-induced NAFLD	Citrulline	Prevented hypertriglyceridemia and attenuated liver fat accumulation	Jegatheesan *et al* [150]
Sprague-Dawley rats MetS; *in vitro* and *in vivo* studies	Nanoformulations of herbal extracts	Decrease the lipid profile, inflammation, oxidative damage, and insulin resistance in *in vitro* and *in vivo* models of MetS-related complications	Nouri *et al*[151]
MetS humans	Pharmabiotics	Improves gut microbiota profile wich influence serum lipid levels, BP, neuroendocrine cells and immune functions *via* regulating the metabolism of the host	Nguyen *et al*[152]
MetS; *in vivo* studies	Seaweed-derived bioactive components	Modulate the gut microbiota by reversing the Firmicutes/Bacteroidetes ratio, increasing the relative abundance of beneficial bacteria and decreasing the abundance of harmful bacteria; these compounds increase the production of short-chain fatty acids and influence glucose and lipid metabolism	Zang *et al*[153]
HFr induced adiposity	Valsartan; Amlodipine	Both treatment reduced triacylglycerol storage in adipocytes by inhibiting PU.1	Chou *et al*[154]
Hepatic steatosis induced by HFr diet; Wistar rats	β-catenin	Mediates the effect of GLP-1 receptor agonist on ameliorating hepatic steatosis	Gao *et al*[155]
NAFLD and nonalcoholic steatohepatitis Humans	Acetyl-CoA Carboxylase inhibitors	Therapeutic target for MetS as a key regulatory role in fatty acid synthesis and oxidation pathways	Chen *et al*[156]

BP: Blood pressure; HFr: High-fructose; FFA: Free fatty acids; NAFLD: Non-alcoholic fatty liver disease; HF: High-saturated fat; LDL-C: Low-density lipoprotein cholesterol; MetS: Metabolic syndrome.

its effects are related to the supply of excess energy and that other equally energy-dense nutrients can cause the same metabolic alterations as fructose[157].

Two meta-analyses showed that isocaloric replacement of other carbohydrates with fructose produced better glycemic control in people with diabetes and had no effect on markers of NAFLD in healthy subjects. However, as stated by the authors of these studies, the data should be taken with caution because the studies were conducted in small populations and over relatively short periods of time[158,159]. Likewise, in another study where the effects of isocaloric intake of glucose and fructose were analyzed in healthy, centrally overweight men, no change in hepatic TG or markers of liver damage were observed. However, it should be noted that the carbohydrates were consumed for a period of two weeks [160].

In contrast to the above studies, in a study conducted with 37 overweight adult individuals, a small decrease in intrahepatic fat was observed with a fructose-restricted diet compared to an isocaloric diet without fructose restriction, although no difference was observed between serum lipid content and glucose tolerance. As in the aforementioned studies, the major limitation of this study was its short duration of six weeks[161]. Consistent with the above study, in another study in 94 lean healthy men where daily carbohydrate intake was increased by daily intake of fructose or sucrose-sweetened beverages for 7 wk, an increase in hepatic lipid synthesis was observed, whereas this was not observed in individuals who ingested glucose-sweetened beverages[162]. A recent meta-analysis has concluded that the effects of fructose on adiposity are rather related to the source of fructose and the energy level at which it is consumed. Intake of sugar-sweetened beverages in amounts above 100 g/d or providing more than 20% of energy was found to

result in moderate increases in adiposity. On the contrary, the consumption of fructose sources such as honey, dried fruits, fruits and 100% fruit juice in amounts above 100 g/d or providing more than 20% of the energy do not show harmful effects, and even have moderate beneficial effects[163].

On the other hand, an umbrella review of existing meta-analyses of the effects of sugar consumption on health outcomes showed an association between consumption of sugar-sweetened beverages at hypercaloric levels and an increased risk of MetS[164]. This has led to the recommendation to minimize consumption of sugar-sweetened beverages [165]. This is consistent with the conclusions of some reviews evaluating the effects of fructose consumption in children. The idea that fructose consumption has a negative effect on the development of NAFLD is not without controversy also in children and adolescents[166], which is due in part to the fact that there were only four studies evaluating the effect of fructose or sucrose restriction on liver fat in young people as of 2023[167]. For example, a recent study where biopsies from obese and lean adolescents were analyzed showed that patients with non-alcoholic steatohepatitis have higher sugar intake and that the severity of liver disease correlates with the amount of total carbohydrate and added sugars ingested [168]. In the light of this and other observations, restriction of added sugar intake in the management of NAFLD in at-risk children and adolescents has been suggested like in adult populations[168,169].

CONCLUSION

The fast-food industry offers highly palatable products that are attractive to children. In general, these products consist of a meal containing HF and a sugar-containing beverage, with fructose as the main component of such beverages. Studies in animals administered HF + HFr diets mimic the consumption of UPC products offered by fast-food establishments better than studies using HF-only or HFr-only diets. Future studies should address the effects of dietary, physical, and pharmacological interventions on the metabolic health of the pediatric population, as cumulative evidence in animal studies shows that HF + HFr diets produce more deleterious effects on health than HF-only or HFr-only diets. This is because of the differential effects of fructose and saturated fats, as, in contrast to HF diets, fructose has a powerful ability to promote both hepatic DNL and uric acid synthesis, both leading to altered blood lipid levels and promoting insulin resistance. Moreover, HFr exacerbates the effects of HF alone by enhancing mitochondrial dysfunction, ROS production, and inflammation, which are the main factors that lead to insulin resistance, diabetes, and CVD. This raises several questions about whether the effectiveness of an intervention targeting alterations in MetS is equal when tested only in the context of HF consumption and when it is tested only in the context of HFr intake. For example, are FDA-approved drugs for the treatment of obesity in children equally effective in the alterations elicited by HF + HFr compared to alterations produced by HF-only or HFr-only? When a child frequently eats fast food, is restricting only the calories provided by fat equally effective as restricting the calories provided by sugar only? What are the more effective prebiotics, probiotics, and obesity drugs that target the alterations elicited by the combined intake of HF + HFr? These and other questions deserve further research using animal models to develop interventions that better target the metabolic alterations produced by combined fat and fructose consumption, to decrease early onset MetS-associated diseases and delay as much as possible the development of MetS later in life.

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FOOTNOTES

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REVIEW

Type 2 diabetes in children and adolescents: Exploring the disease heterogeneity and research gaps to optimum management

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Abstract

Over the past 20 years, the incidence and prevalence of type 2 diabetes mellitus (T2DM) in children and adolescents have increased, particularly in racial and ethnic minorities. Despite the rise in T2DM in children and adolescents, the pathophysiology and progression of disease in this population are not clearly understood. Youth-onset T2DM has a more adverse clinical course than is seen in those who develop T2DM in adulthood or those with T1DM. Furthermore, the available therapeutic options are more limited for children and adolescents with T2DM compared to adult patients, mostly due to the challenges of implementing clinical trials. A better understanding of the mechanisms underlying the development and aggressive disease phenotype of T2DM in youth is important to finding effective prevention and management strategies. This review highlights the key evidence about T2DM in children and adolescents and its current burden and challenges both in clinical care and research activities.

Key Words: Type 2 diabetes mellitus; Children and adolescents; Pathophysiology; Heterogeneity; Complications; Treatment options; Barriers

Core Tip: The incidence and prevalence of type 2 diabetes mellitus (T2DM) in children and adolescents have dramatically increased over the past 20 years. Accumulating evidence suggests that youth-onset T2DM presents unique characteristics, demographics, and disease progression compared to adult-onset T2DM. In addition, the available therapeutic options for children and adolescents with T2DM are inadequate. T2DM in children and adolescents is becoming a public health concern worldwide. Research programs should aim to overcome the challenges seen with clinical studies in this population to address the unmet need of optimal management of T2DM in children and adolescents.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) has usually been regarded as a disease with onset in middle age to older ages. However, the incidence and prevalence of T2DM in children, adolescents, and young adults are increasing worldwide. In North America, a study among individuals aged < 20 years confirmed the presence of T2DM in all racial and ethnic populations and observed a high prevalence, especially among American Indian and Indigenous peoples[1]. Over the last few decades, the rising frequency, greater severity, and earlier onset of childhood obesity in conjunction with sedentary lifestyles and an increasing occurrence of intrauterine diabetes exposure are key drivers of young-onset T2DM. T2DM in children and adolescents differs from T1DM and more closely resembles the pathophysiology in adult patients: insulin resistance and non-autoimmune failure of β-cells. In people who develop T2DM during childhood or adolescence, a more aggressive course is frequently noted than those who develop T2DM in adulthood with a faster decline in β-cell function, high incidence of treatment failure, and accelerated development of complications[2,3]. Moreover, the available therapeutic options for children and adolescents with T2DM are inadequate. Until recently, pharmacological treatments recommended by the regulatory bodies were limited to metformin and insulin. Taking together, young-onset T2DM is an emerging disorder with unique challenges in clinical care as well as research programs[4], leaving huge knowledge gaps in pathophysiology, clinical course, and optimization of treatment.

In this review, we examine the epidemiology of T2DM in children and adolescents and its complicated course globally, with a focus on disease heterogeneity. The clinical courses of youth who develop T2DM are compared with adults who develop T2DM. The impact of young-onset T2DM on the important microvascular and macrovascular complications is also explored. Finally, we investigate the possible mechanisms to elucidate the observed aggressive metabolic phenotype in individuals with youth-onset T2DM and discuss available treatment strategies and the complexities that hinder their implementation in this population.

T2DM TRENDS IN CHILDREN AND ADOLESCENTS

T2DM in children and adolescents is often underrecognized because of its prolonged subclinical course and lack of awareness regarding the increasing risk among patients and providers. Consequently, national population-based disease registry data might not adequately reflect the frequency and growing trends of T2DM in this population. Nevertheless, the prevalence of T2DM in the 10- to 19-year-old population has doubled over the past two decades in the United States [5]. It is present in nearly 2 persons per 1000 among Black and American Indian youth. The prevalence of youth-onset T2DM will double to quadruple by 2050 as estimated by the SEARCH for Diabetes in Youth study. The incidence of T2D has similarly doubled among adolescents in the United States over this period, from 9 to 18 cases per 100000 per year, with an incidence that is particularly high in Black and American Indian youth[6]. Similarly, T2DM in youth is increasing in incidence and prevalence worldwide. In a review of country-specific prevalence and incidence of youth-onset T2DM, it was found that some of the more economically developed countries, such as the United States and China, have the highest reported rates worldwide[7]. The analysis also noted that data for T2DM in children and adolescents are still scarce across the globe. The number of incident cases of T2DM in youth is more than 2-fold higher in China and 1.4-fold higher in India than in the United States based on the larger population of these countries, but with the United States showing the highest reported incident rates in a more recent worldwide survey among children and adolescents aged under 20 years[8]. In general, the prevalence of T2DM in youth is lower in Europe than in other developed countries[7]. Data on youth-onset T2DM are scarce in South America and Africa. The escalating prevalence of obesity in children and adolescents has likely triggered an increased rate of T2DM[5]. However, this alone does not seem to fully explain the increase in T2DM.

PATHOPHYSIOLOGY

Risk factors

All of the traditional risk factors for T2DM are also involved in young-onset T2DM but in an amplified manner. Obesity is the single most important risk factor, with more than 90% of patients suffering from young T2DM being obese[9]. Early onset obesity or greater cumulative exposure to obesity are more important risk factors for diabetes[10]; however, the risk is reduced if weight normalizes before puberty[11]. Positive family history and female sex are considered to be non-modifiable risk factors, with more than 80% of United Kingdom adolescents having a family history of T2DM[9]. In cohort studies, the female sex predominates until age 25 years after which it equalizes and the male sex predominates after 40 years of age[12]. Ethnicity and socioeconomic status also play important roles in determining the prevalence. Asian, Middle East and North Africa, Hispanic, and Black ethnic populations have a disproportionately high prevalence of diabetes in adolescents[13], and low middle and middle socioeconomic index countries have the highest age-standardized incidence compared with low socioeconomic index[14]. Other risk factors can be prenatal exposure, maternal malnutrition, and diabetes, which increase the risk of diabetes in the offspring. The SEARCH study showed that maternal obesity (odds ratio [OR]: 2.8, 95% confidence interval [CI]: 1.5–5.2) and maternal gestational diabetes (OR: 5.7, 95%CI: 2.4–13.4) were associated with risk of youth-onset T2DM compared with controls that persisted even after adjustment for age, socioeconomic factor, and ethnicity[15]. The Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study group reported an association of maternal diabetes history with faster glycemic progression and worsening β-cell function in offspring with youth-onset T2DM over 12 years of follow-up[16]. In the SEARCH Case-Control Study, the odds of T2DM were significantly lower among those who were breastfed in infancy (OR: 0.26, 95%CI: 0.15–0.46). Nevertheless, the association was confounded by current body weight[17]. Additionally, some endocrine-disrupting chemicals, such as perfluoroalkyl and polyfluoroalkyl substances, are associated with an increased risk of T2DM at a young age[18].

Rapid progression

Although obesity and insulin resistance are critical initial factors, progression to glycemic failure is heralded by evidence of impaired β-cell function. Adolescents with T2DM have rapid progression to glycemic failure compared with adults [19]. Their pathogenesis involves an accelerated rate of -cell function loss[20] and lower insulin sensitivity than adults [21]. In the RISE study, the rate of loss of β-cell function was 25%–30% in young people *vs* 7% in those with adult-onset T2DM[22]. The exact etiology for this accelerated β-cell function loss is not known, but excess growth hormone during puberty and obesity-induced insulin resistance and glucolipotoxicity are postulated to have a role[23]. In the TODAY study[24] for T2DM in young people (< 20 years), the initial hemoglobin A1c (HBA1c) and β-cell reserve were independent predictors of glycemic durability; participants with lower β-cell function experienced earlier treatment failure. There were no significant differences in markers of insulin sensitivity between adult and young-onset T2DM. A similar finding was observed in the RISE study[22] and unlike adults, early insulin treatment did not ameliorate β-cell function loss, suggesting that inadequate glycemic control is not contributing to this faster progression. In addition to β-cell dysfunction, higher insulin resistance was noted in young people *vs* adults with T2DM[21] and also with impaired glucose tolerance, which persisted after adjustment of body mass index (BMI), race, and sex[25].

Role of genetics in heritability

Based on family and twin studies, the heritability of T2DM in children ranges between 30% and 70%[26]. The first genome-wide association study for T2DM in young people was published in 2021, which included 9067 participants from multiethnic backgrounds and consisted of 3006 young people and 6061 adult controls[27]. Seven genome-wide significant loci were identified, including a novel locus (rs10992863) in PHD finger protein 2. The remaining six loci (transcription factor 7-like 2 [TCF7L2], melanocortin 4 receptor, cell division cycle protein 123, KCNQ1 channel, insulin-like growth factor-binding protein 2, and solute carrier family 16 member 11) were previously reported as adult loci. Among them, TCF7L2 has one of the strongest effects on the risk of T2DM among common variants (odds ratio [OR] of about 1.4). The T2DM liability variance was 3.4 times higher for common variants and five times higher for rare variant associations compared with adult-onset T2DM, indicating higher genetic contribution for young-onset T2DM. These findings suggest that adults and young-onset T2DM persons have overlapping genetic architecture, but the role of genetics is probably higher for disease risk in adolescents than in adults.

DIFFERENTIATION FROM OTHER TYPES OF DM

During the adolescent period, elevated blood glucose levels can be due to multiple etiologies. As T2DM is a disease of exclusion, one must reasonably exclude other etiologies before jumping to a diagnosis. Common differentials are T1DM, maturity-onset diabetes in the young, diabetes due to pancreatic disease, latent autoimmune diabetes in the young, endocrinopathies (acromegaly, Cushing's), lipodystrophies, mitochondrial disease, and syndromic diabetes. Detailed history taking, focused clinical examination, and a few laboratory investigations are required to clinch the diagnosis. T2DM is typically considered in pubertal youth with obesity, a family history of T2DM, features of the metabolic syndrome, and/or absent islet autoantibodies. A detailed approach to the diagnosis of T2DM in adolescents is illustrated in Figure 1.

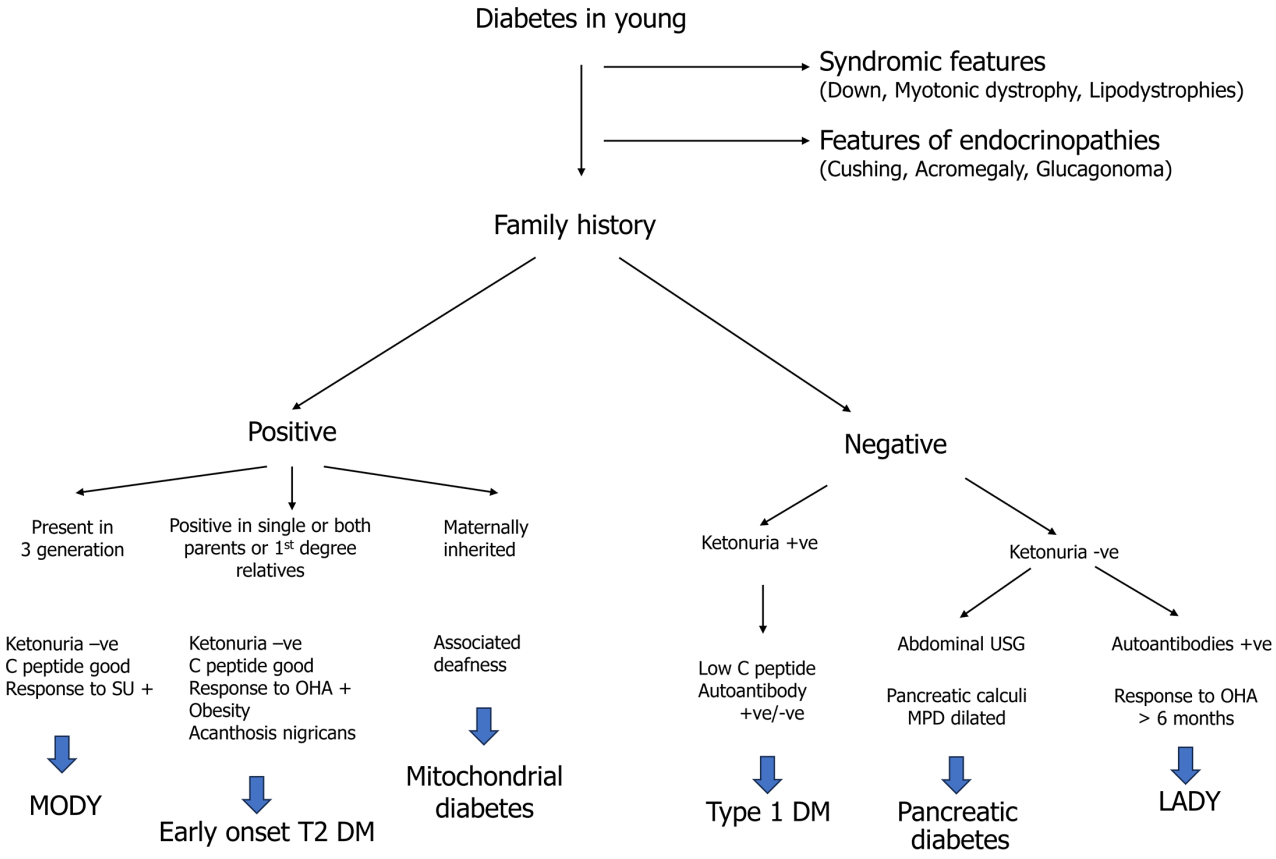

Figure 1 Approach to etiological diagnosis of diabetes in adolescents. LADY: Latent autoimmune diabetes in young; MODY: Maturity onset diabetes in young; MPD: Main pancreatic duct; OHA: Oral hypoglycemic agent; SU: Sulfonylurea; USG: Ultrasonography.

COMPLICATIONS AND CO-MORBIDITIES IN YOUNG-ONSET T2DM

There is a high prevalence of diabetes-associated complications and comorbidities in those with young-onset T2DM - both at the time of diagnosis and beyond. The implications of these complications are of particular importance in this age group because of their effects on education and jobs, psychological consequences, and increased health-care expenditure. Also, women of reproductive potential with DM need special care and attention. Several studies suggest that for people of the same age, the risks of macrovascular and microvascular complications are higher for people with early-onset *vs* later onset T2DM[28].The risks are also higher for young-onset T2DM compared to T1DM of the same duration[29]. With improved healthcare, there has been a significant reduction in the complications and mortality of people living with T2DM; these improvements have not been evident in the youngest subgroup. Rather, there has been a resurgence of complications from 1995 to 2015 among people with T2DM between 18 and 44 years of age from 1995 to 2015[30]. After 15 years of follow-up, about 80% of adolescents in the TODAY study had developed a minimum of one microvascular complication[31].

The proposed hypotheses behind the increased risk of complications could be a more aggressive pathophysiology, poorly controlled hyperglycemia, more prolonged exposure to glycemic burden, the concurrent presence of other metabolic risk factors, an unrecognized period of untreated or inadequately treated hyperglycemia before the diagnosis and the effects of obesity and inflammation since a large proportion of those with young-onset TDM are overweight or obese. There are other factors such as socioeconomic factors, mental health issues with reduced self-management capacity, reluctance to undergo repeated screening tests, or reduced engagement with health services with infrequent follow-up.

Macrovascular complications

An Australian study showed that individuals with young-onset T2DM had a higher risk of coronary artery disease and stroke compared to individuals of similar aged individuals with T1DM[31]. Studies have shown that those with young-onset T2DM have up to 50% higher cumulative risk of cardiovascular disease (CVD) than those with later-onset T2DM. Also, at any age, the risk of adverse cardio-renal outcomes was higher than in the late-onset group, but these differences were primarily driven by the longer duration of diabetes in the young-onset T2DM group[32].

Although overt CV events are not very common in youth with T2DM, it is a well-established fact that the process of atherosclerosis starts early during childhood. Studies have revealed the presence of subclinical vascular disease in adolescents with obesity and T2DM in the form of elevated aortic pulse wave velocity or increased carotid intima-media thickness (CIMT) compared with normoglycemic youth. In the SEARCH study, those with young-onset T2DM had

greater degrees of arterial stiffness than those with T1DM, which was unrelated to the duration of diabetes or glycemic control[33]. Generalized obesity as well as abdominal adiposity were found to be determinants of coronary artery calcifications in those with young-onset T2DM[34]. In the TODAY study, BMI and blood pressure were associated with adverse cardiac measures[35]. Overall, there appears to be more significant vascular dysfunction and a higher chance of progression to overt CVD among youth with T2DM. However, as in adults, there is no recommendation to routinely screen for subclinical atherosclerotic CVD in young-onset T2DM.

Microvascular complications

Nephropathy: The SEARCH study revealed greater rates of microvascular complications, including diabetic nephropathy, retinopathy, and peripheral neuropathy, in youth with T2DM compared to those with T1DM[36].

The progression from microalbuminuria to macroalbuminuria is also higher in young-onset T2DM compared to T1DM [37]. The risk is particularly high for Asian, Native American, and Pima Indian people, suggesting a possible influence of ethnicity[38]. A Japanese study showed that among individuals < 20 years of age, the incidence of nephropathy was higher in those with T2DM than in T1DM and the differences persisted even after adjustment for disease duration[39]. Interestingly, in this study, the incidence of nephropathy slowly declined over several decades in T1DM, whereas the incidence remained high in patients with young-onset T2DM. Another study reported worse renal survival (that is, remaining free of end-stage kidney disease) in T2DM compared with T1DM, with 55.0% survival after 20 years in the T2DM group while in the T1DM group, renal survival persisted at 100%[40]. However, there are inadequate data regarding the risk of microvascular complications in early-onset T2DM compared to later-onset T2DM. One study demonstrated a higher risk of microalbuminuria in young-onset T2DM than later or adult-onset T2DM (hazard ratio [HR]: 1.2, 95%CI: 1.1-1.4)[41]. A recent meta-analysis reported the prevalence of albuminuria to be 22.17%, with higher risk among Pacific Islander, Indigenous, and Asian youth compared to White youth[42].

It should be noted that the process of albuminuria and hyperfiltration may start before the onset of diabetes in youth with T2DM and may be related to early onset of vascular dysfunction in the obese[43,44]. In children and adolescents, estimated glomerular filtration rate (eGFR) can be calculated by the Schwartz equation using serum creatinine levels and height of the patient. However, this formula could underestimate hyperfiltration, which is common in young-onset T2DM. eGFR estimation by formula using a combination serum creatinine and serum cystatin C may be preferable[38].

Retinopathy: The prevalence of diabetic retinopathy in young-onset T2DM is reportedly between 2% and 40%, and varies according to the testing method used for the patient and the duration of diabetes[45,46]. Although chances increase with disease duration, retinopathy has also been seen at the time of DM diagnosis. In the TODAY study, retinopathy in young-onset T2DM detected by digital fundus photography was seen in 13.7% of patients at a mean duration of diabetes of 4.9 years, although there was no evidence of macular edema or proliferative retinopathy[45].

Age is a significant predictor of retinopathy in T1DM but not young-onset T2DM, with a 5% increased risk of developing retinopathy for every 1-year increase in age[47]. Studies have identified more marked subclinical structural and functional retinal abnormalities in adolescents with young-onset T2DM compared to T1DM, despite a shorter overall duration of T2DM[48].

The data regarding the prevalence of retinopathy in early- *vs* late-onset T2DM of same duration is conflicting. One study reported a higher prevalence of retinopathy in individuals with young- *vs* usual-onset DM after ≥ 10 years' disease duration[49]. In this study, longer duration of diabetes was a significant predictor of disease, while age of onset was not. However, in another study on Pima Indian people, retinopathy was relatively lower in patients with young- rather than later-onset T2DM[50]. In a recent meta-analysis, the most significant increase in the prevalence of T2DM was observed at more than 5 years after diagnosis[51]. Overall, available studies suggest that having younger onset T2DM increases the risk of retinopathy more than having T1DM of same duration, but the differences in risk between young-onset and usual onset T2DM remain unclear.

Akin to adults, children and youth with T2DM should be screened for retinopathy at diagnosis and then periodically thereafter, with more frequent monitoring in those with inadequate glycemic control. Fundoscopy has been found to be less sensitive in detecting retinopathy than stereoscopic fundus photography in children.

Neuropathy: Studies on neuropathy in young-onset DM are relatively few. In the SEARCH study, the prevalence of diabetic neuropathy calculated by the Michigan Neuropathy Screening Instrument (MNSI) was 26% in young-onset T2DM. However, in the MNSI, small-fiber dysfunction was not assessed. The prevalence was 21% in an Australian study using thermal and vibration threshold testing for small and large fiber neuropathy respectively[47,52]. Additionally, more than 50% had autonomic neuropathy tested using pupillary reactivity[47].

In the SEARCH study, those with young-onset T2DM had almost double the age-adjusted prevalence of peripheral neuropathy than those with T1DM (17.7% *vs* 8.5%)[36]. However, there were no differences in the prevalence of cardiac autonomic neuropathy between the two groups. In another study on young people aged < 18 years, although no differences were noted in the prevalence of peripheral and autonomic neuropathy, the mean duration of disease in those with T2DM was only 1.3 years compared to 6.8 years for those with T1DM[47]. There have been no systematic studies comparing the risk of neuropathy in young- to late-onset T2DM. One Australian study showed that people with T2DM diagnosed between 15 years and 30 years of age have a greater severity of neuropathy compared to those diagnosed between 40 years and 50 years of age and with a similar duration of DM[29].

The prevalence of neuropathy in young-onset T2DM is at least as frequent as in adults. Thus, diagnosing neuropathy using appropriate screening tests should be at least as frequent in youth as in adulthood - at diagnosis and annually. The neuropathy screening tests should be predominantly aimed at the detection of at-risk foot for diabetic foot ulcer and can be conducted easily using clinical parameters such as foot inspection, assessment of pedal pulses and testing for pinprick

sensation, 10-g monofilament and vibration using a 128-Hz tuning fork, and ankle jerks, all of which involve no or inexpensive equipment. Novel risk factors that have been identified for the development of peripheral neuropathy and cardiac autonomic neuropathy in children with DM include central obesity, smoking, puberty, and eating disorders. Therefore, young T2DM with any of these represent a priority group for neuropathy screening[53]. In an Indian study of 4555 individuals with childhood and adolescent-onset diabetes, 19.5% had T2DM while 71.4% had T1DM. Age-adjusted incidence of retinopathy was 52.9/1000 person-years in T1DM, which was slightly higher than that in T2DM (49.8/1000 person-years). However, the rates of nephropathy and neuropathy were higher in youth with T2DM compared to those with T1DM (6.2 *vs* 13.8 and 8.8 *vs* 24 in T1DM *vs* T2DM, respectively)[54].

Mortality

A study on first nation Canadian adolescents with T2DM found a striking mortality rate of 9% over a follow-up period of 9 years[55]. In an Australian study comparing standardized mortality rate (SMR) in patients with T2DM diagnosed between 15 years and 30 years of age with that of those diagnosed between 40 years and 50 years, the investigators found an inverse relationship between age of diabetes onset and SMR, which was the highest for T2DM diagnosed between 15 years and 30 years of age (SMR 3.4 [95% confidence interval [CI]: 2.7-4.2]), with the highest SMR of more than 6 in early midlife. Notably, SMR for the older-onset group was similar to the non-diabetic background population[3]. The excess risk of death progressively declined until it reached values similar to those of the background population in those who were diagnosed with T2DM beyond 69 years of age. A Swedish study showed up to two- to three-fold increased risk of all-cause mortality in people with diabetes who were aged less than 55 years compared to those who were older. When adjusted for glycemia and albuminuria, excess mortality persisted in those who were younger than 55 years even when the hemoglobin A1c (HbA1c) was < 7% and in the absence of albuminuria[56].

In a large registry from Australia, those with young-onset T2DM had higher all-cause mortality, CV mortality, and stroke but slightly lower cancer-related mortality[57]. Earlier age of diagnosis of diabetes by 10 years, was associated with about 30% higher risk for all-cause mortality and a 60% elevated risk of CV mortality. A Danish study reported that younger age at diagnosis of T2DM increased mortality, but this association was markedly weaker in women than in men [58]. The decline in mortality over several decades with improved healthcare was also evident in men with diabetes but not women. The life expectancy for young-onset diabetes diagnosed between 20 years and 40 years of age is reduced by 14 years in men and 16 years in women compared with those without T2DM, and this reduction in life expectancy is greater than that of T1DM, which is about 12 years[57].

Comorbidities

Hypertension and dyslipidemia: In a United Kingdom study comparing young-onset T2DM to T1DM, the former cohort showed an increased prevalence of obesity, hypertension, as well as dyslipidemia, although they also had an older age of onset ($P < 0.0005$) despite having similar glycemic control in both groups. In another hospital-based study, the hypertension prevalence among T2DM patients was 59.5%, with the most common being stage 1 hypertension (30.95%). The risk of hypertension was higher among those aged between 50 years and 60 years, those with longer duration of T2DM, having BMI ≥ 25 kg/m^2, those with poor glycemic control, and smokers[59]. In a recent meta-analysis of 60 studies involving 3463 participants, hypertension prevalence was 25.33%, with higher risk among male participants than females [42].

Investigators of the TODAY study group found that at the end of follow-up of 13.3 ± 1.8 years, the incidence of hypertension was 67.5% and that of dyslipidemia was 51.6%. There was development of at least one macrovascular or microvascular complication in 60.1% of the participants, and at least two complications in 28.4% of the participants and the independent risk factors for the development of complications included hyperglycemia, hypertension, and dyslipidemia[60].

Studies in individuals with familial hypercholesterolemia have demonstrated that statins reduce CIMT with similar efficacy in youth as in adults[61]. However, a multicenter study in T1DM failed to show any effect of statins on CIMT, although low-density lipoprotein cholesterol was lowered[62]. There is not enough longitudinal data on statin use in youth-onset T2DM. However, the fact that dyslipidemia in youth will track into adulthood is well known, and the presence of diabetes with dyslipidemia could potentially increase the CV risk associated with the latter by several-fold. Therefore, a reasonable approach to managing dyslipidemia should be aligned with the general recommendations for dyslipidemia in diabetes.

Obstructive sleep apnea: Obstructive sleep apnea (OSA) is associated with obesity and insulin resistance in adults and children and is established as a risk factor for future CVD[63-65]. OSA can affect glycemic control in individuals with diabetes, and treatment of OSA might lead to improved glycemic profile, HbA1c, and insulin sensitivity as well as inflammatory markers, as has been seen in studies in adult T2DM[66,67]. The implications in young-onset T2DM remain to be explored. A detailed sleep study is expensive but evaluation for OSA using some general questions about sleep quality, snoring, apneic spells, early morning headaches, and excessive daytime sleepiness can be easily done. A pediatric Epworth sleepiness scale has been validated with a score of 10 or more indicative of excessive daytime sleepiness in children[68].

Polycystic ovarian syndrome: Overweight and obese adolescent girls have higher prevalence of polycystic ovarian syndrome (PCOS) compared with normal weight adolescent girls[69]. Adolescent girls with PCOS and obesity have a high prevalence of impaired glucose tolerance (30%) and T2DM (3.7%)[70]. Up to 21% of adolescent girls in the TODAY cohort had oligomenorrhea, and these girls had higher androgen levels but lower sex hormone-binding globulin and estradiol levels[71].

In girls with T2DM and PCOS, metformin in conjunction with lifestyle modification can improve not only metabolic dysfunction but also improve menstrual regularity as well as reduce hyperandrogenism[70]. However, in the TODAY study, all of the girls were on metformin and differences between effects of the three treatment groups (metformin alone, metformin along with lifestyle changes, and metformin with rosiglitazone) on menses or sex steroids could not be demonstrated[71]. Although some of the hormonal combined oral contraceptive pills (COCPs) have been associated with adverse metabolic status and CV risk, COCPs still form the cornerstone of therapy for hyperandrogenism and anovulation in PCOS and they have no contraindications in young women with T2DM. While pelvic ultrasound-detected polycystic morphology is not indicative of a diagnosis of PCOS in adolescents, it can be diagnosed based on the presence of oligo- or amenorrhea with clinical or biochemical evidence of hyperandrogenism.

Non-alcoholic fatty liver disease: Non-alcoholic fatty liver disease (NAFLD) can be seen in up to 50% of children with T2DM[72]. Longitudinal studies revealed that after 20 years, 6% of non-diabetic adolescents with NAFLD died or required a liver transplant[73]. The figures might be even worse for those with young-onset T2DM and concomitant NAFLD but data are lacking in this regard.

In a multicenter study on youth with NAFLD, 23.4% had prediabetes and 6.5% had T2DM[74]. Furthermore, greater NAFLD histologic severity has been seen in young-onset T2DM compared to adults, with a higher risk of progression to hepatic fibrosis, cirrhosis, and liver failure[74,75]. While transaminase levels have good sensitivity for the detection of advanced stages of hepatitis or fibrosis, they have poor specificity for NAFLD and it is necessary to rule out non-NAFLD causes of chronic liver disease. It is recommended to evaluate young T2DM for the presence of possible NAFLD by measuring alanine transaminase and aspartate transaminase at diagnosis and annually thereafter. Liver ultrasound can detect liver fat > 30% but its sensitivity is poor with lesser degrees of fat infiltration and in morbidly obese patients. Liver biopsy is still considered the gold standard, and magnetic resonance spectroscopy is the non-invasive test with highest sensitivity for detecting liver fat[76]. It should be noted here that the non-invasive scores for fibrosis assessment such as fibrosis-4 and tools like transient elastography are not validated in children or adolescents and should not be used.

Glycemic durability

The rate of loss of β-cell function is more accelerated in young-onset T2DM with about β-cell function loss per year being 25%-30% in young people compared to 7% per year for those having adult-onset T2DM[22]. This accelerated rate of β-cell loss persists beyond youth and a rapid progression to glycemic failure can be seen up to the age of 40 years[77]. The loss is particularly steep during adolescence due to the effects of growth hormone surges as well as gonadal steroids leading to a decrease in insulin sensitivity. In the TODAY study, participants who experienced treatment failure had significantly reduced β-cell function. These findings were similar to those observed in the RISE study and large population-level studies in Europe and China. However, poor glycemic durability could not be ameliorated by early insulin treatment, indicating it was not related to poor glycemic control alone[22,23,77].

In one study, adults with early-onset T2DM had an 80% higher likelihood of requiring insulin therapy compared to those with usual-onset T2DM, although they had a similar average time to initiation of insulin (about 2.2 years)[41].

Mental health

Mental health problems including depression and anxiety are commonly seen in people living with diabetes and the risk might be particularly higher in the younger people. Notably, in another registry-based study, about 40% of hospitalizations among young individuals with T2DM were attributable to mental health issues[78]. In a study on Swedish people with young-onset T2DM, the risk of bipolar disorder, anxiety, depression or stress-related events was higher by up to 4-fold[79]. Among youth with T2DM, younger age at diagnosis was found to be an independent predictor for depression [80]. It is important to assess psychiatric problems such as depression, diabetes-related distress, body image disorders, and eating disorders in all children and adolescents with T2DM. While there is a validated questionnaire for assessing diabetes distress in children such as the Problem Areas in Diabetes-Child score, they have mostly been used in children with T1DM who have other factors like fear of needles and a high risk for life-threatening complications[81]. Fear of hypoglycemia should be assessed. A lot of the issues can be handled with timely identification and appropriate counseling. Socioeconomic factors adding to their distress such as food security, housing stability, child labor, school drop-outs, and exam-related stress should be kept in mind when devising a treatment plan after detailed discussion with the children and should ideally include the family.

Cancers

Evidence suggests a higher frequency of diabetes-related cancers in those with young-onset T2DM compared to adult-onset T2DM[82]. In the Nurses' Health study, early-onset T2DM was associated with increased risk of early-onset cancers by 1.47-times, of obesity-related cancers by a 1.75-times, and of diabetes-related cancers by a 2.11-times, all of which were higher than the rates observed in usual onset T2DM across all ages[82,83].

Pregnancy outcomes

In the TODAY study reporting the outcomes of 260 pregnancies among 141 women with young-onset T2DM, 65% of the women reported complications during their pregnancy. About one-fourth (25.3%) suffered pregnancy loss while preterm birth was seen in 32.6% of pregnancies. One-third of pregnant women had HbA1c higher than 8%. Congenital abnormalities were seen in up to 10% of pregnancies, of which 3.7% resulted in stillbirths, which was three times higher than the national rates for the United States during that time. Of the offspring, 7.8% were small for gestational age, 26.8% were born large for gestational age while 17.9% of infants were in the macrosomic range[84]. In the National Pregnancy

data from the United Kingdom, pregnant women with T2DM had increased perinatal deaths than women with T1DM in all HbA1c categories[85]. The risk is particularly high for adolescent mothers with T2DM in whom as high as 38% of women experienced pregnancy loss in a Canadian study[55]. Much of the risk can be eliminated or reduced with appropriate pre-conception counseling, meticulous glycemic control, and folic acid supplementation (5 mg/d) combined with close monitoring of the mother and fetus. Unfortunately, studies have shown that compared to women with T1DM, a significantly higher proportion of women with young-onset T2DM receive potentially teratogenic medications, and < 50% had knowledge or used adequate contraception[86].

Recommendations on complications screening in young-onset T2DM

Currently, there is dearth of guidelines by societies that focus on the management issues of young-onset T2DM. A position statement to this regard was released by American Diabetes Association (ADA) in 2018 and another consensus guideline from International Society of Pediatric and Adolescent Diabetes (ISPAD) in 2022, the salient points of those are summarized in Table 1[87,88]. Overall, in addition to glycemic status assessment the investigations that should be done at diagnosis and annual review should include lipid profile, urine for albuminuria, serum creatinine levels, retinopathy screening, neuropathy screening with foot check-ups as well as liver function tests. Preferably, one should also assess for OSA in all patients, for possibility of PCOS in adolescent and young girls along with a psychological assessment for emotional well-being and quality of life. Although most guidelines recommend that complication screening in T1DM should start after 5 years of diagnosis or at puberty, whichever is earlier in T1DM, the same conditions may not be appropriate in T2DM given that there would be an unknown duration of hyperglycemia before T2DM is diagnosed.

Given the fact that youth-onset T2DM has a more aggressive presentation and earlier appearance of complications, and the rising prevalence of young-onset T2DM, it is expected to portend a significant clinical and socioeconomic burden with high financial burden. Complications would result in both increased direct costs of hospitalization and indirect medical costs due to work absenteeism, reduced productivity while working, reduced productivity at work place, loss of jobs and disability-adjusted life years (DALYs) due to disease-related disability and also premature deaths. Data on the socioeconomic burden of diabetes in youth-onset T2DM are lacking but extrapolating the data from adults, it can be hypothesized that there is a significant increase in health-care costs due to delayed diagnosis of complications and comorbidities in young-onset T2DM, thus making it necessary for timely and periodic screening tests for complications [89]. Instead of focusing on costly investigations, complication screening should start with inexpensive clinic-based tests such as anthropometry, clinical neuropathy assessment, fundoscopy for retinopathy, history and examination for oligomenorrhoea, and hyperandrogenism for PCOS and validated questionnaire for diabetes-distress and excessive daytime sleepiness in children. Laboratory investigations may be limited to those with abnormalities detected on screening.

Using a similar logic, children and young adults who have complications such as atherosclerotic CVD, cardiomyopathy, peripheral arterial disease, kidney disease, or neuropathy must undergo screening for prediabetes or diabetes as a first-line investigation, irrespective of their age. While these are rare, comorbidities such as obesity, dyslipidemia, and PCOS are commonly encountered in adolescents. Available guidelines recommend screening for prediabetes or diabetes in overweight or obese children or adolescents with additional risk factors such as hypertension, dyslipidemia, and PCOS. Screening for diabetes preconceptionally and gestational DM screening in adolescent pregnancy should proceed in the same lines as during adulthood.

MANAGEMENT

Lifestyle interventions

Guidelines from the ADA, ISPAD, and American Heart Association advocate a lifestyle modification along with pharmacotherapy in youth-onset T2DM[90-92]. Dietary modification targeting a daily caloric deficit and enhanced nutrient intake is an integral part of the management of youth with T2DM[93]. Diet modification should be advised for the entire family and emphasizing healthy parenting practices related to diet should be the goal, while avoiding excessively restricted food intake[91]. The ADA recommends adding 4-5 sessions involving 30-60 min vigorous physical activity weekly to regular school physical education sessions for children and adolescents with T2DM and obesity[87]. Nevertheless, weight reduction remains a challenge in the management of T2DM and obesity in youth. Barriers to achieving recommended weight loss in are multifactorial. Intrinsic biological factors are there that hinder weight loss, while the lack of engagement is related to family education, motivation, accessibility of social support, and diverse socioeconomic barriers [94]. The rate of attendance in lifestyle sessions was only 60% of the planned sessions in the TODAY study[95]. However, participants who attended more than 75% of the program duration achieved higher body weight loss and improvement in body composition. Despite the encouraging results in adults, the TODAY trial demonstrated that intensive lifestyle interventions plus metformin did not improve weight-related outcomes compared to the group that received metformin only[96]. In the TODAY trial, only 53.6% of participants met the physical activity target, and this failure might have contributed to the suboptimal response in the metformin plus lifestyle intervention group. The efficacy of lifestyle modification in T2DM youth may be less than expected and inability to improve of fitness, HbA1c levels or weight may have a physiologic basis[97]. Although diet and exercise can reduce BMI and alleviate the risk of diabetes related complications, these lifestyle interventions should always be used in conjunction with pharmacotherapy for T2DM in children and adolescents because of the aggressive nature of the disease.

Table 1 Screening and management of complications in young-onset type 2 diabetes mellitus

Complication	American diabetes association 2018[87]	International society for paediatric and adolescent diabetes 2022 [91]
Cardiovascular disease	Intensive lifestyle interventions focusing on weight loss, dyslipidemia, hypertension, and dysglycemia are important to prevent overt macrovascular disease in early adulthood. Routine ECG, echocardiography, stress tests or other screening tests for cardiovascular disease are not warranted in the absence of cardiac symptoms	
Retinopathy	Screening for retinopathy should be performed by dilated fundoscopy or retinal photography as soon as possible after diagnosis and then annually. Less frequent examination (every 2 yr) may be considered for those with adequate glycemic control and a normal eye examination	Screening of youth with T2DM at the time of initial diagnosis and annually by an ophthalmologist or optometrist by a comprehensive eye examination with dilated pupils or retinal photograph. More frequent examinations are required if retinopathy is of higher grade, progressing and if there is suboptimal glycemic control
Neuropathy	Screening for the presence of neuropathy by foot examination at diagnosis and then annually, including inspection, assessment of foot pulses, pinprick and 10-g monofilament tests, testing of vibration perception using a 128-Hz tuning fork, and ankle reflexes	Foot examination (including sensation, vibration sense, light touch and ankle reflexes) at diagnosis and then annually is recommended to detect at risk feet
Nephropathy	UACR to be obtained at diagnosis and then annually thereafter. Elevated UACR (> 30 mg/g creatinine) should be confirmed on two out of three samples. eGFR should be determined at the time of diagnosis and annually thereafter. Those with nephropathy should undergo continued monitoring with yearly UACR, eGFR, and serum potassium. Referral to nephrology is recommended if etiology is uncertain or if there is worsening UACR, or decline in eGFR	Albuminuria screening should occur at diagnosis and annually thereafter using three first morning urine collections. If UACR is > 30 mg/g (3 mg/mmol) and BP is elevated or UACR is > 300 mg/g (30 mg/mmol) irrespective of BP, ACEi, or ARB should be started and BP normalized. If albuminuria is present, serum potassium and renal function should be evaluated annually. Renal function to be evaluated using calculated eGFR from validated formulas cystatin C measurement is currently not recommended as it shows high variability and is affected by age, sex, BMI, and diabetes control. A repeat UACR may be helpful 6 mo after the start of ACEi or ARB to ensure albuminuria is normalized. Non-diabetes-related causes of renal disease should be considered and consultation with a nephrologist obtained if severely increased albuminuria (UACR > 300 mg/g or 30 mg/mmol) or if hypertension is present
Hypertension	BP should be measured at every visit and optimized if necessary. If BP is > 95th percentile for age, sex, and height, increased emphasis should be placed on lifestyle management. Antihypertensive therapy should be initiated if BP is not optimized by 6 mo. ACEi or ARB) should be initial therapeutic agents. Other BP–lowering agents may be added if needed to optimize BP	BP should be measured at diabetes diagnosis and at every subsequent visit, in the seated position. ABPM can be considered if there is suspicion of white coat hypertension or to confirm hypertension. Echocardiographic evaluation is recommended in youth with confirmed hypertension to assess for left ventricular target organ injury
Dyslipidemia	Lipid testing after initial glycemic control has been achieved and repeated annually thereafter. Optimal goals are: LDL-C < 100 mg/dL (2.6 mmol/L), HDL-C > 35 mg/dL (0.905 mmol/L), triglycerides < 150 mg/dL (1.7 mmol/L). Dietary counseling using the AHA Step 2 diet. If LDL-C remains above goal after 6 mo of dietary intervention, to initiate statins with goal of LDL-C < 100 mg/dL. If triglycerides are > 400 mg/dL (4.7 mmol/L) fasting or > 1000 mg/dL (11.6 mmol/L) non-fasting, to optimize glycemia and begin fibrate, with a goal of < 400 mg/dL (4.7 mmol/L) fasting to reduce risk of pancreatitis	Testing for dyslipidemia should occur once glycemic control has been achieved or after 3 mo of initiation of medication, and annually thereafter unless abnormal
NAFLD	Evaluation for NAFLD (by measuring ALT and AST) should be done at diagnosis and annually thereafter. Referral to gastroenterology should be considered for persistently elevated or worsening transaminases	Liver enzymes (ALT, AST, and GGT) should be evaluated at T2DM diagnosis and annually thereafter, preferably sooner if abnormal
Psychosocial	Assessment of social factors, including food and housing stability, financial constraints. Appropriate patient-appropriate validated tools to assess mental health issues including diabetes distress, depression and disordered eating behaviours, and referral to specialty care if needed. At every visit, to check medication adherence. Effects of medication on body weight must be considered. Screening for smoking and alcohol use at diagnosis and at regular intervals	Youth with T2DM should be screened for psychological co-morbidities including depression, diabetes distress, and disordered eating at diagnosis and at regular follow-up intervals. Providers should specifically consider household food security, housing stability, and family financial resources when devising a treatment plan with the youth and family
OSA	OSA screening should be done at each visit, and referral to a pediatric sleep specialist for evaluation and a polysomnogram, if indicated, is recommended. OSA should be treated when documented	Youth with T2DM should be screened for symptoms of OSA at diagnosis and annually thereafter unless there is excessive weight gain, which requires an earlier review of OSA symptoms. OSA can be initially evaluated using general questions about snoring, sleep quality, apnea, morning headaches, daytime sleepiness, nocturia, and enuresis. If symptoms are suggestive of OSA, the diagnosis of OSA is confirmed by a sleep study and referral to a sleep specialist. Nocturnal pulse oximetry can be an initial useful evaluation

		if there is limited access to a sleep study
PCOS	Adolescents with T2DM should be evaluated for PCOS including laboratory studies when indicated. Metformin in addition to lifestyle modification can be used to improve the menstrual cyclicity and hyperandrogenism in girls with T2DM. Weight loss and metformin may improve the menstrual disorder. If hormonal contraception is commenced, effects on metabolic risk should be considered in agent-selection. Oral contraceptives, if required for treatment of PCOS, are not contraindicated for girls with T2DM	PCOS screening should occur at diagnosis in pubertal girls and yearly after that with an evaluation of menstrual history and evidence of hyperandrogenism (hirsutism and/or moderate to severe acne and/or total testosterone measurement). PCOS is diagnosed based on the presence of oligo- or amenorrhea with clinical or biochemical evidence of hyperandrogenism (total testosterone) after exclusion of other possible causes. Pelvic ultrasound is not recommended for the diagnosis of PCOS within 8 yr post-menarche
Pregnancy	Pre-conception counseling should be incorporated into routine diabetes clinic visits for all females of child-bearing potential, starting from puberty	

ABPM: Ambulatory blood pressure monitoring; ACEi: Angiotensin convertase enzyme inhibitors; AHA: American Heart Association; ALT: Alanine transaminase; ARB: Angiotensin receptor blocker; AST: Aspartate transaminase; BP: Blood pressure; ECG: Electrocardiogram; eGFR: Estimated glomerular filtration rate; GGT: Gamma glutamyl transaminase; HDL-C: High-density lipoprotein cholesterol; Hz: Hertz; LDL-C: Low-density lipoprotein cholesterol; NAFLD: Non-alcoholic fatty liver disease; OSA: Obstructive sleep apnea, PCOS: Polycystic ovarian syndrome; T2DM: Type 2 diabetes mellitus; UACR: Urine albumin/creatinine ratio.

Pharmacological treatment-approved options

The treatment of young individuals with T2DM remains a challenge. Pharmacological treatment options approved by the regulatory bodies were limited to metformin and insulin until 2019, when liraglutide once daily, a glucagon-like peptide 1 receptor agonist (GLP-1 RA) gained indication for children aged 10 years or above based on an acceptable safety data and a -1.3% placebo-corrected reduction in HbA1c after 52 wk of treatment[98]. In the subsequent 3 years, the once-weekly GLP-1 RAs exenatide[99] and dulaglutide[100] were approved by the United States Food and Drug Administration (FDA) for use in children and adolescents who have T2DM. In Europe, exenatide has received approval in 2023 and dulaglutide is expected to gain approval in 2024. The indication of the (SGLT-2) inhibitor dapagliflozin was extended to children aged 10 years or more subsequent to a small study of young people, aged 10-24 years in Europe[101]. The placebo-corrected reduction in HbA1c was -0.85% for exenatide, -1.4% for dulaglutide, and -0.75% for dapagliflozin. In a recent study of metformin and/or insulin treated youth (aged 10–17 years) with T2DM having mean baseline HbA1c about 8%, dipeptidyl peptidase (DPP-4) inhibitor linagliptin did not provide a clinically significant HbA1c reduction when added to metformin or insulin therapy, and SGLT-2 inhibitor empagliflozin only showed a modest effect on HbA1c in a randomized clinical trial (DINAMO study)[102]. Similar safety profiles of empagliflozin and linagliptin were observed as found in adults with T2DM. Diabetic ketoacidosis episodes were not reported. Of note, empagliflozin has recently been approved to manage T2DM in children 10 years or older based on results from DINAMO trial. The findings with empagliflozin and linagliptin are similar to those from earlier studies in youth-onset T2DM with dapagliflozin (SGLT-2 inhibitor) and sitagliptin (DPP-4 inhibitor)[101,103]. Use of sulfonylureas, DPP-4 inhibitors, or thiazolinidiones is not approved for the treatment of T2DM in children and adolescents.

Approach to pharmacotherapy of T2DM in children and adolescent

Initial and subsequent therapy: Metformin is the initial treatment of choice for T2DM in children and adolescents together with healthy lifestyle changes[90,91]. If HbA1c < 8.5% in absence of ketosis or hyperglycemic symptoms may be initiated on metformin at 500 mg a day with weekly slow titration to 1000 mg twice daily. Insulin replacement is indicated in combination with metformin in youth with symptoms or HbA1c ≥ 8.5%. Once daily intermediate- or long-acting basal insulin (starting dose 0.25-0.5 units/kg) is necessary in the face of ketosis or acidosis[91]. In general, transition to metformin only can be obtained within the following 2-6 wk. If the goal of initial treatment (HbA1c < 7.0%) is not attained with metformin monotherapy in adolescents, addition of basal insulin or GLP-1 RA may be considered. With higher HbA1c values (> 9%), the preferred option is basal insulin. If the glycemic target is not achieved on a combination of metformin and basal insulin (up to 1.5 units/kg), initiation of mealtime insulin should be considered[91]. An algorithm on the drug treatment of T2DM in children and adolescents is provided in Figure 2[104].

Unmet needs in drug therapy

In contrast to the several therapies available to adults with T2DM, metformin and liraglutide remain the only non-insulin treatments formally approved in the United States for use in children and adolescents with T2DM. Metformin monotherapy fails to achieve glycemic control in about half of adolescents with T2DM, especially among adolescents with severe metabolic abnormalities at diagnosis[24,105]. The RISE Consortium data demonstrated that pediatric patients with recent-onset T2DM had continued deterioration of β-cell function regardless of metformin therapy[10]. Data are limited with regard to the use of liraglutide in children and adolescents with T2DM. Despite the FDA approval of liraglutide in 2019 for pediatric T2D, the uptake of GLP-1RAs in this population has been slow due to injectable way of administration and cost. Furthermore, contrary to findings from adult studies, the pivotal GLP-1RA trials in pediatric T2DM do not demonstrate significant weight loss. In addition, the long-term safety and CV and kidney impact of GLP1 RAs have not been investigated in youth-onset T2DM. Although numerous formulations of insulin are commonly used in children and adolescents with T2DM, they have not been studied for the particular indication of pediatric T2DM. Dapagliflozin was

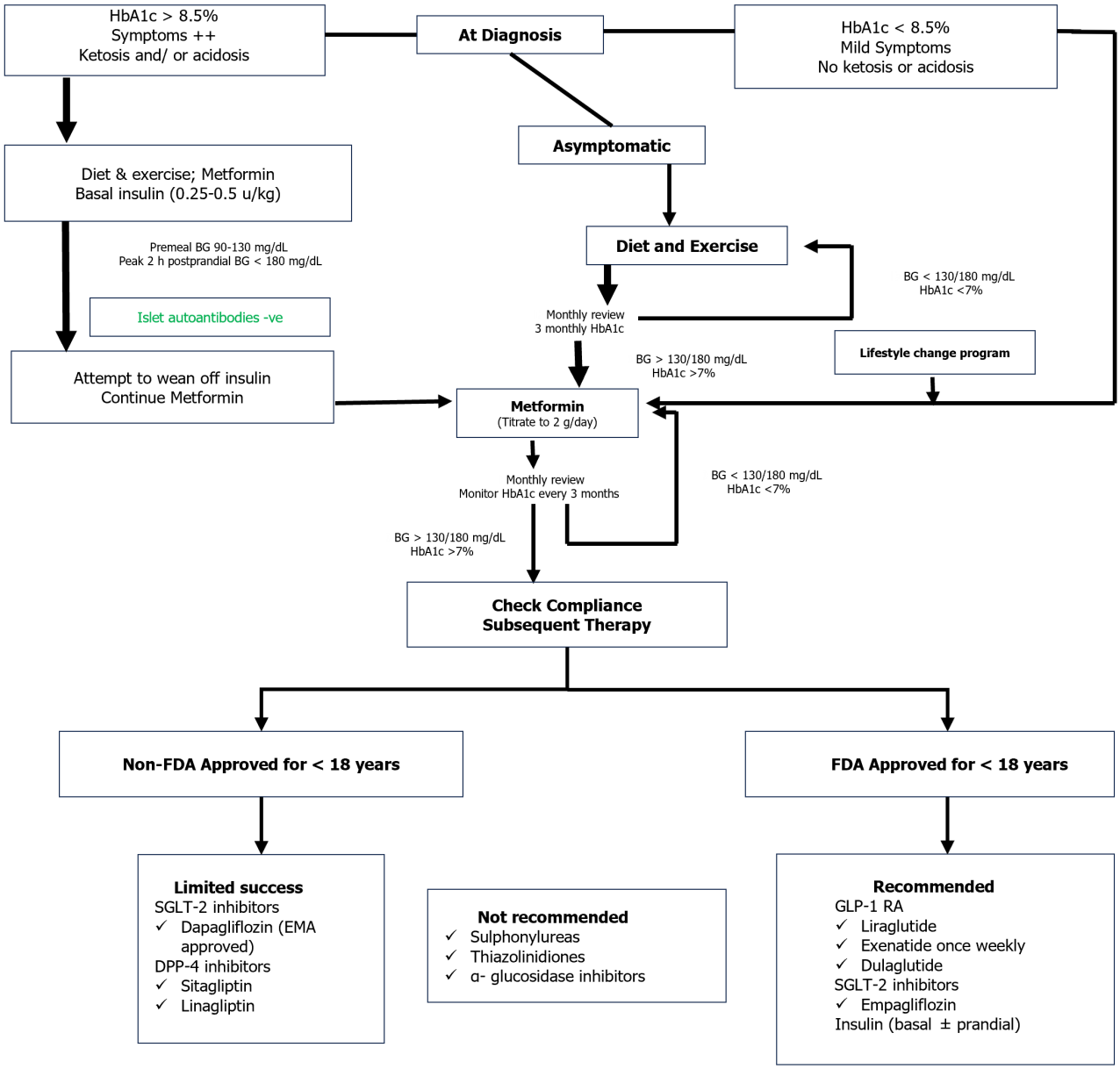

Figure 2 Approach to treatment of type 2 diabetes in children and adolescents. Modified from Karavanaki *et al*[104].

the first oral glucose-lowering medication since metformin to be approved in Europe for children (≥ 10-years-old) and young adults with T2DM. Given the adverse cardio- and microvascular profile[41,106] of many young individuals with T2DM, the non-glycemic benefits of dapagliflozin may become increasingly important in the management of youth-onset T2DM. However, due to the lack of published data, SGLT-2 inhibitors are not yet widely used for the treatment of T2DM in children and adolescents. Obesity is the key pathophysiological driver of T2DM and development of CV risk factors in most children and adolescents. Therefore, studies testing semaglutide and tirzepatide, which have shown impressive weight loss together with robust effects on glycemic control in adults with T2DM, are now needed in young people with T2DM to get these drugs approved as future treatments. Overall, the therapies currently available are limited and often inadequate. Although promising results have been shown by some treatments in short-term, it is imperative to ensure that long-term benefits of these therapeutic options are evidently showed in youth with T2DM. Future research should aim to surmount the challenges seen in clinical trials in this population in order to address the unmet need for the best possible management of T2DM in children and adolescents.

Bariatric surgery

As per current international guidelines, metabolic bariatric surgery (MBS) can be recommended in adolescent (10-19 years of age) T2DM patients with a BMI of more than 35 kg/m^2 or more than 120% of the 95th percentile of age and sex matched population[107]. Among the adolescent populations, vertical sleeve gastrectomy (VSG), laparoscopic adjustable gastric band and the roux-en-Y gastric bypass (RYGB) are the commonly performed surgeries[108]. In a meta-analysis of 29 cohort studies, a total of 4970 adolescents with at least 5 years follow up after bariatric surgery were included. A remarkable remission rate of 90% (95%CI: 83.2–95.6) for T2DM following MBS was reported in this study[109]. When the

metabolic effects of MBS were compared between adolescent and adult populations, the chance of remission of T2DM was found to be significantly higher in adolescents than adults (86% *vs* 53%; risk ratio: 1.27; 95%CI: 1.03 to 1.57) even with similar degree of weight loss[110]. In the 3 years follow up of Teen- Longitudinal Assessment of Bariatric Surgery (LABS) study, 76% remission was found even among pre-diabetes adolescents who underwent MBS[111]. In the adolescent population, both RYGB as well as VSG showed comparable T2DM remission rates[112,113]. Thus, MBS can be used as a tool for early intervention for prevention as well as better control of dysglycemia in adolescents. On the other hand, patients undergoing MBS at early age are at higher risk for both micro as well as macronutrient deficiencies[114]. Moreover, as maximum bone accrual occurs at this age, bone health can also be affected due to the risk of nutrient deficiency associated with MBS[115]. Further studies evaluating the long-term safety data of MBS performed in adolescents are needed in the future[116]. Reoperation can also be necessary either due to complications (hernia, leak, band slip) or failure (inadequate weight loss or resolution of co-morbidities) of primary surgery[117]. Limited expertise and availability, cost of therapy, lack of insurance coverage, stigma, and lack of awareness and family support are the common barriers to performing MBS in adolescents[118,119]. Medically correctable obesity, substance abuse, current or planned pregnancy within 12 to 18 mo, eating disorders, the chance of non-adherence to post-operative recommendations, and poor family support are considered relative contraindications to MBS[108]. Overall, as the chance of remission of T2DM is significantly high with MBS in adolescents, it should be considered in carefully selected morbidly obese adolescents with T2DM. Among the minimally invasive newer procedures, intragastric balloon treatment and aspiration therapy are now being tried in adolescent obesity[120,121].

Glycemic target

As per ADA recommendations, HbA1c should be measured every 3 mo and the HbA1c target for children or adolescents with T2DM should be < 7%[87]. However, ADA also recommends a more stringent target of < 6.5% if that can be achieved without causing any significant adverse effects including hypoglycemia[87]. The lower HbA1c target is preferred in young T2DM patients because of the following reasons. Firstly, the risk of complications due to diabetes is higher in younger patients because of cumulatively longer duration of exposure to dysglycemia[45,122]. Second, young T2DM have a greater degree of β-cell dysfunction[123]. Moreover, they are prone to rapid deterioration of beta cell function. In TODAY study, HbA1c cut-off as low as prediabetes range (> 6.3%) was associated with a higher risk of rapid loss of glycemic control within 48 mo of follow-up[124]. Thus, more stringent control can help to preserve β-cell function. Thirdly, with the advent of newer molecules like SGLT-2i as well as incretin-based therapies, the risk of hypoglycemia is much less when compared to intensive treatment with traditional sulphonylureas and insulin[125]. Moreover, the risk of hypoglycemia is reported to be very low in adolescents even when insulin therapy has been used[126]. Thus, in young T2DM patients, stringent glycemic control is possible without increasing the risk of hypoglycemia significantly. To summarize, the HbA1c target in adolescents with T2DM should be on the lower side. However, the target should be individualized depending on the risk of hypoglycemia. The frequency and targets for self-monitoring of blood glucose monitoring should also be individualized[87].

Diabetes remission

As per the international expert group consensus report, 'remission' of T2DM is considered if HbA1c remains < 6.5% for at least 3 mo without any glucose lowering pharmacotherapy[127]. The HbA1c testing should be done at least 3 mo after any surgical or pharmacological intervention or after at least 6 mo from the initiation lifestyle-based intervention[127]. The studies which evaluated the chance of T2DM remission in adolescent patients are mostly limited to lifestyle and surgical interventions. In a small study by Willi *et al*[128], ketogenic very low calorie diet (VLCD; 680-800 Kcal/d) had been reported to be effective in compliant children with T2DM allowing withdrawal of exogenous insulin and oral antidiabetic medications. However, in TODAY study done in T2DM adolescents, lifestyle interventions (VLCD 1200-1500 Kcal/d with 200-300 min of weekly moderate exercise) failed to show any additional benefit when combined with metformin[24]. Moreover, adherence rate, long term durability and adverse effects of VLCD in children and adolescents with T2DM are not well studied. On the other hand, the effect of MBS on induction of remission of T2DM in adolescents is encouraging. In the Teen-LABS study, among 29 adolescents who underwent MBS, 95% reported remission from diabetes at 3 years[111]. In the same study, 76% remission rate had been reported in adolescents with pre-diabetes. In the meta-analysis by Wu *et al*[109], 90% diabetes remission rate was reported after at least 5 years of undergoing MBS. The MBS are found to be more effective if inducing remission if done earlier than later in life. Moreover, remission occurs very rapidly following MBS even before significant weight loss suggesting additional mechanisms of diabetes remission beyond sustained weight loss[129].

PREVENTION

One of the chief drivers as well as a major modifiable risk factor for young-onset T2DM is excess body weight starting from childhood. The risk starts in utero with the nutritional status of the mother along with the fetal birth weight having important implications on the metabolic health of the child. Thus, interventions that reverse or reduce obesity in children and adolescents at the community level are essential in preventing young-onset T2DM. In this regard, school-based health interventions are gaining importance in addressing and mitigating environmental and behavioral factors that increase the risk for the development of obesity and T2DM.

A wide range of prevalence data on childhood obesity (BMI > 95th centile) in T2DM has been reported- from 64.5% in Asian populations to 89.9% in European populations[130]. Important evidence regarding the impact of lifestyle interventions for youth with T2DM comes from the TODAY study. While the goal was to achieve a 7%-10% decrease in weight with lifestyle changes, the addition of metformin monotherapy to lifestyle intervention was not associated with any increase in the duration of metabolic control beyond that with metformin[96]. Youth with T2DM receiving metformin plus lifestyle intervention demonstrated short-term weight loss and body composition improvement but the changes were not sustained over a long period. Irrespective of treatment, sustained weight losses above 7% led to improvements in HbA1c, high-density lipoprotein cholesterol, and C-peptide levels. Family-based behavioral weight management programs have been found to have some positive impact on weight and metabolic risk factors of school-going children without diabetes but were not very effective in adolescents and children who had greater degrees of obesity[131]. A comprehensive pediatric lifestyle intervention should involve the family and employ evidence-based behavioral strategies that have been demonstrated to develop sustainable changes in nutrition and physical activity. Therefore, young-onset T2DM who are overweight/obese should receive appropriate comprehensive lifestyle programs, involving their family members, targeting to achieve a 7%–10% reduction in body weight. Nutrition advice should include healthy eating patterns with increased consumption of nutrient-dense and decreased consumption of calorie-dense, nutrient-poor foods, like sugar-sweetened beverages. Physical activity must include at least 30-60 min of moderate to vigorous physical activity at least 5 d every wk along with strength-training activities for a minimum of 3 d per wk[87]. A meta-analysis found the short-term efficacy of very-low calorie in reducing weight in obese people aged below 18 years, but no long-term follow-up has been undertaken[132]. Younger people have shown better uptake of behavioral interventions in the digital format[133]. Data regarding the efficacy and safety of pharmacotherapy for weight loss in the youth are limited. Most anti-obesity medications except orlistat and liraglutide are not yet approved for use in children. Overall, there is limited evidence base to support diabetes screening in all adolescents or young adults with obesity which would not be a very cost-effective approach[134]. Some guidelines recommend screening for dysglycemia in obese youth who have additional co-morbidities like hypertension[91]. Several risk scores to identify individuals at risk for and needing screening for diabetes have been developed and validated in different countries, including clinical and polygenic factors [135]. The trajectories from normoglycemia to T2DM might differ in young people, thus narrowing the window of opportunity to screen at later ages. Another target group for preventive strategies especially includes women with a history of gestational diabetes. A study showed that the adjusted hazards in women with gestational diabetes for progression to T2DM were 71.9 per 1000 person-years[136]. Such women should receive timely and appropriate advice regarding lifestyle, nutrition, and monitoring.

BARRIERS AND SOLUTIONS

The development of best practices for managing youth-onset T2DM is limited by gaps in the understanding of glucose metabolism abnormalities during adolescence and development of complications and the long-term outcomes of young-onset T2DM. Even the definitions of prediabetes and T2DM in children and adolescents are not evidence-based, as they have only been extrapolated from glycemic indices predicting microvascular complications in adults. Hyperglycemia appears to be temporary in some youth, and rapid loss of glycemic control on oral monotherapy is seen in others requiring insulin treatment[137]. Therefore, a better understanding of disease heterogeneity is needed to envisage disease trajectory that would allow customized approaches to treatment. The long-term outcomes for early-onset dyslipidemia, hypertension, kidney disease, NAFLD, and CV dysfunction are largely not known[138]. Research is required to improve understanding of what diabetes-related complications may require pediatric-specific approaches.

Recent studies suggest that pediatric T2DM differs from adult-onset T2D in a variety of ways and adolescents with T2DM display more insulin resistance and glycemic failure compared with adults[139]. Consequently, there is an urgent need for an expanded set of treatment options. However, the social and environmental complexities surrounding youth with T2DM hinder recruitment into and completion of clinical trials[138]. As a consequence, trial recruitment for youth with T2DM has had limited success as a relatively new endeavor. Other barriers to study implementation include a relatively small number of available study participants (compared with adults) with an increasing number of trials competing for that restricted pool of available patients, restrictive eligibility criteria, the difficulty of participants/caregivers repeatedly taking time off from school or work, and the small number of research sites with resources dedicated to pediatric T2DM trials[140-142].

Knowing the realities of the epidemiology, disease heterogeneity, and socioeconomic challenges of youth-onset T2DM, as well as the clinical research experience to date, it is imperative to find solutions to this situation in order to progress in understanding the pathophysiology of T2DM in children and adolescents and successful completion of research programs for this population. Research recommendations and possible solutions to barriers are summarized in Table 2.

CONCLUSION

T2DM in children and adolescents is an emergent public health concern globally that presents with distinctive characteristics, demographics, and disease progression in comparison to adult-onset T2DM. It shows lower rates of response to oral pharmacotherapy than in adults. Given the relatively recent emergence of T2DM in children and adolescents, evidence base about prevention, optimal treatment approaches, and monitoring of this population is lagging behind the steady rise in cases. The available treatment options to manage youth-onset T2DM and prevent the development of

Table 2 Research gaps and possible solutions

Knowledge gaps in youth-onset T2DM
1 Future studies are needed with larger and more diverse samples to understand the unique aspects of T2DM in children and adolescent
2 What are the physiological barriers to exercise seen in obese youth and youth with T2DM? Are lifestyle interventions successful, durable, and sufficient?
3 What are effective ways to increase compliance with lifestyle interventions and medication in adolescents with T2DM?
4 What medications (alone or in combination) achieve durable glycemic control in youth-onset T2DM? Should disease-modifying therapies (SGLT2 inhibitors and GLP-1 RAs) be deployed earlier in the treatment algorithms?
5 What is the optimal approach to management of comorbidities and complications in youth-onset T2DM?
Possible solutions to barriers
1 Prioritization of clinical and translational research addressing the gaps in knowledge regarding the unique physiological features of youth-onset T2DM
2 Increased explorations of the psychological and socioeconomic aspects of youth-onset T2DM
3 Collaboration among academic leaders, government and charitable sponsors, industry, and regulatory agencies to delineate research strategies
4 Increasing research and infrastructure capacity for youth-onset T2DM through the development of research centers of excellence those are uniquely staffed and maintained
5 Increasing the proportion of youth with T2DM who participate in clinical drug trials through trial designs appropriate for the typical youth with T2DM and creative strategies to overcome barriers to care and research

GLP-1 RA: Glucagon-like peptide 1 receptor agonist; SGLT-2: sodium-glucose cotransporter-2; T2DM: Type 2 diabetes mellitus.

complications are limited. However, two more GLP-1RAs have already been approved. The trials of oral semaglutide (Pioneer Teens) and tirzepatide (SURPASS-PEDS) in type 2 diabetic children and adolescents are ongoing. We hope that additional therapies will not only help in the optimization of glycemic control but also slow the progression of disease and decrease long-term co-morbidities and complications. T2DM prevention in youth is another priority. Detection of children and adolescents who are at the highest risk of developing T2DM will aid the development of more efficient interventions to prevent or delay the disease onset. A focus on improving knowledge regarding the pathophysiology as well as biology and environment of youth-onset T2DM, finding the optimal approach to working with this population, and utilizing more realistic and effective study designs and interventions suitable to this population is needed to help improve the care of children and adolescents with T2DM across the world.

FOOTNOTES

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Krishna Kumar Govindarajan

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Abstract

The choledochal cyst (CC) can be better termed as biliary tract malformation because of the close association of embryology and etiology in the causation of CC. Contrary to Babbitt's postulation of reflux, damage and dilatation, reflux was not demonstrable as the causative factor in all varieties of CC. High pressure in the biliary system, otherwise termed ductal hypertension, is put forth as an alternative to explain the evolution of CC. The forme fruste type, which does not find a place in the standard classification, typifies the ductal hypertension hypothesis. Hence a closer, in-depth review would be able to highlight this apt terminology of biliary tract malformation.

Key Words: Choledochal cyst; Biliary tract; Biliary dilatation; Ductal hypertension; Common channel; Pancreatobiliary malunion

Core Tip: The biliary tract malformation has undergone a metamorphosis from its previous nomenclature of choledochal cyst owing to a variety of reasons. The etiology, embryopathology and the current classification require revisiting due to the same. The review looks at the same in detail.

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INTRODUCTION

Choledochal cyst (CC) accounts for 1/1000 to 1/150000 live births, with high incidence

in Asia, especially Japan. The majority (80%) would present by ten years of age. Less than 1% of the benign biliary tract disorders are due to CC, accounting for the relative rarity in adults. Being a disease of children, CC shares a closer link with embryo pathogenesis[1,2].

The classification of CC into various types considers the dilatation/segment of the biliary tract involved. This may have an etio-pathological association. The old school of thought (Babbitt) is subject to debate as the postulation does not satisfactorily explain the different types of CC. Hence a review of embryology is in order to understand the drift to the current thinking[1,2].

THE OLD THINKING

The traditional concept referred to inflammation as the initiating factor, leading to damage and dilatation. Based on contrast reflux into the pancreatic duct during intraoperative cholangiography, Babbitt suggested that pancreatic enzyme backflow into the bile duct initiates inflammatory damage to the bile duct resulting in progressive dilatation with CC formation. According to this pancreatic reflux-induced etiology, Amylase levels may need to be elevated to justify the presence of reflux[3]. But the cyst Amylase level measurements do not universally show an increase. Rather, what is known, is an inverse co-relation of the CC size with the cyst pressure[4]. Hence, an increase in the intraluminal pressure arising from distal bile duct obstruction was shown as the offending factor in the causation of CC. Relative stenosis of the distal bile duct could be the primary factor which can cause elevation of pressure with resultant intraductal hypertension [5]. Not only does this go against traditional thinking but it also sets up the stage for reconsideration of etiology (Figure 1).

NEED FOR RECONSIDERATION

The ductal plate malformation is the embryological basis of Caroli's disease. At the time of bile duct formation, initial thin plates (single layer) of bile duct around the portal vein get reinforced (double layer), resulting in robust bile ductules after extensive resorption. In case of failure to complete resorption with partial/incomplete retention of the primitive ductal plate can lead to large dilated segments. When this occurs at the level of the segmental ducts or intermediate-size ducts, the resultant insult can be either Caroli's disease or Caroli's syndrome, respectively[6].

A different mechanism must be highlighted at this juncture for the evolution of the CC types I/II/IV. The pancreatic duct and common bile duct join together to form a common channel, which is sandwiched between the sphincter of Oddi (entry into D2) and the sphincters of choledochus and pancreaticus. Thus the common channel is usually a short length of 'uneventful' passage. Trouble brews when the common channel lengthens for various embryological factors, as the anomalous junction of the biliary and pancreatic with abnormal sphincters lets loose the tight compartmentalization of fluid travel, resulting in reflux of pancreatic contents across into the bile duct. The stage is now set for reflux, inflammation and damage. Thus the presence of a long common channel would lead to a vicious cycle of stasis and dilatation, ultimately evolving into a CC. Should an anomalous proliferation of the biliary epithelium occur during fetal life, this common channel will become longer than the length of the sphincter (which remains constant) with the accompaniment of reflux[7].

ODDITIES FROM THE PREVIOUS CLASSIFICATION

Choledochocele or Type III CC stands apart from the other counterparts due to a different mechanism of pathophysiology. It appears to be a misfit in the Biliary Tract malformation group due to its behaviour and presentation. It has the lowest incidence of malignancy and is associated with divisum pancreas rather than abnormal pancreato biliary malunion, unlike the rest of the Biliary Tract malformations[8,9].

In addition, Caroli's disease, in view of its familial association and link to hepatic fibrosis, is possibly a separate and different type from the rest of the biliary tract malformation and it may have to stand out as a different entity. Of note embryologically is its relation to ductal plate malformation which pursues another recognizably different pathway[10].

CURRENT UNDERSTANDING

The hallmark of CC, namely gross biliary ductal dilatation, can sometimes not be present but may have clinically significant pain abdomen, as identified in a unique subtype of CC, namely the 'forme fruste' type of CC. The presence of marginal dilatation or absent dilatation of the extrahepatic bile duct with malunion of the pancreato-biliary junction is referred to as the 'forme fruste' type of CC. The striking relief of symptoms (pain abdomen) post -operatively, after the bile duct is separated from the pancreatic duct during surgical reconstruction (excision of bile duct and roux en y hepatico-jejunostomy) has firmly established the association of ductal hypertension in this subtype[11]. A combination of long common channel with a minimal dilated common bile duct (< 10 mm) noted on an intra-op cholangiogram or Endoscopic Retrograde Cholangio Pancreatography helps in the identification of this condition[12].

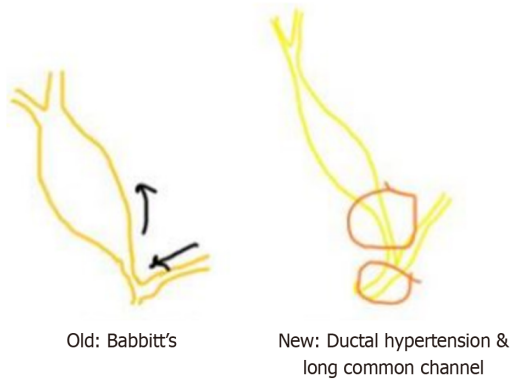

Figure 1 The traditional Babbitt's reflux hypothesis and the recent ductal hypertension hypothesis.

Hamada *et al*[13] considered the dilatation of bile duct by grouping them into two categories, one with biliary dilatation in combination with abnormal pancreato-biliary junction (congenital biliary dilatation or CC) and the other with only abnormal pancreato-biliary junction.

The elaboration of abnormal pancreato-biliary junction or pancreato-biliary malunion is out of scope as it deserves an in-depth and focussed review.

REVISIT CLASSIFICATION?

Todani's classification of CC dates back to 1977, which attempts to put together the various types as per the understanding of the causation of CC relevant at that point in time. Over a period of time, newer studies have changed the understanding of the etiology of CC, prompting a relook into the traditional classification. Not only the surgical treatment but also the malignancy risk is different among the CC types suggesting a newer regrouping logically agreeable to the etiology as well[14]. A simpler Kings' college classification which is a management-based classification has been proposed as an alternative[15,16]. But none are widely adopted in current clinical practice.

MORE FOR THE FUTURE-MOLECULAR MECHANISMS

Varied etiology owing to molecular mechanisms has been put forth recently. Analysis of the molecular characteristics has shown that the cystic and fusiform types of CC have different molecular mechanisms of pathogenesis[17]. A higher rate of harmful alleles in genes involved in soft tissue disorders and conditions related to tissue overgrowth may be linked to the formation of the stenotic distal bile duct, ultimately resulting in proximal dilatation and CC[18]. Further studies are required to characterise in detail the genetic polymorphisms involved in the pathogenesis of CC.

CONCLUSION

CC appears to be a misnomer and biliary tract malformation, the current terminology is apt and justifiably in line with the evolving etiopathology. In continuum, the same logic is applicable to the Alonso Lej classification of CC as well, which needs to give way to a system of modified sorting of the various types of Biliary Tract malformation.

FOOTNOTES

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MINIREVIEWS

Mast cell activation syndrome: An up-to-date review of literature

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Abstract

Mast cells are a subtype of white blood cells and are involved in the immune system. These cells contain many chemical substances called mediators, which are involved in the allergic response. The fact that mast cells play a role in many events that require urgent intervention, especially anaphylaxis, has led to a more detailed study of these cells. The diseases also caused by dysfunctions of mast cells have been examined in many circumstances. For instance, mast cell activation syndrome is known as an augmented number of cells due to decreased cell death, resulting in clinical symptoms affecting many systems. The main common symptoms include flushing, hypotension, urticaria, angioedema, headache, vomiting and diarrhea. Although the underlying mechanism is not yet clearly known, we aim to review the literature in a broad perspective and bring together the existing knowledge in the light of the literature due to the diversity of its involvement in the body and the fact that it is a little known syndrome.

Key Words: Mast cell; Mast cell activation syndrome; Tryptase; Histamine

Core Tip: Mast cell activation syndrome is a rare disease that is mostly diagnosed based on clinical symptoms. It is a disorder that should be considered with specific signs and symptoms of mast cell activation in individuals with skin, gastrointestinal, cardiovascular, respiratory, and neurological system involvement.

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INTRODUCTION

Mast cells have normal physiologic functions such as homeostasis, tissue repair, angiogenesis, and their role in the innate/acquired immune system[1]. Mast cell activation and liberation of mediators are needed for the maintenance of normal physiological processes[2]. However, there are also abnormal conditions, in which this process is not regulated and, like mast cell activation syndrome (MCAS), causes manifestations in different organ systems in the body. Therefore, MCAS is a disorder that should be considered and diagnosed clinically with specific signs and symptoms of activation in individuals with skin, gastrointestinal, cardiovascular, respiratory, and neurological system involvement[1,3,4].

MCAS is a rare disease that is mostly diagnosed based on clinical symptoms. Here, we summarize recent developments about this disease, which is rapidly increasing in the frequency of diagnosis today, in the light of current literature data. We also review the epidemiology, pathogenesis, and diagnosis of MCAS in a broader context and to examine the signs and symptoms that occur due to its clinical relationship with other systems.

HISTORICAL BACKGROUND

Mast cells were first described in 1863 as granular cells observed in a study using frog mesenteries and in 1877, following this examination, named the as yet unidentified cells 'mastzellen'. A few years later, the relationship of mast cells with a pathological condition was first understood when they were found scattered in this area while examining the lesions of a case with urticaria pigmentosa (UP). The certainty that mast cells were associated with any disease only emerged in 1949 discovered the existence of mast cells in many organs in the autopsy of a one-year-old child who passed away from cachexia[5]. This was the first report of systemic mastocytosis (SM). In 1988, Travis *et al*[6] designed a classification scheme for mastocytosis and this was accepted by the scientific world as the first scheme by the National Institutes of Health of United States in 1991.

In 2010, a conference was organized to elaborate on this topic to modernize the classification and diagnostic criteria for mast cell disorders, especially MCAS. Mastocytosis was already well known, but MCAS also required to be described and some criteria established. The classification into primary, secondary, and idiopathic MCAS was first proposed at this conference for diagnostic purposes[1]. Two years later, Valent *et al*[7] published an update of the criteria based on learning and observation of the disease. In 2016, the World Health Organization published a second updated classification of mastocytosis[7].

EPIDEMIOLOGY

Epidemiologic studies are not yet sufficient to verify the incidence and prevalence of both mastocytosis and MCAS. The incidence of mastocytosis is currently thought to be 1/10000[8,9]. It is equally distributed by sex and can occur at any age. Epidemiologic studies on MCAS have not yet been conducted sufficiently and the frequency of these syndromes is much more hard to guess[10]. The incidence and prevalence of cases with monoclonal MCAS (MMCAS) and idiopathic MCAS are not well known and there is insufficient data in the literature[11,12].

PATHOGENESIS AND MECHANISMS INVOLVED

An increase in cell number due to decreased apoptosis, an abnormal activation in response to microenvironmental triggers, rather than neoplasia of mast cells have a role in the development and classification of the syndrome. The disease can be categorized into three main groups: Primary, secondary, and idiopathic[13].

In MCAS, the pathological behavior of mast cells is not due to abnormal mast cell proliferation but to chronic abnormal constitutive and reactive activation[5]. This abnormal activation may occur as a result of a change in the activation threshold, abnormal expression of receptors and mediators inducing an allergic immune response, changes in the tissue environment affecting the expression and function of mediators, or mutations in the regulatory genes of the cells[14,15]. Although the genetic basis is not fully understood, mutations and alternative variants have been detected in the c-KIT

receptor (CD117+), which is responsible for the proliferation of mast cells in tissue *via* stem cell factor[12].

The best-defined and most focused physiopathologic role of mast cells is the allergic reaction caused by an abnormal response of mast cells to harmless antigens. Mastocytosis and MMCAS are both described as having clonal mutations in mast cells[16,17]. Although the pathogenic mechanisms of mastocytosis are comparatively well characterized, the mechanism of MMCAS has not been explained. Moreover, no mutations have been found in idiopathic MCAS and not enough is known about its pathogenesis[18,19].

PARAMETERS USED AS MCAS DIAGNOSTIC CRITERIA

The MCAS criteria were first recognized in 2012 and remain to be developed by an international consensus group. Three criteria are considered, all of which must be met for a diagnosis of MCAS to be made[20].

In the case of clinically episodic, recurrent, severe (mostly like anaphylaxis), and systemic (including at least two organ systems) typical MCAS symptoms; the criteria used in the diagnosis are following: (1) Serum tryptase level, one of the markers of MCAS in the laboratory, exceeding 120% + 2 ng/mL above the serum baseline value of the individual; and (2) clinical reaction to anti-mediator drugs that inactivate mast cell mediators or preclude their release, and finally iii) primary (clonal) and/or secondary diseases of mast cell activation ruled out[4].

The standard laboratory diagnostic marker for MCAS is serum tryptase, with a normal serum level defined to be between 0 and 11.4 ng/mL in adults. Studies have shown that blood samples should be taken within 1 to 4 h of the beginning of symptoms and that basal levels should be evaluated in advance during a symptom-free period of at least 24-48 h after complete recovery. However, some studies have suggested that normal tryptase levels do not diagnostically rule out MCAS[1,6,21].

In addition, other mediators such as histamine, prostaglandin D2, chromogranin-A, leukotriene E4, and urinary metabolites of histamine and 11-beta-prostaglandin are not well known about the increased levels required for the diagnosis of MCAS. These mediators are also thought to be less specific for the diagnosis of MCAS[6].

Biologic agents used therapeutically, such as antihistamines, leukotriene modifiers, mast cell stabilizers, cyclooxygenase inhibitors, or omalizumab, have been identified as supporting the diagnosis as a recognized reaction to drugs that act specifically on MCAS[19,22].

MCAS CLASSIFICATION

Clonal mast cell disorders are considered primary MCAS if they are due to the existence of c-KIT mutations (generally involving the D816V mutation or expression of CD25, CD2, or CD30 on mast cells). If mast cell activation is owing to an allergic or other hypersensitivity disorder, then it is considered secondary MCAS (non-clonal)[12,23]. In addition, if there is no clonality and no other specific reason can be recognized, then MCAS is accepted as idiopathic (Figure 1). Combined types of MCAS have also been defined in which cases have both primary and secondary MCAS features and are categorized as mixed MCAS[24].

CLINICAL SYMPTOMS AND SIGNS IN DIFFERENT ORGANS AND SYSTEMS IN MCAS

This syndrome involves different systems and organs in the body, resulting in different clinical symptoms and signs (Figure 2)[3]. Common symptoms typically include symptoms suggestive of allergies such as flushing that may increase, decrease, and travel throughout the body, fatigue, cognitive dysfunction, irritation of the eyes, nose, mouth, and throat, lymph node inflammation, nausea, reflux, headache, dyspnea, palpitations, abdominal pain, diarrhea/constipation, anxiety, and mood disorders. In addition, dermatographism, fibromyalgia-type pain, joint hypermobility, benign growth anomalies, interstitial cystitis, menorrhagia, dysmenorrhea, vulvovaginitis, sensory neuropathy, dysautonomia, and various metabolic endocrinologic abnormalities can be seen affecting many organs and systems. Here, different system involvement will be discussed below one by one.

Cardiovascular system involvement

Common symptoms of MCAS include hypotension, tachycardia, syncope or near syncope, blood pressure changes, shock, and chest pain. In addition, postural orthostatic tachycardia syndrome (POTS) has some similar clinical symptoms as in MCAS, although the etiology is not fully known, suggesting that there may be a relationship between them and they may have a common pathogenesis[25]. Although the pathogenesis is not yet fully known, several pathophysiologic processes have been suggested to be involved in this syndrome. The first likely pathophysiologic mechanism is incomplete sympathetic neuropathy[26]. Some cases have been observed to have incomplete sympathetic denervation and irregular epinephrine reactions in the lower extremities[27]. As a result, inadequate vasoconstriction is thought to increase heart rate with sympathetic activation in response to congestion in the legs. Clinically, orthostatic intolerance symptoms should be present in patients with POTS, which mostly affects women of reproductive age[25].

POTS is characterized by symptoms of palpitations, chest pain, heart discomfort, headache, blurred vision, and dizziness. POTS, which has been described by many researchers with different names until today, is now characterized as 'orthostatic intolerance syndrome'. The pathognomonic feature of orthostatic intolerance is the manifestations of

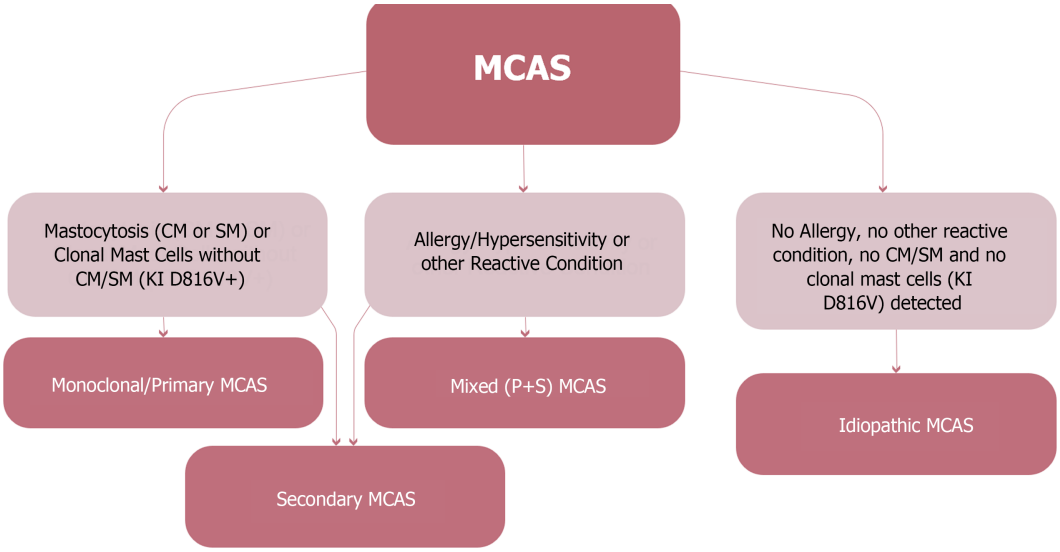

Figure 1 Classification of mast cell activation syndrome. MCAS: Mast cell activation syndrome; SM: Systemic mastocytosis; CM: Cutaneous mastocytosis.

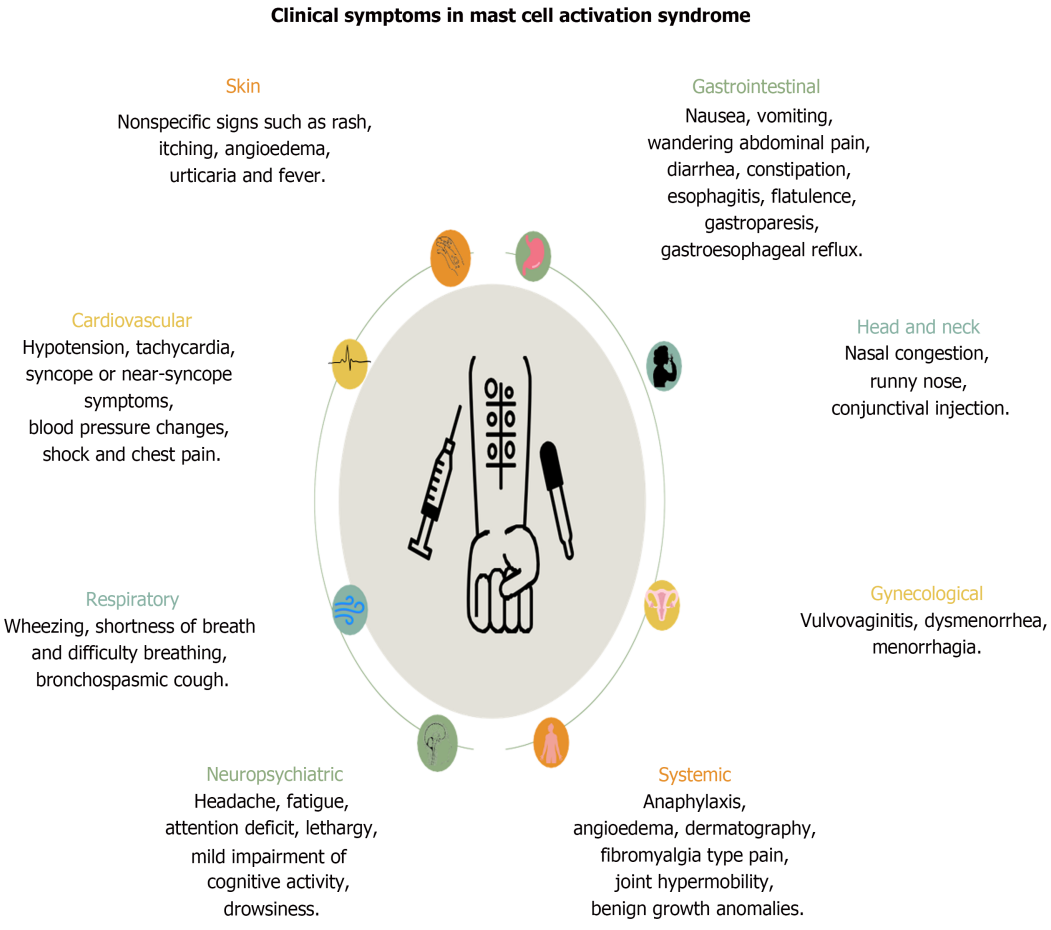

Figure 2 Clinical manifestations in different systems in patients with mast cell activation syndrome.

symptoms while upright and relief when in supine positions[25].

The first study targeting to investigate a relationship between MCAS and POTS was conducted in by Shibao *et al*[26]. Since flushing is a symptom in both POTS and MCAS, it is thought that MCAS may contribute to the pathogenesis of POTS. Since studies on the relationship between these two are very limited, more data are needed to better characterize the mechanisms. As a result, it is believed that more research should be done on patients who meet the criteria for both diseases.

Dermatologic involvement

Cases with MCAS often manifest flushing caused by the vasodilating effects of histamine and other mediators[28]. Flushing may be caused by exercise, alcohol, temperature, and emotional changes. Angioedema may occur especially on the lips and tongue. The flushing seen in mast cell disorders is episodic, lasts longer, and it is usually not accompanied by sweating[29]. Among other clinical findings, nonspecific urticaria, pruritus, *etc.* may occur[30].

Respiratory system involvement

Upper and lower respiratory tract symptoms are common in patients with MCAS. These symptoms include nasal congestion, nasal itching, shortness of breath, wheezing, bronchoconstriction, bronchospastic cough, throat swelling, and rhinorrhea[1,23]. Angioedema of the upper respiratory tract may also be seen, but it is rare[4].

Gastrointestinal tract involvement

General effects of MCAS on the gastrointestinal tract include nausea, vomiting, wandering abdominal pain, abdominal tenderness, gastroesophageal reflux, dysphagia, atypical chest pain, diarrhea, constipation, esophagitis, intestinal cramps, bloating, malabsorption, mouth sores, gastroparesis, and angioedema[31].

Irritable bowel syndrome (IBS) may be observed in some patients in association with MCAS. IBS is a common gastrointestinal disorder affecting the quality of life in a large quantity of the population. It is characterized by abdominal pain, alternating constipation, and diarrhea. It has been noted that impaired intestinal barrier function in IBS is caused by mast cell activation due to stress response. One of these stress responses is certain components in the food ingested, such as plant-derived substances. These components regulate mast cell activation[32]. Mast cell numbers have been shown to increase in the terminal ileum, jejunum, and colon of patients with IBS. At the same time, biopsies performed on atopic IBS patients showed a higher proportion of mast cells compared to non-atopic patients[33]. Mast cell numbers were found to be directly proportional to the severity of symptoms in IBS and it is thought that mast cell mediators may cause symptoms. The rise in the count of mast cells in the colon is one of the most consistent changes observed in IBS[34]. In addition, the severity of pain observed in patients with IBS is associated with the number of mast cells located close to enteric nerves. The released mediators activate enteric nerves[32,35].

Small intestinal bacterial overgrowth (SIBO) is common in MCAS. SIBO is a condition in which colon bacteria overgrow in the small intestine. It occurs as a result of anatomical abnormalities as well as motility, and metabolic, systemic, and immune system disorders. In this condition, intestinal symptoms include nausea, anorexia, and bloating due to malabsorption and impaired small intestinal motility. These clinical symptoms are probably caused by invasive strains. Studies have shown the presence of 3 main organisms, *E. coli*, *Klebsiella*, and *Aeromonas species*. The diagnosis of SIBO is made when the bacterial count exceeds 10^3 organisms /mL in a patient with clinical symptoms[36]. The relationship between SIBO and MCAS is as follows; SIBO causes activation of mast cells and increase in T lymphocytes. T lymphocytes in turn secrete microparticles that again activate mast cells. Activated mast cells and T lymphocytes release cytokines that increase intestinal permeability[37]. This leads to a vicious cycle in which intestinal permeability is constantly impaired and inflammation is constantly increased[36,38].

In mice, abdominal surgeries such as colorectal surgery as well as laparoscopic procedures are known to increase the release of mast cell mediators in peritoneal fluid, extracellular matrix thickness and the risk of intra-abdominal adhesions. This suggests that it may increase the likelihood of intra-abdominal adhesions such as postoperative ileus in humans[38].

Bone-joint system involvement

Ehler-Danlos syndrome (EDS) is frequently associated with MCAS. Patients are increasingly being admitted to hospitals with EDS and MCAS. EDS is primarily presented by skin hyperextensibility, joint hypermobility, and tissue fragility. It is caused by a group of genetic disorders. Extensive gastrointestinal tract involvement also occurs in this syndrome. Esophagitis, gastroesophageal reflux, abdominal pain, and IBS are common gastrointestinal symptoms[35]. Patients with EDS have been reported to have increased gastrointestinal symptoms seen in MCAS. It is also thought that mast cell activation may be the cause of other symptoms seen in patients with EDS[18]. It is recommended that patients with EDS should also be screened for MCAS during evaluation. In addition, it is thought that IL-17A, which is highly produced as a result of mast cell activation disorder in MCAS, causes focal bone loss[27]. Therefore, it has been reported that osteoporosis may occur at an early age in patients with MCAS and EDS[26].

Allergic symptoms and disorders

Anaphylaxis is a severe systemic hypersensitivity reaction and increased levels of mast cell mediators such as tryptase and histamine have been detected during attacks[39]. Mast cells seem to be the primary cells that trigger anaphylaxis in humans[40]. In addition, activation of basophils results in histamine and mediators such as LTC-4 may be secreted and indirectly contribute to the development of symptoms. To date, no specific biomarker has been identified to follow suspected patients, classify the severity of reactions, or manage the disease. The underlying mechanisms leading to idiopathic anaphylaxis are not fully understood, but several theories have been proposed to explain the pathogenesis[41]. Researchers have observed that peripheral blood from patients with idiopathic anaphylaxis has higher mast cell counts in culture compared to healthy controls[42].

In another study, the relationship between MCAS and anaphylaxis was examined through some molecules *via* the ERK 1/2 pathway. Extracellular signal-regulating kinases (ERK 1/2) contribute to allergic responses by regulating degranulation, eicosanoid creation, and cytokine expression by mast cells, but the mechanisms emphasizing their positive effects on FcεRI-dependent signaling have not been fully elucidated. It has recently been demonstrated that mast cell activation and anaphylaxis are negatively regulated by AMP activated protein kinase (AMPK a type of serine-threonine kinase that

is a central regulator of energetic metabolism). However, little was known about the association between ERK1/2-mediated positive and AMPK-mediated negative regulation of FcεRI signaling in mast cells. In one study, ERK1/2 activated FcεRI signaling in mast cells by abolishing the AMPK-dependent negative regulatory axis[43].

In conclusion, the possible relationship between anaphylaxis and MCAS has been examined through many theories and studies, but more clinical trials are needed to say anything definitive[29].

Neuropsychiatric symptoms and disorders

Headache, fatigue, weakness, lethargy, lack of attention, feelings of exhaustion, lethargy, and mild impairment of cognitive activity are common symptoms in cases with primary and secondary mast cell activation (*i.e.* with mastocytosis and also with allergic disorders). These complaints might be owing to the psychosomatic effects of mast cell-mediated mediators, drugs, or (in adults) having a chronic disease[44]. Subjective neuropsychiatric symptoms alone should not be taken into consideration for mast cell disorders. Fairly, individual complaints must be along with signs and complaints affecting other organ systems before mast cell disorders can be thought of.

According to various studies and investigations, mast cells are considered to make a significant contribution to the pathophysiology of migraine[45]. Mast cells are found in the meninges and are thought to be included in the pathophysiology of migraine through events such as sequential neuropeptide release and vasodilation leading to mast cell degranulation[46,47]. Mast cells discharge hundreds of various mediators such as histamine, tryptase, and leukotrienes, and degranulation of meningeal mast cells contributes to the activation of the trigeminal vascular afferent pathway[48]. This is assumed to be one of the underlying mechanisms of migraine and pain. A recent study has examined the link between the parasympathetic nervous system, mast cells, and migraine, with research suggesting that endogenous acetylcholine activates meningeal mast cells and thus contributes to migraine pathophysiology[49]. However, although the data obtained are enlightening, further studies are required to explain the complex interaction between the autonomic nervous system, mast cells, and connective tissues of the meninges, cerebral vasculature, and other structures significant in the pathophysiology of migraine headaches[50].

MASTOCYTOSIS-MCAS RELATIONSHIP

Mastocytosis is divided into two categories: Systemic (SM) and cutaneous mastocytosis (CM). In SM, abnormally proliferating mast cells affect the skin, bone marrow, and other organs, causing various symptoms. Common symptoms include itching, abdominal cramps, and tachycardia[16]. CM is more common in young children and usually resolves spontaneously in puberty. In CM, mast cells gather in the skin but not in other organs. However, systemic symptoms may still be observed. This is because mediators secreted from activated mast cells accumulated in the skin are released into the circulation[29].

The symptoms of SM and MCAS are similar. Both conditions have symptoms of mast cell activation, such as facial flushing, abdominal cramps, and hypotension due to degranulation. Mastocytosis is mast cell proliferation with infiltration of dermis (CM) or other tissues and organs (SM). MCAS is increased and inappropriate activation of mast cells without clonal proliferation. The differences between these two conditions are as follows: (1) Patients with SM have an elevated baseline serum tryptase level, typically >20 ng/mL. In cases with MCAS, baseline serum tryptase levels are normal or slightly elevated; (2) multifocal mast cell aggregates are observed. It is characteristic to observe these aggregates in the bone marrow of cases with SM, but not in MCAS; and (3) it is characteristic to usually observe UP called maculopapular CM (MPCM) in patients with CM. However, UP/MPCM-like lesions are not observed in MCAS[17].

THE RELATIONSHIP BETWEEN PEOPLE WITH LONG-TERM CORONAVIRUS DISEASE 2019 AND MCAS

Long-term coronavirus disease 2019 (COVID-19) is an outcome of immune dysregulation. T and B cell deficiency, hyperactivity of innate immune cells, and an increase in proinflammatory cytokines are observed[7]. This dysfunction leads to a constant inflammatory reaction, pathogen reactivation, endothelial damage, host-microbiome dysfunction, and autoimmunity. Risk factors for the development of long-term COVID-19 include female gender, type 2 diabetes, autoimmune diseases, connective tissue, and allergic disorders[51,52].

People with long-term COVID-19 disease have major cardiac, neuropsychiatric, and pulmonary complaints. Multisystem disorders such as myocardial inflammation, POTS, dystonia, and myalgic encephalomyelitis/chronic fatigue syndrome can develop. Immune system effects include recurrent infection, autoimmunity, urticaria, allergic rhinitis, and asthma. MCAS is thought to be the possible mechanism underlying these effects[26,53].

Mast cells are the key producers of the inflammatory cytokines of COVID-19. A persistent inflammatory state with prolonged COVID-19 causes abnormal mast cell activation. One of the reasons for this is the maturation of mast cells in the pulmonary perivascular space[54]. Another cause is the discharge of substance P from immune cells due to severe acute respiratory syndrome coronavirus 2 infection. The secretion of this substance increases the stimulation of the G-protein X2 receptor and predisposes to MCAS formation[55,56].

It is thought that symptomatic improvement will be achieved with the treatment of MCAS in long-term COVID-19 cases. It is thought that stabilization of mast cells and reduction in related symptoms will be achieved by histamine blockade[57,58].

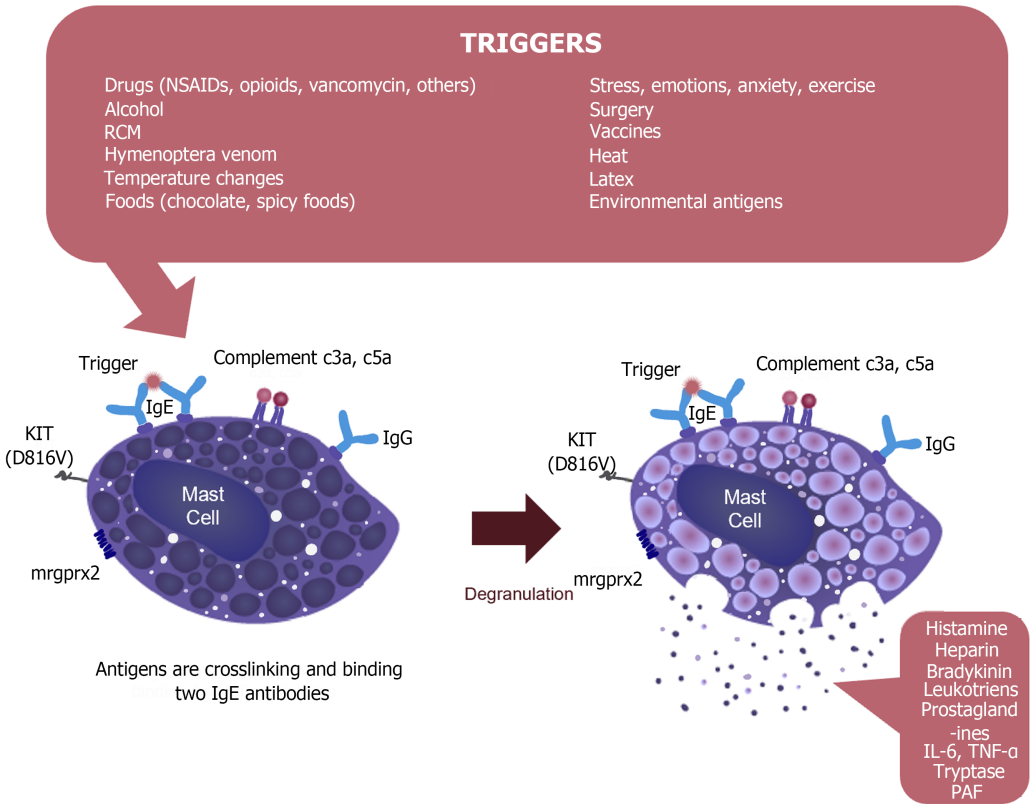

Figure 3 The mechanism of mast cell activation and release of mast cell mediators.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of MCAS includes numerous diseases and nonspecific signs and symptoms: Infectious diseases, gastrointestinal (IBS, gastrinoma, VIPoma, eosinophilic gastroenteritis or esophagitis, inflammatory bowel disease, food intoxication, *etc.*), cardiovascular (endocarditis or endomyocarditis, pulmonary embolism, aortic stenosis with syncope, myocardial infarction), endocrinological (medullary thyroid carcinoma, pheochromocytoma, carcinoid), neuropsychiatric disorders (anxiety/panic attacks, vasovagal syncope), urticaria and angioedema types, drug-induced itching/rash, vasculitis, and atopic dermatitis[59]. In addition, the differential diagnosis should include two disorders in which there is chronic systemic elevation of mast cell pre-performed mediators in granules and depends on the function of the granules without over activation of mast cells, namely histamine intolerance and hereditary alpha tryptasemia.

A thorough physical examination, together with a thorough history and laboratory evaluation of specific biomarkers, can help differentiate these disorders from MCAS[60].

TREATMENT PLANS OF MCAS

First, it is of paramount importance to explain to patients and their parents to elude any agents or interactions that could trigger anaphylactic or allergic reactions (Figure 3)[18]. MCAS cases should also be counseled to take prophylactic anti-mediator therapy (*e.g.* histamine receptor blockers) throughout their lives and to carry at least two self-administered epinephrine autoinjectors after being instructed on how to utilize these injectors in case of illness[41].

In cases with reaction-inducing (IgE-dependent) anaphylaxis and thus secondary MCAS, immunotherapy is generally suggested because its potential side effects are better known. The frequency of life-threatening MCAS events can be significantly reduced after inactivation of neoplastic mast cells. Aspirin has formerly been suggested as a potential therapeutic tool for the therapy of anaphylaxis in patients with SM[61], but it has been observed that the doses of aspirin required to prevent MCAS must be high and are not tolerated in many cases. Other medications contain mast cell regulatory agents and corticosteroids[7,62].

In addition, there is also an emerging class of drugs targeting mast cells, namely broad-acting tyrosine kinase inhibitors *e.g.* midostaurin or avapritinib. Some of these medications, such as midostaurin, not only inhibit mast cell proliferation but also block IgE-dependent allergic stimulation of mast cells. Hence, midostaurin might be an encouraging treatment to subdue the effects of MCAS, especially in cases with primary MCAS[19,63].

Another proposed interpretation of MCAS treatment is the use of specific IgE (*e.g.*, omalizumab) in secondary MCAS cases with underlying IgE-dependent allergy. Furthermore, in cases with mixed MCAS (primary + secondary MCAS), multiple specific treatment modalities may be necessary after an individualized medicine approach[18]. For instance, cases with high levels of neoplastic mast cells, advanced SM, and severe IgE-dependent allergy may require a drug

directed against the c-KIT mutation or combined treatment with cladribine and omalizumab to control MCAS events[18, 62].

CONCLUSION

Although the mast cell is an obligatory cell for life, issues related to its activation may also be associated with disorders such as MCAS that may present with a wide variety of clinical findings and complaints. Awareness of this disorder should be increased so that MCAS can be easily differentiated from other diseases and treatment plans should be well known.

FOOTNOTES

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ORIGINAL ARTICLE

Case Control Study

Diagnostic significance of complete blood cell count and hemogram-derived markers for neonatal sepsis at Southwest Public Hospitals, Ethiopia

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Abstract

BACKGROUND

Neonatal sepsis is defined as an infection-related condition characterized by signs and symptoms of bacteremia within the first month of life. It is the leading cause of mortality and morbidity among newborns. While several studies have been conducted in other parts of world to assess the usefulness of complete blood count parameters and hemogram-derived markers as early screening tools for neonatal sepsis, the associations between sepsis and its complications with these blood parameters are still being investigated in our setting and are not yet part of routine practice.

AIM

To evaluate the diagnostic significance of complete blood cell count hemogram-derived novel markers for neonatal sepsis among neonates attending public hospitals in the southwest region of Oromia, Ethiopia, through a case control study.

METHODS

A case control study was conducted from October 2021 to October 2023 Socio-demographic, clinical history, and laboratory test results data were collected using structured questionnaires. The collected data were entered into Epi-data 3.1 version and exported to SPSS-25 for analysis. Chi-square, independent sample *t*-test, and receiver operator characteristics curve of curve were used for analysis. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

In this study, significant increases were observed in the following values in the case group compared to the control group: In white blood cell (WBC) count, neutrophils, monocyte, mean platelet volume (MPV), neutrophils to lymphocyte ratio, monocyte to lymphocyte ratio (MLR), red blood cell width to platelet count ratio (RPR), red blood width coefficient variation, MPV to RPR, and platelet to lymphocyte ratio. Regarding MLR, a cut-off value of ≥ 0.26 was found, with a sensitivity of 68%, a specificity of 95%, a positive predictive value (PPV) of 93.2%, and a negative predictive value (NPV) of 74.8%. The area under the curve (AUC) was 0.828 ($P < 0.001$). For WBC, a cut-off value of ≥ 11.42 was identified, with a sensitivity of 55%, a specificity of 89%, a PPV of 83.3%, and a NPV of 66.4%. The AUC was 0.81 ($P < 0.001$). Neutrophils had a sensitivity of 67%, a specificity of 81%, a PPV of 77.9%, and a NPV of 71.1%. The AUC was 0.801, with a cut-off value of ≥ 6.76 ($P = 0.001$). These results indicate that they were excellent predictors of neonatal sepsis diagnosis.

CONCLUSION

The findings of our study suggest that certain hematological parameters and hemogram-derived markers may have a potential role in the diagnosis of neonatal sepsis.

Key Words: Complete blood count; Hemogram-derived marker; Neonate; Sepsis; Ethiopia

Core Tip: It is try to show the importance of complete blood cell count and hemogram-derived markers for the neonatal sepsis, which are simple and accessible relative culture especially in developing countries like Ethiopia.

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INTRODUCTION

A clinical condition known as neonatal sepsis is defined by signs and symptoms of infection and the presence of bacteremia within the first month of life[1]. Neonatal sepsis is the leading cause of mortality and morbidity in newborns [1,2]. It is characterized by the body's systemic inflammatory response to infection. When this occurs within the first 28 d of life, it is referred to as "neonatal sepsis"[3]. Evaluating a baby for suspected infection in neonatal sepsis nurseries is a challenging clinical task[4]. There are two types of neonatal sepsis. Type 1 is known as early-onset sepsis (EOS), which occurs within 0-7 d. The risk factors for EOS include trans-placental, ascending, or intrapartum transmission during the perinatal period before or during birth, up to postnatal day 3[5,6].

Blood culture is the current gold standard for diagnosing newborn sepsis[7]. Prematurity problems, infection, postnatal fluid changes, late umbilical cord clamping, sampling sites, method of delivery, and sample collection timing are all typical factors that alter the neonate's hematological profile. This profile distortion may also be caused by hypothermia, hypoxia, and other prematurity-related issues[8]. Preeclampsia and intrauterine growth restriction, together with prolonged hypoxia, may increase the formation of reticulocytes while decreasing the number and total mass of megakaryocytes and blunting platelet function[9,10].

According to studies from Asia and Africa, around five million neonates die each year, with 1.6 million (20%) dying as a result of sepsis[11]. In wealthy countries, the prevalence of neonatal sepsis ranged from 1 to 5 per 1000 live births[12]. In developing countries, however, the incidence of newborn sepsis has increased from 1.8 to 18 per 1000 live births, with a death rate of 12-68 per 1000 live births[13]. Sepsis also leads to hospitalization and death. The global hospitalization rate for sepsis is predicted to be 19.4 million people, with 14.1 million only surviving hospitalization[14,15]. According to studies, neonate sepsis is a third-stage illness that causes neonatal mortality with a 1%-20% mortality rate after a premature delivery and neonatal encephalopathy (perinatal asphyxia and trauma)[16].

Current diagnostic approaches for neonatal sepsis are poor, resulting in an inability to lead clinical treatment on time, compromising its therapeutic impact. According to a report, the global newborn sepsis death rate ranged from 1.0%-5.0% [17]. According to a 2021 estimate, there were around 2797879 live births and 29608 sepsis cases in 14 middle-income nations. In the total time frame, the random-effects estimation for newborn sepsis incidence was 2824 cases per 100000 live births, with an estimated 17.6% dying[18]. In Ethiopia, the disease is still a major cause of morbidity and mortality[19, 20]. The most prevalent diagnosis in the neonatal intensive care unit (NICU) is sepsis, and antibiotics are the most commonly utilized medications in the NICU. The prospects are bleak if antibiotic treatment is postponed until overt clinical symptoms appear. However, overuse of antibiotics can result in a variety of negative effects, including antibiotic resistance. Resistant infections are now responsible for three out of every ten newborn sepsis deaths[21].

Currently, one of the most difficult difficulties confronting clinicians is the early detection of neonatal sepsis[22-24]. If the diagnosis of neonatal sepsis is delayed and treatment is ineffective, it can lead to systemic problems and a high fatality risk. Although blood culture is considered the gold standard diagnostic technique for newborn sepsis, it has several limitations. These are inaccessible in the majority of impoverished nations, have technological issues, and take more than three days to see at least the first preliminary result, with a positivity yield of 30%-70%[25,26]. As a result, the left percent of neonates with sepsis could not be diagnosed by blood culture; the diagnosis of neonatal sepsis is based on clinical assessment, and management is also based on empirical treatment protocol, which usually results in unnecessary hospitalization, increased irrational use of antibiotics, and additional costs for the family[25,26]. Although platelet, lymphocyte, and neutrophil counts are analyzed in a single mode, they may serve as clinical markers of underlying infections such as sepsis and associated immune dysfunctions[27-29]. In recent years, the platelet to lymphocyte ratio (PLR), neutrophil to lymphocyte ratio (NLR), immature to total neutrophil ratio, and immature to mature neutrophil ratio have been identified as prospective markers of systemic inflammation and infectious illness prognosis[27-30].

Because newborns' defense barrier function and immune system development are still insufficient, and the condition of neonatal sepsis advances rapidly, early identification and accurate treatment are critical to lowering death. The NLR and PLR markers derived from blood analysis are now gaining attention in the study of inflammation-related disorders. Furthermore, some research suggests that the NLR and PLR, can be employed as prognostic indicators for cancer and cardiovascular disease[31,32]. Adult sepsis studies revealed that NLR might be employed as a biomarker for assessing systemic inflammation[33]. According to one study, PLR is a good measure for assessing patients' inflammatory response and disease activity[34].

Researchers and previous understandings show a progressive increase in newborn sepsis mortality, morbidity, and economic burden around the world, specifically in developing nations. Though not a replacement for blood culture, hematological parameters and hemogram-derived markers have been proposed as indicators of neonatal sepsis. Though several studies have been conducted to test the usefulness of hematological parameters and hemogram-derived novel markers in neonatal sepsis diagnosis, there is not yet used very commonly in routine practice and results vary extensively among studies. Even though assessment and use of the hemogram-derived markers have infinite considerations in the forecast of diagnosis of neonatal sepsis patients in a simple, rapid, and inexpensive manner. To the best of our knowledge, no published article is in our perspective. Therefore, the goal of this study was to assess complete blood count (CBC) parameters and hemogram-derived novel markers convenience for neonatal sepsis diagnosis among neonates admitted at the NICU of Southwest Shoa Public Hospitals, Ethiopia from October 2021 to October 2023.

MATERIALS AND METHODS

Study design, period, and area

A retrospective case control study was conducted at Southwest Shoa Public from October 2021 to October 2023. Tulu Bolo General Hospital and Waliso General Hospitals are the hospitals found in Oromia Regional State and are found in Southwest Shoa Zone central Ethiopia, 47 km apart, southwest of Addis Ababa.

From both hospitals, Waliso referral hospital is located in Woliso town which is the capital city of Southwest Shoa zone at 114 km in the direction of the Southwest from Addis Ababa. It has a latitude and longitude of 8 32′N 37 58′E with an elevation of 2063 m above sea level. Currently, this hospital serves around 1.1 million people by having many departments such as a clinic for patients with tuberculosis, an rapid antiretroviral therapy (ART) clinic, an adult outpatients department, NICU, under-five outpatient departments, antenatal care (ANC), postnatal care, *etc*.

Tulu Bolo General Hospital is located in Southwest Shoa 90 km from Addis Ababa. Tulu Bolo has a latitude and longitude of 8 40′N 38 13′E with an elevation of 2193 m or 7195 feet above sea level. Also offers chronic care, NICU, emergency services, ART services, surgical, dental, medical services, ophthalmology, pediatrics, gynecology and obstetrics, radiology, physiotherapy, pathology services, pharmacy and laboratory services, and others. Both hospitals are currently giving followup services for neonates that include ICU services, physical examinations, and laboratory services such as determination of CBC parameters, serological, and chemistry. This study obtained needed information in those hospitals' central laboratories providing hematology and immunohematology, parasitology, microbiology, clinical chemistry, and serology services.

Study population, inclusion, and exclusion criteria

The study population of this study consisted of neonates aged 0-28 d. The cases group who showed clinical signs and symptoms of sepsis upon admission to NICU, or who developed sepsis during their hospitalization within the same age interval. The control group consisted of neonates within the same age range who did not show any sign of sepsis. Both groups met the inclusion criteria during the study period. The case group was comprised of neonates aged 0-28 d who were diagnosed with sepsis based on the systematic inflammatory response syndrome (SIRS) criteria. These criteria included the occurrence of at least two of the following: A fever greater than 38 °C or less than 36 °C, a heart rate greater than 90 beats per minute, a respiratory rate greater than 20 breaths per minute, a partial pressure of CO_2 less than 32 mmHg, a white blood cell (WBC) count greater than 12000 or less than 40000 per liter. Additionally, these neonates had stayed in the hospital's ICU for more than 24 h.

The control group consisted of neonates aged 0-28 d who were seen in the out-patient and in-patient departments and did not meet the SIRS criteria in their medical records. These neonates had normal total leukocyte counts, and had not been diagnosed with any infectious disease.

Both the cases and control groups had CBCs taken up to 24 h before admission to the ICU, and this data was archived in the hospital's database system. All sociodemographic and clinical data were recorded in the computerized system of the hospitals during the study period. To minimize the influence of confounding factors, neonates in both groups with a history of hematological disease, those receiving chemotherapy, glucocorticoids, or antibiotics were excluded.

Additionally, patients with inaccessible or incomplete file information, patients with genetic disease, metabolic disease, congenital heart disease, perinatal asphyxia, and neonates with congenital and chromosomal anomalies were also excluded.

Sample size and sampling technique

The figure shows the study participant sampling procedure and processes (Figure 1).

Data collection procedure

Patient information, including age, gender, clinical history, and vital signs (such as body temperature, heart rate, respiratory rate, and systolic and diastolic arterial pressure) was obtained from the medical record department of Southwest Public Hospitals. The cases consisted of patients who were diagnosed with sepsis based on clinical features and laboratory examination findings by a specialist doctor. The control group consisted of healthy neonates who underwent a general check-up, and had no diagnosis of infectious diseases, with normal leukocyte counts on laboratory examinations.

All hematological parameter values were obtained from archived data on a hematological analyzer. The values for NLR, monocyte to lymphocyte ratio (MLR), red blood cell (RBC) width to platelet count ratio (RPR), mean platelet volume (MPV) to platelet count ratio (MPVPCR), and PLR were calculated by the lymphocyte count, dividing the red blood distribution width by the platelet count, calculating the lymphocyte to monocyte count ratio (LMR), dividing the platelet count by the lymphocyte count, calculating the MPV to platelet count, and calculating the MLR count.

Data quality management

To ensure data quality, questionnaires in English were translated into the local language and then retranslated back into English to ensure accuracy and consistency. Two data collectors (two clinical nurses) received a half-day training session on study objectives, data collection procedures, and the importance of maintaining confidentiality to reduce technical and observation bias.

To ensure the quality of the socio-demographic and clinical data, daily checks were conducted by on-site supervisors to ensure completeness and consistency. Codes were used to protect the confidentiality of participants' test results, and all records were stored in a secure, and inaccessible location. Feedback and corrections were provided as necessary throughout the data collection process.

Statistical data analysis and interpretations

Collected data was checked for completeness and consistency, then entered into Epi-Data version 3.1 (Epi-Data Association, Denmark), and analyzed using Statistical Package for Social Sciences (SPSS) software version 25 (inclusion body myositis SPSS Statistics, United States). Histograms, Kolmogorov-Smirnov, and Shapiro tests were used to check the normality of the data distribution. Results for categorical variables were presented as frequency and percentage. Statistical differences for these variables were determined using the chi-square test.

Since continuous parameters followed a normal distribution in the goodness-of-fit model test, the independent sample *t*-test was used. Data was reported as mean ± standard deviation. Receiver operating characteristic (ROC) curves were constructed to determine the sensitivity, specificity, cut-off value, area under the curve (AUC), positive predictive value (PPV), and negative predictive value (NPV) of hematogram-derived markers in distinguishing neonates with sepsis from neonates without sepsis. A P-value < 0.05 was considered statistically significant.

RESULTS

Socio-demographic and clinical characteristics of the study participants

Sociodemographic and clinical characteristics data showed that there were no significant differences in age, sex, birth weight, ANC visit, history of diarrhea, respiratory distress, reddish orogastric tube, cardiovascular disturbances, hypoglycemia, abdominal distention, and place of delivery between neonates diagnosed with sepsis and those without sepsis (P > 0.05). However, there were significant differences in the two groups in terms of the residence of mothers, blood pressure, body temperature, gestational age, mode of delivery, history of jaundice, seizure, chorioamnionitis, neonatal reflex, premature membrane rupture, and prolonged premature rupture of membrane (P < 0.05) (Table 1).

High blood pressure, high body temperature, a history of jaundice, seizures, chorioamnionitis, abnormal neonatal reflex, premature membrane rupture, and prolonged premature rupture of the membrane are clinical factors that may be induced in neonates due to sepsis. Some of these factors are directly linked to the alteration of CBC values and hemogram-derived markers, while others are indirectly linked to the alteration of CBC values and hemogram-derived markers.

Table 1 Socio-demographic and clinical characteristics of the study participants, *n* (%)

Variables	Categories	Suspected neonatal sepsis	Control group	*P* value
Age in days (mean ± SD)		11.48 + 8.15	11.36 + 8.58	0.834
Sex	Male	206 (79.2)	162 (77.1)	0.585
	Female	54 (20.8)	48 (22.9)	
Residence of mothers	Urban	143 (55)	95 (45.2)	0.035
	Rural	117 (45)	115 (54.8)	
Birth weight in grams	< 1500 grams	27 (10.4)	21 (10)	0.979
	1500-2500 grams	109 (41.9)	87 (41.4)	
	> 2500 grams	124 (47.7)	102 (48.6)	
Blood pressure in mmHg	Systolic	137.57 + 10.86	148.87 + 11.077	< 0.001
	Diastolic	96.79 + 9.29	105.94 + 7.672	
Body temperature in ^{0}C	Hypothermia	29 (11.2)	17 (8.1)	< 0.001
	Fever	210 (80.8)	112 (53.3)	
	Normal	21 (8.1)	80 (38.1)	
ANC visit	< 4 visit	108 (41.5)	82 (39)	0.584
	> 4 visit	152 (58.5)	128 (61)	
Gestational age	28-32 wk	108 (41.5)	0 (0)	< 0.001
	33-36 wk	124 (47.7)	61 (29)	
	37-41 wk	28 (10.8)	149 (71)	
Delivery place	Home	38 (14.6)	26 (12.4)	0.483
	Hospital/health facilities	222 (85.4)	184 (87.6)	
Mode of delivery	Spontaneous vaginal delivery	8 (3.1)	162 (77.1)	< 0.001
	Cesarean section	146 (56.2)	17 (8.1)	
	Forceps extraction	106 (40.8)	31 (14.8)	
Respiratory distress	No	104 (40)	74 (35.2)	0.29
	Yes	156 (60)	136 (64.8)	
Reddish orogastric tube	No	106 (40.8)	74 (35.2)	0.22
	Yes	154 (59.2)	136 (64.8)	
Jaundice	No	99 (38.1)	100 (47.6)	0.037
	Yes	161 (61.9)	110 (52.4)	
Seizure	No	107 (41.2)	109 (51.9)	0.02
	Yes	153 (58.8)	101 (48.1)	
Diarrhea	No	101 (38.8)	82 (39)	0.96
	Yes	159 (61.2)	128 (61)	
Cardiovascular disturbances	No	100 (38.5)	74 (35.2)	0.472
	Yes	160 (61.5)	136 (64.8)	
Hypoglycemia Abdominal distention	No	103 (39.6)	74 (35.2)	0.33
	Yes	157 (60.4)	136 (64.8)	
Chorioamnotic	Negative	107 (41.2)	176 (83.8)	< 0.001
	Positive	153 (58.8)	34 (16.2)	
Premature rupture membrane	Negative	218 (83.8)	192 (91.4)	0.014
	Positive	42 (16.2)	18 (8.6)	

Duration of premature rupture membrane	< 18h	11 (26.2)	13 (72.2)	0.001
	> 18h	31 (73.8)	5 (27.8)	
Neonatal reflex	Intact	88 (33.8)	148 (70.5)	< 0.001
	Depressed	172 (66.2)	62 (29.5)	
Age at onset of sepsis	Early onset sepsis	134 (51.5)	-	
	Late-onset sepsis	126 (48.5)	-	
Pulse rate	100-145 b/m	67 (25.8)	101 (48.1)	< 0.001
	146-180 b/m	193 (74.2)	109 (51.9)	

Data are expressed as frequency (percent), and mean ± SD. ANC: Antenatal care; SD: Standard deviation.

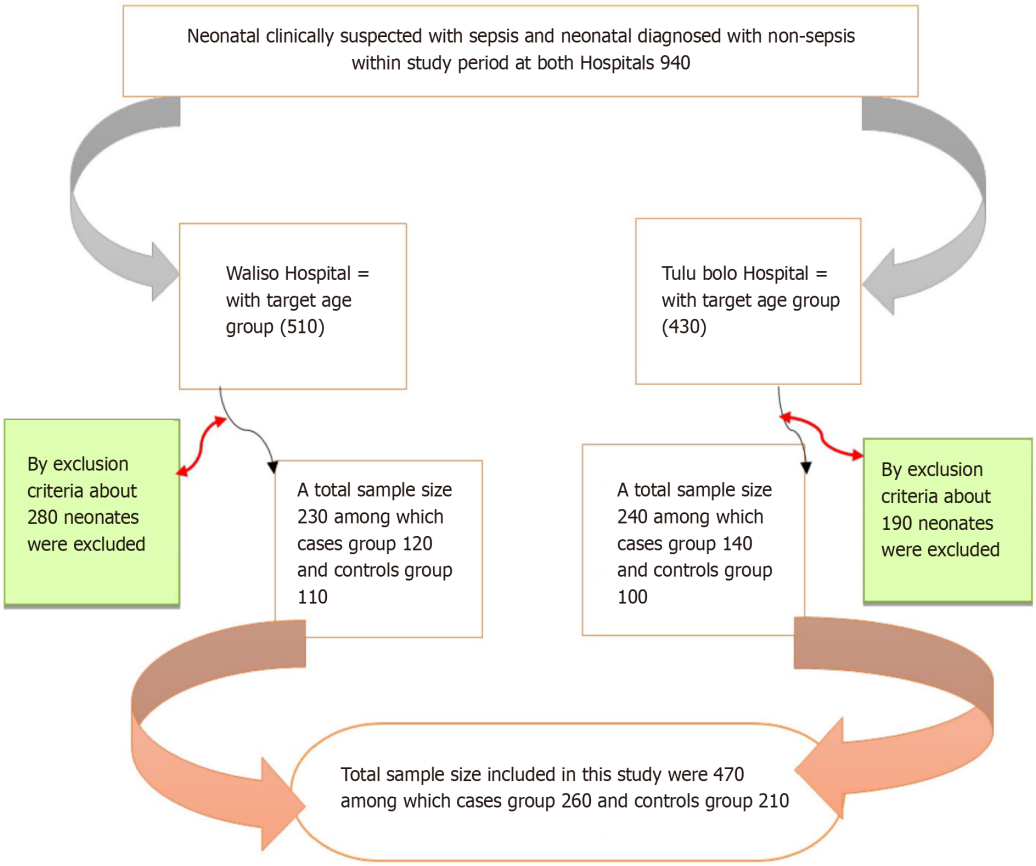

Figure 1 Sampling techniques and procedures for neonates suspected with sepsis attending Southwest Shoa Public Hospitals Oromia, Ethiopia.

Determination of hematological parameters and hemogram-derived novel markers among participants

Regarding the hematological parameters and novel markers derived from the hemogram, there is a significant increase in the values of RBC distribution width coefficient variation (RDWCV), total white cell count, neutrophils, MPV, NLR, MLR, RPR, MPVPCR, and PLR, in the case group compared to the control group ($P < 0.05$). Conversely, the values of RBC count, absolute lymphocyte count, and platelet count were significantly decreased in the case group compared to the control group ($P < 0.05$). However, the values of absolute eosinophil count and LMR did not show significant differences between the two groups ($P > 0.05$) (Table 2).

Determination of predictive values of hemogram-derived novel markers as indicators for neonate sepsis among neonates admitted at Southwest Shoa Public Hospitals, Oromia, Ethiopia, by ROC analysis.

An analysis was conducted to determine the efficacy of novel markers derived from the hemogram in predicting neonatal sepsis among neonates admitted to public hospitals in Southwest Shoa, Oromia Ethiopia. The results showed that a WBC cut-off value ≥ 11.42 had a sensitivity of 55%, specificity of 89%, PPV of 83.3%, NPV of 66.4%, and an AUC of 0.81 ($P < 0.001$). This indicates that WBC can differentiate between neonates suspected of having sepsis and control neonates.

Table 2 Comparisons of complete blood count values and hemogram derive novel markers between neonates diagnosed with sepsis and neonates without sepsis

Hematological parameters and hemogram-derived markers	Suspected neonatal sepsis	Control group	*P* value
RBC	3.58 ± 0.8	4.46 ± 0.61	< 0.001
RDWCV	14.9 ± 2.6	13.27 ± 1.67	< 0.001
WBC	12.58 ± 3.75	8.35 ± 2.77	< 0.001
NEUTR	8.66 ± 3.05	5.2 ± 2.37	< 0.001
LYMPH	1.097 ± 0.64	2.23 ± 1.11	< 0.001
MONO	2.08 ± 1.59	0.29 ± 0.69	< 0.001
EOS	0.67 ± 0.38	0.6 ± 0.35	0.44
PLT	189.78 ± 56.56	244.7 ± 95.3	< 0.001
MPV	13.43 ± 5.3	10.35 ± 1.06	< 0.001
NLR	8.29 ± 18.02	3.96 ± 5.59	< 0.001
PLR	449.11 ± 996.92	161.2 ± 165.4	< 0.001
MLR	6.14 ± 16.88	0.17 ± 0.57	< 0.001
RPR	0.085 ± 0.03	0.06 ± 0.02	< 0.001
LMR	10.59 ± 20.84	53.9 ± 91.36	0.061
MPVPCR	0.081 ± 0.05	0.05 ± 0.02	< 0.001

Data are expressed as mean ± SD, $P < 0.05$ is significant. RBC: Red blood cell; RDWCV: RBC distribution width coefficient variation; WBC: White blood cell; NEUTR: Neutrophils; LYMPH: Lymphocyte; MONO: Monocyte; EOS: Eosinophils; PLT: Platelet count; MPV: Mean platelet volume; NLR: Neutrophils to lymphocyte ratio; PLR: Platelet to lymphocyte ratio; MLR: Monocyte to lymphocyte ratio; RPR: RBC width to platelet count ratio; LMR: Lymphocyte to monocyte count ratio; MPVPCR: MPV to platelet count ratio; SD: Standard deviation.

Similarly, neutrophils can distinguish between neonates diagnosed with sepsis and neonates without sepsis with a sensitivity of 67%, specificity of 81%, PPV of 77.9%, NPV of 71.1%, and AUC of 0.801. The cutoff value for neutrophils is ≥ 6.76 ($P = 0.001$).

The MLR can also differentiate between neonates diagnosed with sepsis and neonates without sepsis. The cutoff value is ≥ 0.26 with a sensitivity of 68%, specificity of 95%, a PPV of 93.2%, NPV of 74.8%, and an AUC of 0.828 ($P < 0.001$).

Furthermore, the analysis revealed that the NLR can distinguish between neonates diagnosed with sepsis and those without sepsis. The cutoff value is ≥ 4.29, with a sensitivity of 57%, specificity of 80%, PPV of 74%, NPV of 65%, and AUC of 0.74 ($P < 0.001$). The PLR can differentiate between neonatal sepsis and non- neonatal sepsis at a cutoff value of ≥ 903.7, with a sensitivity of 90%, specificity of 100%, PPV of 100%, NPV of 90%, and an AUC of 0.69 ($P < 0.001$).

The RPR can distinguish between neonates diagnosed with sepsis and those without sepsis at a cut-off value of ≥ 0.051% with a sensitivity of 82%, specificity of 44%, PPV of 59.4%, NPV of 71%, and AUC of 0.586 at ($P < 0.001$). However, LMR was unable to differentiate between neonates diagnosed with sepsis and those without sepsis in our data from the ROC analysis (Table 3 and Figure 2).

DISCUSSION

Early diagnosis and therapy are crucial in preventing morbidity and mortality caused by neonatal sepsis. However, there is no excellent biomarker available for accurately evaluated the sensitivity and specificity of diagnostic markers for neonatal sepsis including hematological parameters and hemogram-derived markers.

However, the results of these studies vary widely, the widespread attention given to the use of hemogram derived markers for predicting the diagnosis of neonatal sepsis in a simple, rapid, and inexpensive manner; to the best of our knowledge, no published article is in our perspective.

Current studies have shown that MPV is a marker of activated platelets and is associated with various inflammatory conditions such as diabetes mellitus, cardiovascular disease, peripheral artery disease, and cerebrovascular disease. Increased MPV levels are associated with a low degree of inflammatory status. The MPVPCR has been reported to be prognostic indicator of long-term mortality in several diseases, including ischemic cardiovascular diseases, sepsis, and nonalcoholic fatty liver disease. The RPR has been found to project the severity of liver fibrosis in nonalcoholic fatty liver disease[35]. According to this study, the values of RDWCV, WBC, absolute neutrophil count, and absolute monocyte count, MPV, NLR, MLR, RPR, PLR, and MPVPCR were significantly increased in the case group compared to the control

Table 3 Determination of predictive values of some complete blood count values and hemogram-derived markers for neonate sepsis

Hemogram derived markers	Sensitivity	Specificity	PPV	NPV	Cut-off value	AUC	95%CI	*P* value
WBC	55	89	83.3	66.4	> 11.42	0.81	(0.722-0.848)	< 0.001
NEUTR	67	81	77.9	71.1	> 6.76	0.801	(0.763-0.84)	< 0.001
MONO	67	95	93.1	74.2	> 0.86	0.782	(0.74-0.825)	< 0.001
NLR	57	80	74	65	> 4.29	0.740	(0.695-0.785)	< 0.001
PLR	90	100	100	90	> 903.7	0.69	(0.642-0.737)	< 0.001
MPVPCR	47	92	85.5	63.4	> 0.187	0.718	(0.673-0.764)	0.394
MLR	68	95	93.2	74.8	> 0.26	0.828	(0.791-0.865)	< 0.001
RPR	82	44	59.4	71.0	> 0.051	0.586	(0.534-0.638)	0.001
LMR	84	53	64.1	76.8	< 0.4	0.169	(0.132-0.206)	< 0.001

Data are expressed as percentage, frequency, $P < 0.05$ is considered as significant. PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve; 95%CI: At 95% confidence interval; WBC: White blood cell; NEUTR: Neutrophils; MONO: Monocyte; NLR: Neutrophils to lymphocyte ratio; PLR: Platelet to lymphocyte ratio; MPVPCR: Mean platelet volume to platelet count ratio; MLR: Monocyte to lymphocyte ratio; RPR: Red blood cell width to platelet count ratio; LMR: Lymphocyte to monocyte count ratio.

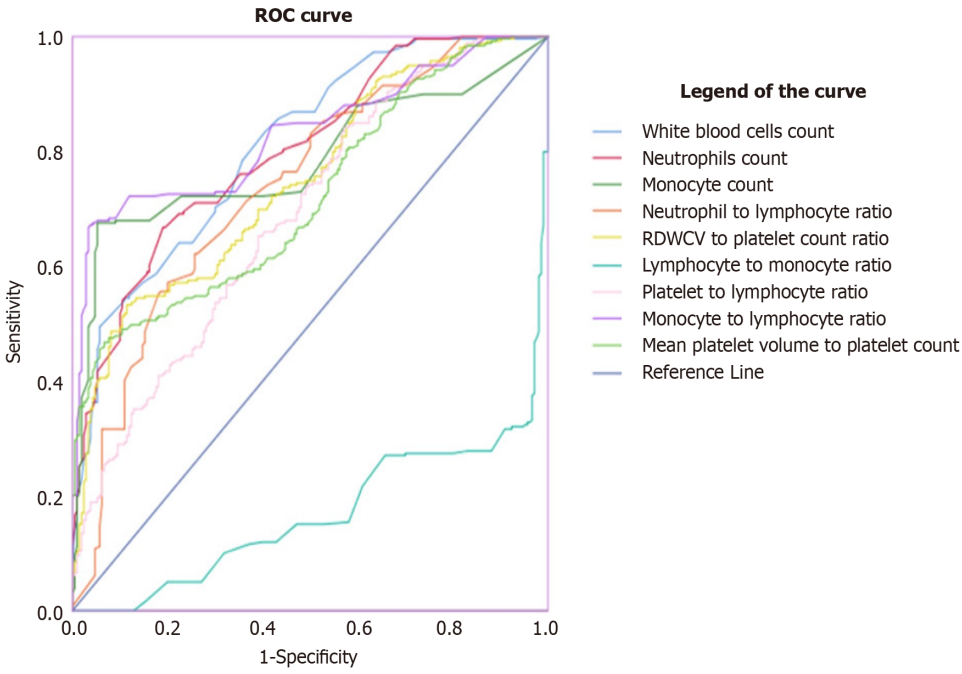

Figure 2 Predictive values of some complete blood count parameters and hemogram-derived markers for neonate sepsis. ROC: Receiver operating characteristic; RDWCV: Red blood cell distribution width coefficient variation.

group. Similar results were found in studies conducted in Turkey[28,36,37], China[34,38], Egypt[39-41], and Indonesia[33,42].

On the other hand, studies conducted in Indonesia[33], Turkey[28], Egypt[41], and China[38] found dissimilarities to our findings. This divergence may be attributed to various reasons, such as the treatment received before the CBC was conducted, the manner in which blood pressure was controlled, and the duration of acquired nephrotic syndrome in both current and previous study participants. Failure to control these risk factors may serve as sources for the rise of WBC, monocyte, neutrophil and their derivatives in nephrotic syndrome patients.

A low RBC count is a sign of sepsis, which can be caused by several mechanisms. These mechanisms include functional iron depletion, diminished erythropoietin production, infection, inflammation, RBC loss during sepsis due to pre-existing clinical conditions (such as cancer, liver disease, or renal impairment, as well as new-onset multi-organ dysfunction, especially of the liver and kidney). Disseminated intravascular coagulation (DIC), pathogen-associated hemolysis, hypoadrenalism, and dietary insufficiency[43].

Similarly, several pathological mechanisms are thought to contribute to low platelet count such as DIC, which leads to the consumption of both platelets and coagulation factors. This paradoxically results in increased bleeding and clotting [44].

The body controls immune-mediated tissue damage by lymphocytes through apoptosis. As a result lymphocyte migration to the site of infection, lymphocytopenia is also observed in sepsis[28]. In line with this concept, our study found that, the values of RBC count, absolute lymphocyte count, and platelet count were significantly decreased in the case group compared to the control group. Consistent findings with our results have also been reported in studies conducted in China[34], Egypt[40,41], and Turkey[37,28].

On the other hand, studies conducted in Turkey[36], Indonesia[42], and Egypt[39] found an increased platelet count. This discrepancy may be due to the fact that in the previous studies the CBC conducted too late. Platelets are recognized as first-line indicators for detecting and responding pathogens, as well as for responding to injury signals in blood vessels and in the extracellular space. Information has revealed that during the initial stage of bacterial infection, there is a significant increase in the number of platelets in the bloodstream, which then deceased disproportionately[45].

Eosinopenia occurs during infection due to increased peripheral eosinophil sequestration, decreased eosinophil production, and eosinophil destruction. The increased release of corticotrophin-releasing hormone in response to inflammatory mediators such as interleukin 1 and 6, and tumor necrosis factor-alpha stimulates the pituitary gland to release adrenocorticotropic hormone, which in turn stimulates the synthesis and release of glucocorticoids that prevent the release of eosinophils from the bone marrow[46,47]. The current study states that the values of absolute eosinophil count; and LMR were not significantly different among the groups. Our study is consistent with the findings of studies conducted in Indonesia[42], Egypt[41], and Turkey[37].

Meanwhile, studies conducted in Turkey[36], and Egypt[39,40] have found that the LMR value is increased in the case group compared to the control group. This inconsistency may be due to the presence of confounding factors and differences in the lifestyle of the caregivers of the participants in the previous and current studies. Lifestyle dynamics could be an unmeasured confounding factor in this context, which could have various effects on multiple organs with cardiovascular and immune implication.

In the current study the ROC analysis showed that WBC, with a cut-off value ≥ 11.42 can differentiate neonates suspected with neonatal septicemia from the control neonates. The sensitivity was 55%, specificity was 89%, PPV was 83.3%, NPV was 66.4%, and the AUC was 0.81 ($P < 0.001$). Similar results have been reported in studies conducted in Egypt[42], China[48], United States[49,50], and Ethiopia[51].

In cases of severe neonatal infections, there is often an increase in the total leukocyte count. This increase may be due to the secretion of growth factors and cytokines that stimulate bone marrow production. However, virus-infected neonates typically have a normal or slightly reduced WBC count[27].

Also, absolute neutrophils were identified as predictors of neonatal sepsis, with a sensitivity of 67%, a specificity of 81%, a PPV of 77.9%, and a NPV of 71.1% with an AUC of 0.801. The cut-off value was determined to be ≥ 6.76 at significance level of $P = 0.001$. Similar findings were reported studies conducted in the Detroit, United States[49], Indonesia[52], California, United States[50], and Ethiopia[51].

Dynamic changes in neutrophils and lymphocytes occur during neonatal sepsis. These cells are mobilized from the bone marrow to the site of infection. The apoptosis process these cells is also affected. During sepsis, the lifespan of neutrophils increases due to decreased apoptosis, which is mediated by decreased levels of caspase3 level and activation of NF-kB. Neutrophils play a crucial role as the first-line of defense against invading microbes. They function through phagocytosis and are regulated by various factors at different stages[53].

Monocytes and lymphocytes are types of leukocyte cells that contribute to the immune system and are typically examined in a CBC. Lymphocytes play a role in the adaptive immune system. In the past, decreased lymphocyte counts, or lymphocytopenia, were used as indicators of bacteremia. In cases of sepsis and septic shock, lymphocytopenia can occur due to the migration and redistribution of lymphocyte into the lymphatic system, as well as increased lymphocyte apoptosis[54]. Monocytes, on the other hand, function as antigen-presenting cells and produce cytokines in response to infection. Infection stimulate the immune system, leading to an increase in monocytes and a decrease in lymphocytes[54,55].

As a result of this condition, the MLR is amplified. Therefore, at a cut-off value of ≥ 0.26 as identified as a potential indicators of neonatal septicemia, with a sensitivity of 68%, specificity of 95%, PPV of 93.1%, NPV of 74.8%, and an AUC of 0.828 ($P < 0.001$). Similar results were found in studies conducted in Indonesia[56] and United States[50].

Additionally, the ROC analysis demonstrated that the NLR can differentiate between neonates diagnosed with sepsis and those without sepsis. The cut-off value for NLR was found to be ≥ 4.29, with a sensitivity of 57%, a specificity of 80%, a PPV of 74%, and a NPV of 65%. The AUC was calculated to be 0.74, with a significance level of $P < 0.001$. Similar findings were reported in studies conducted in China[38,57], Egypt[39], Turkey[37,28], and California, United States[50].

Sepsis is a systemic inflammatory response disease triggered by infection, and inflammation plays a crucial role in its imitation and development. WBCs and their subpopulations are essential components of the immune system and provide defense against pathogen infections. Numerous clinical studies have shown that neutrophil counts, lymphocyte counts, and the NLR are predictive factors for sepsis. The NLR, in particular, is considered to be more reliable than absolute neutrophil or lymphocyte counts as it takes both into account. The NLR has gained significant attention due to its potential as a new risk factor for sepsis[58].

Studies have suggested that the renowned RPR is effective in human treatment for evaluating and predicting the degree of fibrosis and inflammation in several conditions, including hepatic cirrhosis, acute pancreatitis, severe burns, and acute kidney injury. It has been proposed that high RPR levels are associated with sepsis diagnosis and prognosis in adult and neonatal human patients. In all studies, non-survivors had higher RPR values than survivors. A recent study evaluated RPR as a tool to assist in diagnosing perinatal disease in neonatal thoroughbred foals. The study concluded that

foals at risk of developing systemic disease had elevated RPR values[50]. The study identified a cut-off value of ≥ 0.051% with a sensitivity of 82%, specificity of 44%, PPV of 59.4%, NPV of 71% ,and an AUC of 0.586 ($P < 0.001$) as potential predictors of neonatal sepsis. Similar findings were reported in studies conducted in California, United States[50], and China[57].

The PLR was identified as a predictor of neonatal sepsis diagnosis with a cutoff value of 903.7, sensitivity of 90%, specificity of 100%, PPV of 100%, NPV of 90%, AUC of 0.69, and $P < 0.001$. Similar reports have been found in studies conducted in China[34,57], Egypt[39,40], Turkey[28,37], and Palembang[59]. Current research also indicates that platelets and lymphocytes play a critical role in the inflammatory progression. The PLR is serve as an indicators of the balance between inflammation and thrombosis. As a result, increased inflammation. Additionally, elevated platelet counts and decreased lymphocyte counts have been shown to be associated with both aggregation and inflammation, thus indicating a higher risk[60].

CONCLUSION

Our study findings suggest that certain hematological parameters and hemogram-derived marker values were significantly increased in the cases group compared to the control group. On the other hand, some hematological parameters were decreased in the cases group compared to the control group, while others showed no significant difference between the groups. Additionally, certain hematological parameters and hemogram derived markers were identified as potential indicators of neonatal sepsis.

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FOOTNOTES

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Institutional review board statement: Before the study began, our study obtained the approved ethical clearance from the local Institutional Review Board of Research (Ref. No BEFO176981/2023, Oromia Health Bureau, Ethiopia).

Informed consent statement: Before the study began, we obtained consent from the local Institutional Review Board of Research (Ref. No BEFO176981/2023, Oromia Health Bureau, Ethiopia) and received ethical clearance from Tulu Bolo General Hospital and Waliso General Hospital administration offices, as well as the Pediatric Intensive Care Unit head offices. Once we obtained permission from the hospital administrator and the head of the pediatrician clinic care unit, we collected the data from record data. We ensured the confidentiality and privacy of the obtained information. Our study adhered to the principle of the Helsinki Declaration (64th WMA General Assembly). Individual patient consent was not required as all data used in this study was acquired retrospectively from the laboratory information system and medical records without any additional blood sampling.

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ORIGINAL ARTICLE

Retrospective Study

Flexible bronchoscopy for foreign body aspiration in children: A single-centre experience

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Abstract

BACKGROUND

The technological evolution of bronchoscopy has led to the widespread adoption of flexible techniques and their use for both diagnostic and therapeutic purposes. Currently, there is an active debate regarding the comparative efficacy and safety of rigid *vs* flexible bronchoscopy in the treatment of foreign body aspiration.

AIM

To evaluate our experience with tracheobronchial foreign body extraction using flexible bronchoscopy and provide a literature overview.

METHODS

This was a single-centre retrospective study. Twenty-four patients were enrolled between January 2017 and January 2023. Medical records of patients aged below 18 years who were admitted to authors' affiliated institution with a suspected diagnosis of foreign body aspiration were collected from hospital's database to Microsoft Excel 2019. Data were analysed using MedCalc Statistical Software.

RESULTS

Patient ages varied from 9 months to 11 years. The median age was 23.5 months, 95% confidence interval (CI) 19.49-44.77. We observed age clustering in children with foreign body aspiration at our institution with three age subgroups: (1) 0-25 months; (2) 40-60 months; and (3) 120-140 months. We expectancy of an organic tracheobronchial foreign body was significantly higher in 0-25 months subgroup than that in older ones when subgroups 40-60 and 120-140 months were combined together (odds ratio = 10.0, 95%CI: 1.44-29.26, P = 0.0197). Successful foreign body extraction was performed in all cases. Conversion to a rigid bronchoscope was not required in any of the cases. No major complications (massive bleeding, tracheo-bronchial tree perforation, or asphyxia) were observed.

CONCLUSION
Flexible bronchoscopy is an effective and safe method for tracheobronchial foreign body extraction in children.

Key Words: Foreign body aspiration; Tracheobronchial foreign body; Paediatric bronchoscopy; Flexible bronchoscopy; Rigid bronchoscopy

Core Tip: Foreign body aspiration is a well-known paediatric emergency issue, with a peak incidence at the age of 1-2 years. According to guidelines, rigid bronchoscopy remains the most widespread and acceptable treatment option for foreign body aspiration, while the role of flexible bronchoscopy is mainly limited to diagnostic purposes. A growing body of research has confirmed the safety and efficacy of flexible bronchoscopy as a therapeutic option. Literature data and our data indicate that flexible bronchoscopy may be considered a competitive alternative to rigid techniques, especially in relation to the distal tracheobronchial tree in young children.

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INTRODUCTION

A known history of paediatric therapeutic bronchoscopy began during the first half of the 20th century. This was associated with the development and usage of appropriate equipment-rigid bronchoscopes, prototype of which was developed in 1904 by famous American otorhinolaryngologist[1]. One of the first documented cases of tracheobronchial foreign body extraction *via* bronchoscopy in children was the extraction of chestnut parts from the youngest described patient, at 13 months of age, by Dr Hill[2], which was described in an article published in 1912. Earlier, in 1911, the "father of bronchoscopy", published a series of 19 cases of foreign body extractions from the tracheobronchial tree in children aged 14 months to 7 years[2].

The equipment and personal experience improved over time and the procedure ceased to be exceptional. For example, 75 foreign bodies of the tracheobronchial tree in children aged ≥ 6 months were extracted *via* rigid technique by Burrington and Cotton[3] over a 5-year period in their late 60 s.

A new era of bronchoscopy began with the development of the flexible fibreoptic bronchoscope in 1967 by the Japanese doctor[1]. The use of flexible fibreoptic bronchoscopes for foreign body extraction was limited to single cases in adults and experimental animal research. Six out of seven successful cases of tracheobronchial foreign body extraction *via* flexible bronchoscope in adults were described in 1977[4] and 267 out of 300 (89%) were described in 1978[5].

In contrast to adults, the use of flexible bronchoscopy in children, especially therapeutic flexible bronchoscopy, is limited by several factors. The main of which is the small diameter of the airways and, therefore, the need for appropriate small-diameter devices. The first flexible bronchoscope made was about 6 mm in diameter which is comparable to the diameter of the trachea of a one-year-old child, not to mention the bronchi. At the end of 1978, Olympus Corporation developed a prototype bronchoscope with a 3.5 mm outer diameter and 1.2 mm working channel (BF3C4). The only diagnostic capabilities of which were described by Wood and Sherman[6], who performed 211 procedures in children ranging in age from newborns weighing 840 g to 14-year-old patients. In the same article, published in 1980, the following statement was found: "Most authorities suggest that flexible bronchoscopy cannot (or should not) be performed on children younger than about ten years", reflecting the dominant opinion of the medical community of that time[6]. Thereafter, the first ultrathin bronchoscope equipped a 2.8 mm insertion tube outer diameter and 1.2 mm working channel was introduced in 2004 (Olympus BF-XP60)[7]. However, even nowadays, paediatric flexible bronchoscopy remains an exclusive add-on to endoscopy, with a unique pathology requiring special equipment and a long educational process.

For a long time, the main indication for bronchoscopy in children was foreign body aspiration. Currently, this remains the primary indication for rigid bronchoscopy in children. Data available from the United States showed that the frequency of choking injuries increased; there were 26 cases per 100000 patients aged 0-19 years for the period 2011-2016, compared with 19 cases per 100000 for the period 2005-2010[8]. The peak incidence of foreign body aspiration is noted at the age of 1-2 years[9-11]. Younger children are at special risk; patients aged below 5 years accounted for 73% of nonfatal injuries and 75% of choking fatalities[8]. The foreign body aspiration-related mortality rate is 1% in children aged from 1 to 15 years and up to 4% in infants, according to Health reports by the Federal Government of Germany[12].

According to current guidelines, removal of the airway foreign body should be performed using rigid bronchoscopy, while flexible bronchoscopy is considered only an auxiliary tool intended mainly for diagnostic purposes (pre- and postoperative examinations)[13,14]. However, flexible bronchoscopy is a well-known, safe, and widely accepted procedure involving several therapeutic modalities.

We hypothesized that this adherence to rigid method may be explained by historical circumstances and accumulated experience of medical community, as well as the lack of better alternatives in the past due to technical limitations of flexible equipment. In this study we aimed to evaluate our experience with tracheobronchial foreign body extraction using flexible bronchoscopy and provide a literature review.

MATERIALS AND METHODS

Study design and participant selection

This was a single centre, retrospective study. Twenty-four patients were enrolled in the study over a 5-year period from January 2017 to January 2023 (Table 1). Medical records of patients aged below 18 years were collected from patients who were admitted to the authors' affiliated national paediatric surgery centre with a suspected diagnosis of foreign body aspiration. This study was approved by the Institutional Review Board of the Republican Scientific and Practical Centre of Paediatric Surgery (IRB No. 8.0). Date: 20/03/2023).

Equipment, experience, and anaesthesiology

To extract the vast majority of foreign bodies, bronchoscopes BF-XP190 (distal end outer diameter 3.1 mm; instrument channel 1.2 mm) and BF-P190 (distal end outer diameter 4.2 mm; instrument channel 2.0 mm) were used; one foreign body was extracted using portable endoscope MAF-GM2 (distal end outer diameter 3.9 mm; instrument channel 1.5 mm) and three more using BF-1TH190 (distal end outer diameter 6.2 mm; instrument channel 2.8 mm). All the endoscopes were manufactured by Olympus Corporation (Tokyo, Japan).

As a working instrument, we used endoscopic grips, such as a retrieval basket, tripod type, forceps, a retrieval net, alligator jaw forceps, rat tooth forceps, and a snare, depending on the type of foreign body and operator's experience. Three experts in gastrointestinal endoscopy (including gastrointestinal foreign body extraction) and bronchoscopy performed the extractions. The extraction was performed under general anaesthesia using artificial airways with a laryngeal mask or an endotracheal tube, under the supervision of a competent anaesthesiologist. Notably, the endoscope should be at least 1 mm thinner than the inner diameter of the endotracheal tube to avoid tube dislocation and equipment damage[13]. Furthermore, the inner endotracheal tube diameter can be too small to extract a large foreign body, without fragmentation. In such cases, our strategy was to extract the foreign body along with the endotracheal tube during planned extubation.

Data analysis

Data were processed using Microsoft Excel 2019 (version 2109, build 14430.20306). Thereafter, the data were analysed using MedCalc® Statistical Software version 22.016 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org, 2023). We used univariate analysis (mean, median) with calculation of confidence interval (CI). Analysis of patient age cumulative frequency distribution was used. Then, we analysed the odds ratio (OR) and relative risk to find the strength of the association between different age groups.

RESULTS

Patient ages varied from 9 months to 11 years. The mean age was 46.7 months (95%CI: 27.91-65.5). The median age was 23.5 months (95%CI: 19.49-44.77).

We observed age clustering in children with foreign body aspiration who underwent endoscopic intervention at our institution. There were three age subgroups: (1) 0-25 months; (2) 40-60 months; and (3) 120-140 months (Figure 1). Fourteen of the 24 patients (58.3%) were aged < 25 months.

Fifty percent of tracheobronchial foreign bodies (12 out of 24) were of inorganic origin, most often plastic toy products or parts thereof. In several cases, a screw, a dental pulp extractor, and a light-emitting diode were found. The remaining 50% of cases were of organic origin (nuts, sunflower seeds, and orange peels). Only four of the 24 foreign bodies were radiopaque and could be seen on radiography.

Most often, 17 of 24 (71%) foreign bodies were localised in the right parts of the tracheobronchial tree, whereas only 7 of 24 (29%) foreign bodies were found in the left parts (Figure 2 and Table 2). In addition, we analysed the origin of the foreign body and its topography after aspiration according to the age subgroup (Table 3).

Analysis of the OR and relative risk of finding topographic differences in foreign body localisation after aspiration between the age subgroups was not statistically significant. However, we found that expectancy of an organic tracheobronchial foreign body was significantly higher in 0-25 months subgroup than that in older ones when subgroups 40-60 and 120-140 months were combined together (OR = 10.0, 95%CI: 1.44-29.26, P = 0.0197).

Successful foreign body extraction was performed in all cases (Table 1). Conversion to a rigid bronchoscope was not required in any of the cases. In 11 of the 24 cases, foreign body extraction was performed using endotracheal tubes of various diameters. No major complications (massive bleeding, perforation of the tracheobronchial tree, or asphyxia) were noted, and no minor complications (transient hypoxia and laryngeal oedema) were recorded.

Table 1 Summary table of patients and clinical features characterizing foreign body extraction from the tracheobronchial tree

Patient	Age (months)	FB localization	Distal end outer diameter of bronchoscope (mm)	Endoscopic instrument	Type of anaesthesia (LMA/ET)
1	53	Right main bronchus	4.2	Retrieval basket	ET, extraction with extubation
2	13	Left main bronchus	3.1	Tripod type forceps	ET, through the tube
3	41	Right main bronchus	4.2	Alligator jaw forceps	LMA
4	122	Intermediate bronchus	3.1	Rat tooth forceps	ET, extraction with extubation
5	25	Left main bronchus	3.1	Retrieval basket	LMA
6	22	Intermediate bronchus	3.1	Retrieval basket	ET, through the tube
7	136	Right lower lobe bronchus	4.2	N/D	LMA
8	125	Right main bronchus	6.2	Tripod type forceps	LMA
9	16	Left main bronchus	3.1 with switching to 4.2	Rat tooth forceps	LMA
10	42	Intermediate bronchus	6.2	Alligator jaw forceps	LMA
11	25	Right upper lobe bronchus	3.1 with switching to 4.2 mm	Rat tooth forceps + retrieval net	LMA
12	50	Left lower lobe bronchus	3.1	Rat tooth forceps	LMA
13	20	Right lower lobe bronchus	3.1	Retrieval basket	LMA
14	13	Left main bronchus	3.1	N/D	LMA
15	20	Left lower lobe bronchus	3.1	Tripod type forceps + retrieval basket	ET, through the tube
16	43	Right upper lobe + lower lobe bronchi	3.1	Retrieval basket	ET, through the tube
17	18	Right main bronchus	3.1	Tripod type forceps	ET, extraction with extubation
18	128	Right lower lobe bronchus	4.2	Retrieval net	ET, extraction with extubation
19	22	Right lower lobe bronchus	4.2	Tripod type forceps + retrieval basket	ET, through the tube
20	131	Right main bronchus	6.2	N/D	LMA
21	20	Right main bronchus	3.1	Retrieval basket	LMA
22	15	Left main bronchus	3.1	Retrieval basket	LMA
23	9	Right upper lobe bronchus	3.9	Alligator jaw forceps	ET, through the tube
24	12	Right main bronchus	3.1	Snare	ET, extraction with extubation

FB: Foreign body; ET: Endotracheal tube; LMA: Laryngeal mask airway; N/D: No data.

DISCUSSION

Foreign body aspiration represents one of the very frequent accidents in children. Young children tend to put small objects into the mouth, they also often eat during activity and move around with food in the mouth. Quite often older siblings or even parents represent a risk as they may give the young child food inappropriate for age, such as nuts, peanuts, seeds[15].

That might be one of the reasons why airway foreign bodies are mostly organic in nature (64%-93%)[10,16,17]. Interestingly, the intuitive expectation of a higher frequency of localisation of foreign bodies in the right parts of the bronchial tree is not always confirmed by literature. The right/Left ratios recorded in various studies were 55%/45% (number of comparisons = 86)[9], 48%/52% (n = 187)[10], 60%/40% (n = 20)[16], 53%/47% (n = 30)[17], and 55%/45% (n = 112)[18], which do not demonstrate a large disparity between the two sides.

The recommendations of the currently existing guidelines favour rigid bronchoscopy as the method of choice for tracheobronchial foreign body extraction[13,14,19]. Some authorities state that flexible bronchoscopy can be used for foreign body extraction but only if the operator has the ability to quickly switch to a rigid bronchoscope[12,13]. Based on

Table 2 Foreign body localization in the tracheobronchial tree (percentage)

Tracheobronchial tree localization	Number of cases	%
Right main bronchus	7	29.2
Left main bronchus	5	20.1
Intermediate bronchus	3	12.5
Right lobar bronchi	7	29.2
Left lobar bronchi	2	8.9

Table 3 Analysis of a foreign body origin and its topography after aspiration

Age (month)	Topography		Origin of foreign body	
	Large bronchi	Lobar and segmental bronchi	Organic (%)	Non-organic (%)
0-25	9	5	10 (71.4)	4 (28.6)
40-60	3	2	1 (20)	4 (80)
120-140	3	2	1 (20)	4 (80)

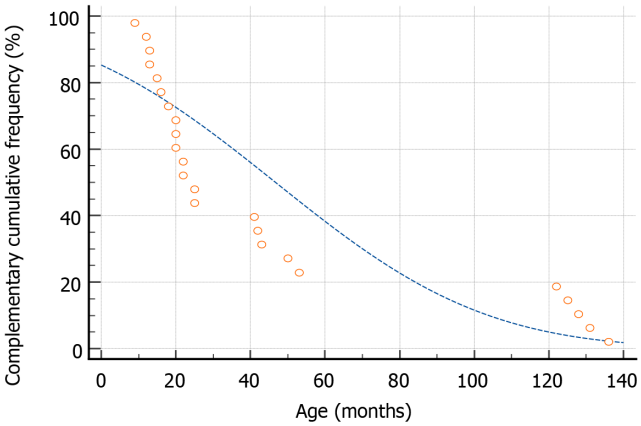

Figure 1 Analysis of patient age cumulative frequency distribution.

a survey of German specialists in paediatric pulmonology in 2017, it was reported that only 20% of medical centres in Germany prefer to use flexible bronchoscopy for foreign body removal[12]. Authors of this survey state that this approach is more common in centres with a relatively small number of manipulations and explained this percentage by the lack of equipment and sufficient expertise and skills in performing rigid bronchoscopy.

Schramm *et al*[12] concluded that rigid bronchoscopy is a more effective and safer method (the success rate of the "primary flexible method" is 73.9%, while the success rate of the "primary rigid method" is 99.4%), which, however, is not consistent with data found in literature (Table 4).

A number of studies have confirmed the safety and effectiveness of flexible bronchoscopy as a therapeutic procedure for foreign body aspiration[10,11,16,20,21], suggesting that it can be a first-line therapy for the extraction of foreign bodies [17,20-23], with up to 100% efficiency in experienced hands[11,24]. Fewer complications were noted in comparison to rigid bronchoscopy, as well as a shorter duration (42 min *vs* 58 min[20] and 36 min *vs* 53 min)[23]. For example, Tang *et al* [22] reported a 91.3% success rate for foreign body removal with a flexible endoscope in a retrospective evaluation of 1027 children. Notably, all the above studies emphasised the importance of suitable equipment and endoscopic tools, as well as sufficient experience and skills of the personnel performing the extraction.

Despite the high success rates for flexible bronchoscopy, it should be recognised that the rigid technique has a high efficiency (97%-99.7%), which has been repeatedly demonstrated in a large number of children[25-27]. Simultaneously, less invasiveness and a shorter duration of manipulation are expected advantages of the flexible technique, which should be reflected in fewer complications. We also actively used rigid bronchoscopy for foreign body aspiration at our centre until 2014; however, the appearance of flexible bronchoscopes of various diameters and appropriate endoscopic tools displaced this method.

A large study published in 2017 involving 197 European centres performing paediatric bronchoscopy provided two interesting facts: (1) 65 of 197 centres had only flexible bronchoscopes and no rigid bronchoscopes at all; and (2) 38 of 148 centres providing 24/7 care for patients with suspected tracheobronchial foreign body aspiration did not have rigid

Table 4 Efficacy of flexible bronchoscopy as a first treatment option in foreign body aspiration

Ref.	Number of patients	Efficacy of flexible bronchoscopy alone (%)	Complications (%)
Ding *et al*[10]	165	97.6 (161/165)	Transient hypoxia and tachypnoea; one severe complication (no specified)
Tenenbaum *et al* [11]	28	100 (28/28)	No/no specified
Kim *et al*[16]	20	90 (18/20)	10 (n = 2), mild laryngeal oedema
Yüksel *et al*[17]	31	93.5 (29/31)	0
Tang *et al*[22]	1027	91.7 (938/1027)	12.9 (transient hypoxia); 2.3 (bleeding, laryngeal oedema, bradycardia, airway leak)
Golan-Tripto *et al* [23]	40	95 (38/40)	0
Suzen *et al*[24]	24	100 (24/24)	8.4 laryngeal oedema
Ciftci *et al*[25]	283	92 (260/283)	3.5 (transient hypoxia and bradycardia); 3.9 (minor bleeding); 3.9 (minor nosebleed); 2.8 (laryngeal oedema)

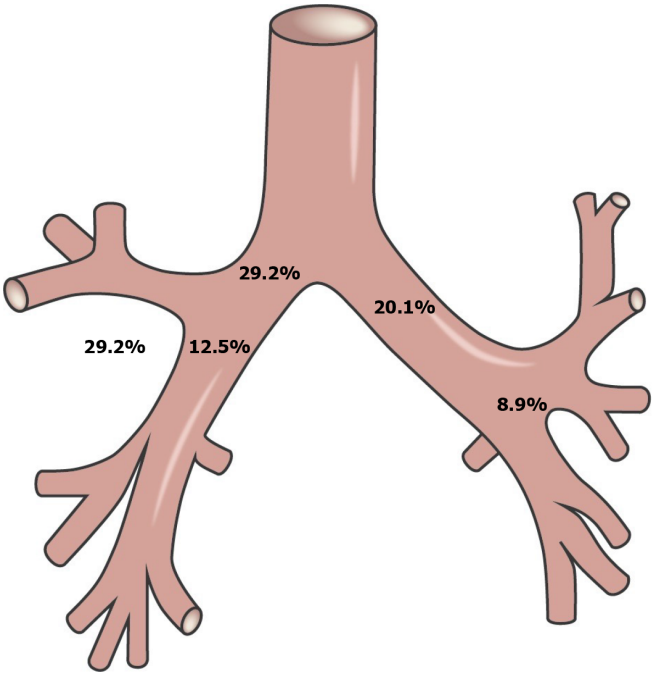

Figure 2 Foreign body localization in the tracheobronchial tree (visualization). 29.2% (inside tracheobronchial tree): Right main bronchus; 20.1%: Left main bronchus; 12.5%: Intermediate bronchus; 29.2% (on white space): Right lobar bronchi (without differentiation); 8.9%: Left lobar bronchi (without differentiation).

bronchoscopes[28].

Our experience indicates that flexible bronchoscopy is a competitive alternative to rigid techniques, particularly in relation to the distal tracheobronchial tree in young children. Despite having a rigid bronchoscope set and the skills to perform this manipulation, we have not used rigid bronchoscopy for the aspiration of foreign bodies since 2017. Furthermore, our commitment to flexible bronchoscopy can be explained by the availability of several appropriate endoscopic instruments for a 1.2-mm working channel.

The main limitation of this study is that the majority of available studies, including this article, were retrospective analyses with a low level of evidence. This information can be manipulated depending on the personal preferences of the expert.

CONCLUSION

Successful bronchoscopy intervention is the result of multidisciplinary teamwork (anaesthesiologists and endoscopy experts). We believe that at least one multicentre randomised controlled trial with the following points should be performed: (1) A large number of patients should be included; (2) all complications (minor and major) with pre-agreed

unified criteria should be noted; (3) a comparison of the procedure duration should be included; and (4) operator experience should be considered. We report that flexible bronchoscopy is an effective and safe method for tracheobronchial foreign body extraction in children.

FOOTNOTES

Author contributions: Sautin A writing original draft, conceptualization and design of the research; literature review and data collecting, text structuring and revising of the paper; Marakhouski K design of the research, performing therapeutic endoscopies, editing of the original draft, critical revision for important intellectual content and statistical analysis; Pataleta A and Sanfirau K analysis of clinical data, performing therapeutic endoscopies. All authors have read and approve the final manuscript.

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Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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ORIGINAL ARTICLE

Observational Study

Gut microbiota in preterm infants receiving breast milk or mixed feeding

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Abstract

BACKGROUND

Preterm birth is the leading cause of mortality in newborns, with very-low-birth-weight infants usually experiencing several complications. Breast milk is considered the gold standard of nutrition, especially for preterm infants with delayed gut colonization, because it contains beneficial microorganisms, such as *Lactobacilli* and *Bifidobacteria*.

AIM

To analyze the gut microbiota of breastfed preterm infants with a birth weight of 1500 g or less.

METHODS

An observational study was performed on preterm infants with up to 36.6 wk of gestation and a birth weight of 1500 g or less, born at the University Hospital Dr. José Eleuterio González at Monterrey, Mexico. A total of 40 preterm neonates were

classified into breast milk feeding (BM) and mixed feeding (MF) groups (21 in the BM group and 19 in the MF group), from October 2017 to June 2019. Fecal samples were collected before they were introduced to any feeding type. After full enteral feeding was achieved, the composition of the gut microbiota was analyzed using 16S rRNA gene sequencing. Numerical variables were compared using Student's *t*-test or using the Mann–Whitney *U* test for nonparametric variables. Dominance, evenness, equitability, Margalef's index, Fisher's alpha, Chao-1 index, and Shannon's diversity index were also calculated.

RESULTS

No significant differences were observed at the genus level between the groups. Class comparison indicated higher counts of *Alphaproteobacteria* and *Betaproteobacteria* in the initial compared to the final sample of the BM group ($P < 0.011$). In addition, higher counts of *Gammaproteobacteria* were detected in the final than in the initial sample ($P = 0.040$). According to the Margalef index, Fisher's alpha, and Chao-1 index, a decrease in species richness from the initial to the final sample, regardless of the feeding type, was observed ($P < 0.050$). The four predominant phyla were *Bacteroidetes, Actinobacteria, Firmicutes*, and *Proteobacteria,* with *Proteobacteria* being the most abundant. However, no significant differences were observed between the initial and final samples at the phylum level.

CONCLUSION

Breastfeeding is associated with a decrease in *Alphaproteobacteria* and *Betaproteobacteria* and an increase of *Gammaproteobacteria*, contributing to the literature of the gut microbiota structure of very low-birth-weight, preterm.

Key Words: Gut microbiota; Human milk; Preterm infant; Proteobacteria; Very low birth weight; 16S rRNA

Core Tip: Gut microbiota in very low-weight preterm infants is characterized by delayed colonization and decreased bacterial species which can lead to complications. In this study, we analyzed it using 16S rRNA gene sequencing in 40 hispanic infants classified into two groups: those receiving breast milk (BM) and those with mixed feeding. A decrease in the counts of Alpha and Betaproteobacteria, with higher counts of bifidobacteria, Bacteroidetes, and Clostridium were observed in the BM group. This study contributes to the literature on the structure of the gut microbial of preterm infants.

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INTRODUCTION

Every year, nearly 15 million preterm infants are born worldwide, of whom 1.1 million die because of childbirth complications. Preterm birth is considered the leading cause of mortality in newborns, with very low-birth-weight infants usually experiencing neurological, gastrointestinal, and respiratory complications[1-4].

Several structural and functional changes occur in the neonatal gastrointestinal tract as a result of their diet[5]. Breast milk has various benefits to this highly vulnerable population, such as lower rates of late-onset sepsis, retinopathy, and necrotizing enterocolitis, compared with infants receiving formula[6]. Breast milk contains beneficial microorganisms, such as *lactobacilli* and *bifidobacteria*[7,8]. Compared with the multiple aerobic and anaerobic conditions of the microbiota of full-term babies, the microbiota of preterm infants is characterized by delayed gut colonization and decreased bacterial species[9].

Bifidobacteria are the predominant intestinal bacteria for the first three–six days of life in full-term breastfed infants[10]. These bacteria represent almost 90% of the total bacterial count in the intestinal microbiota. The guts of formula-fed newborns contain approximately 20% fewer *bifidobacteria* than breastfed newborns and increased Bacteroides, Clostridium, and Enterobacteriaceae[10-12].

Kingdom Bacteria comprises nearly 30 phyla. The main phyla present in the human gut microbiota are *Firmicutes,* which include *lactobacilli,* and *Bacteroidetes* (> 90%), followed by *Actinobacteria,* which include *bifidobacteria, Proteobacteria,* and microorganisms that can be pathogenic for humans, such as *Escherichia*, *Salmonella*, *Vibrio*, and *Helicobacter*[13-15]. While an imbalance in the microbiota can affect the health of a newborn, maintaining it as healthy as possible is essential for their well-being[16]. To better understand how breastmilk influences gut microbiota, we conducted this study, in which we analyzed the gut microbiota composition using 16S rRNA gene sequencing in preterm infants weighing under 1500 g classified into breast milk feeding (BM) and mixed feeding (MF) groups[17].

MATERIALS AND METHODS

Study design and patient groups

An observational, longitudinal, comparative, and prospective study was performed on preterm infants with up to 36.6 weeks of gestation or less at the time of birth and a birth weight of 1500 g or less[18], born at the University Hospital Dr. José Eleuterio González at Monterrey, northeastern Mexico. Preterm infants were included during the period from October 2017 to June 2019. Infants with congenital gastrointestinal anomalies were excluded. Infants who were transferred to another institution or had incomplete files were also excluded. Number of subjects at each stage of study has been described using a flow diagram (Figure 1).

The mothers of infants in the neonatal intensive care unit (NICU) were encouraged to provide breast milk for their preterm babies. Breastfeeding was typically preferred, and formula (NAN® formula for preterm babies, composed of: 3.4 g of protein/100 kcal, DHA-ARA, Medium chain triglycerides, Whey milk, Skim milk powder, vitamins, lactose and Soy lecithin) was used in case the amount of breast milk was insufficient to meet the needs of the infants. As soon as minimal enteral stimulation could be initiated (decision of the attending neonatologist), feeding was started with a minimal stimulus (0.5 mL/kg/h per day for three days) and then increased to 1 mL/kg/h per day until full enteral feeding was achieved (150 mL/kg/d) in all clinically stable newborns weighing under 1 kg. Newborns weighing over 1 kg were started on enteral feeding (1 mL/kg/h per day) until reaching 150 mL/kg/d. No human donor milk or milk fortifiers were used, and no human milk bank was available at our hospital during the study period. Two independent groups were formed based on the availability of breast milk. One group was exclusively fed with their mother's BM, and the other group was fed with a mixed diet including their mothers' breast milk and preterm formula (MF). A total of 40 preterm infants were included, 21 in the BM group and 19 in the MF group.

The study protocol was approved by the Ethics and Research Committee of the School of Medicine, Universidad Autónoma de Nuevo León (registration code PE16-00009). Written informed consent was obtained from the parents of all newborns.

Sample and data collection

Before any intervention (breast milk administration or mixed feeding), a sample of each infant's first bowel movement was collected to analyze the gut microbiome. All preterm infants were followed up during their NICU stay, and a second stool sample was obtained for analysis after full enteral feeding was achieved (defined as 150 mL/kg/d) (Table 1). Stool samples were first obtained by trained NICU nurses using a sterile applicator and then placed in a sterile container. Subsequently, all samples were frozen at -70° C and stored for later analysis. A unique identification number was assigned to each sample. In addition, the demographic and clinical characteristics of the newborns were collected, including the type of delivery, feeding type, gender, weeks of gestation, weight, and morbidities at the NICU (Table 1).

Sample size

Using a formula to compare two proportions for a one-tailed hypothesis with a confidence level of 95%, a statistical power of 90%, and an expected proportion of beneficial bacteria (*bifidobacteria*) of 90% for the breast-milk-fed group and 40% for the group with mixed feeding[16], the number of participants required was 14 per group. Adjusting this value to 10% of possible losses during follow-up determined that the number of patients to include was 16.

Microbiome analysis

All analyses were performed in MR DNA (Shallowater, TX, United States, (http://www.mrdnalab.com). The semiconserved V4 region of the 16S rRNA gene was amplified using previously described primers[19]. The samples were sequenced using Illumina HiSeq Chemistry (Illumina, Inc., San Diego, CA, United States) following the manufacturer's protocol.

Q25 sequence data (sequencing base calls with an error rate of less than 0.3%) derived from the sequencing process were processed using a proprietary analysis pipeline (MR DNA). Sequences were depleted of barcodes and primers, and then short sequences (< 200 bp), ambiguous base calls, and homopolymer runs exceeding 6 bp were removed. Operational taxonomic units were defined after singleton sequences were removed and after clustering at 3% divergence (97% similarity). They were then taxonomically classified using BLASTn against a curated National Center for Biotechnology Information database and compiled into counts and percentage files at each taxonomic level (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

The statistical methods of this study were reviewed by Neri Alejandro Álvarez-Villalobos, MD, from School of Medicine of Universidad Autónoma de Nuevo León. Descriptive statistical analysis was performed for group comparison between BM and MF. Continuous variables are represented as means ± SD. Percentages and frequencies were used for categorical variables. Numerical variables were compared using Student's *t*-test for independent samples with a normal distribution or using the Mann–Whitney *U* test for nonparametric variables. All analyses were performed using IBM SPSS Statistics version 20 (IBM Corp., Armonk, NY, United States).

Dominance, evenness, equitability, Brillouin's index, Margalef's index, Fisher's alpha, Berger–Parker index, Chao-1 index, Simpson's index, Menhinick's index, and Shannon's diversity index were calculated using the paleontological statistics software PAST (version 4.03)[20].

Table 1 Perinatal and clinical characteristics and outcomes of 40 preterm infants, *n* (%)

Characteristics	Type of feeding		*P* value
	Breast milk, *n* = 21	Mixed, *n* = 19	
Gender			
Female	5 (24)	9 (47)	0.113
Male	16 (76)	10 (53)	
Type of delivery			
Vaginal	6 (28)	4 (21)	0.582
Cesarean section	15 (71)	15 (78)	
Prematurity category			
Late preterm (34-36.6 gestational weeks)	0 (0)	2 (10)	0.225
Preterm (28-33.6 gestational weeks)	20 (95)	15 (79)	
Extreme preterm (< 27.6 gestational weeks)	1 (5)	2 (10)	
Apgar score at 5 min			
0–3	0 (0)	0 (0)	0.087
4–6	2 (9)	2 (10)	
7–10	19 (90)	17 (89)	
Gestational weeks, mean ± SD	29 ± 2	30 ± 2	0.103
Birth weight (g), mean ± SD	1,180 ± 217	1276 ± 169	0.132
Birth height (cm), mean ± SD	37 ± 3	38 ± 2	0.338
Intubated at birth	4 (19)	5 (26)	0.581
Days to achieve full enteral feeding, mean ± SD	18 ± 10	15 ± 8	0.346
NICU stay (days), mean ± SD	28 ±19	26 ±17	0.668
Late-onset sepsis	5 (24)	2 (10)	0.413
Retinopathy	1 (5)	0 (0)	0.997
Necrotizing enterocolitis	3 (14)	4 (21)	0.685
Patent ductus arteriosus	6 (29)	5 (26)	0.994
Respiratory distress syndrome	18 (86)	19 (100)	0.239
Bronchopulmonary displasia	4 (19)	3 (16)	0.991
Intraventricular hemorrhage	10 (48)	5 (26)	0.202
Mortality	0 (0)	2 (10)	0.218

NICU: Neonatal intensive care unit.

Statistical comparisons for each sample group were conducted using Kruskal–Wallis pairwise comparisons. Statistical significance was set at $P < 0.050$.

The microbial community structure was analyzed using weighted UniFrac distance matrices. Principal coordinate analysis plots were used to visualize the data in these matrices, and pairwise analysis of similarities (ANOSIM) was used to determine difference in microbial communities between groups.

RESULTS

Patient characteristics

A total of 40 preterm neonates weighing less than 1500 g completed follow-up and were analyzed and divided into two groups: 21 in the BM group and 19 in the MF group. All infants were Hispanic, and 65% (26/40) were male. All neonates received antibiotics (ampicillin and aminoglycosides) for the initial week while participating in the study as part of their NICU treatment, however, there was no use of antibiotics before delivery and mothers didn't present premature rupture

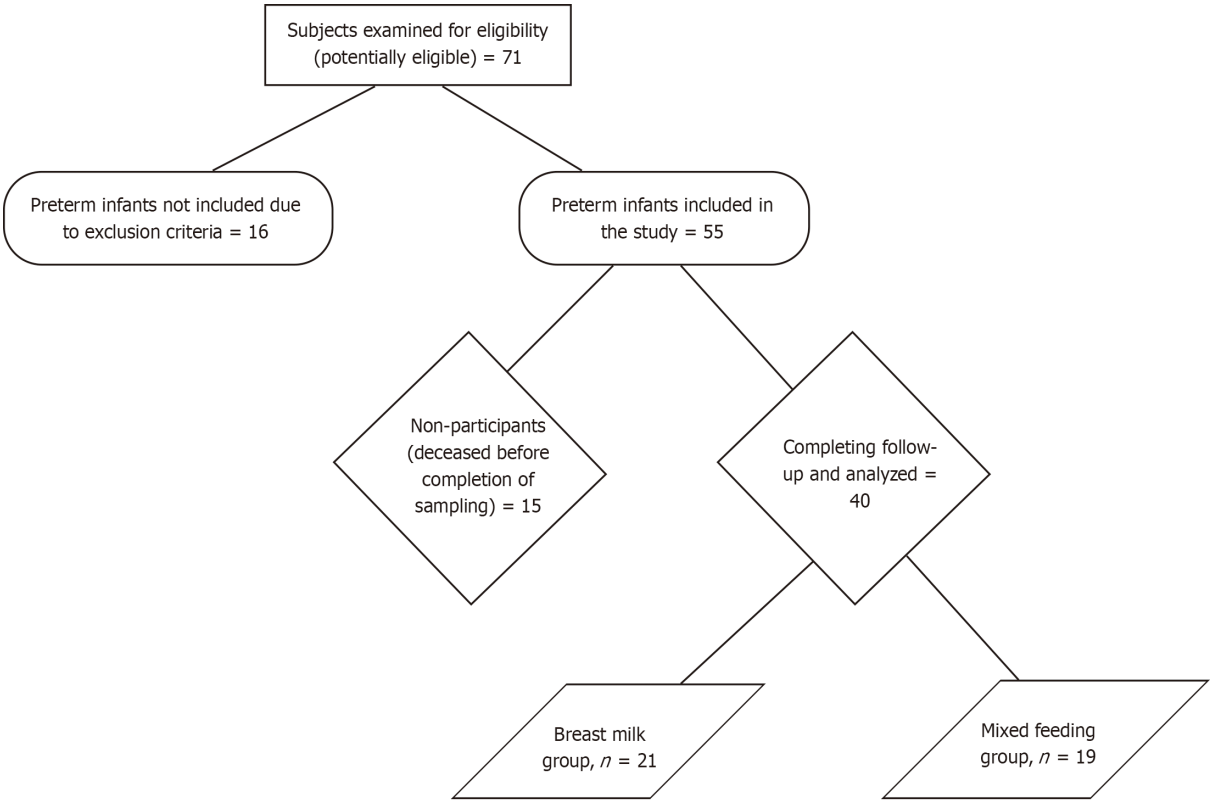

Figure 1 Flow diagram of number of subjects at each stage of study.

of membranes. No significant differences were observed in the demographic characteristics between the two groups. In addition, no differences were observed in the clinical characteristics and outcomes of the infants between groups (Table 1).

Microbiome distribution

After strict quality sequence curation, 3132039 reads were analyzed, and 2940310 reads were pooled. In addition, 2937522 reads identified within the Bacteria and Archaea domains were used for the final microbiota analysis. The mean reads per sample were 36719. For alpha and beta diversity analysis, samples were rarefied by 18000 reads. The data were then multivariate-evaluated to determine the differences between groups.

Data has been deposited in a publicly accessible database: GenBank SRA Accession Number: SRR17156517. BioProject: PRJNA786526 BioSample: SAMN23683919.

No significant differences were observed at the genus level between the BM and MF groups (at both the initial and final time points). A dual hierarchal dendrogram evaluation of taxonomic classification data was constructed to view the data of predominant genera. Samples with similar microbial populations were mathematically clustered, and genera (consortium) were used for clustering. However, no significant difference was observed between the BM and MF groups, given the lack of clustering between groups.

In the BM group, comparisons at the class level indicated higher counts of *Alphaproteobacteria* and *Betaproteobacteria* in the initial compared to the final sample ($P < 0.011$) and higher counts of *Gammaproteobacteria* in the final than in the initial sample ($P = 0.040$) (Table 2). The four predominant phyla were *Bacteroidetes, Actinobacteria, Firmicutes,* and *Proteobacteria,* with *Proteobacteria* being the most abundant (Figure 2). No significant differences were observed between the initial and final samples at the phylum level (Table 2).

Alpha diversity

Species diversity was compared between the initial and final samples for both groups. Significant differences were observed in the Margalef, Fisher's alpha, and Chao-1 indexes in the BM group. Regarding evenness, significant differences were also observed in the Margalef, Fisher's alpha, Chao-1, and Menhinick's index in the MF group ($P < 0.050$) (Table 3).

Beta diversity

The microbial community structure was analyzed using weighted UniFrac distance matrices. However, no significant difference was observed in the phylogenetic assemblage in either group. In addition, no significant difference was observed in the ANOSIM *R* values ($R = 0.007922601$, $P = 0.242$, *Q*-value = 0.24) between the microbial communities of the BM and MF groups.

Table 2 Comparison of relative abundances of classes and phyla between the initial and final samples

	Type of feeding					
	Breast milk, *n* = 21			Mixed, *n* = 19		
	Initial, mean ± SD	Final, mean ± SD	*P* value	Initial, mean ± SD	Final, mean ± SD	*P* value
Class						
Bacteroidia	7.468 ± 15	6.835 ± 14	0.782	1.890 ± 3	2.872 ± 11	0.612
Flavobacteria	0.245 ± 10	0.008 ± 0.004	0.106	0.404 ± 1	0.005 ± 0.005	0.041
Sphingobacteria	0.030 ± 0.09	0.001 ± 0.001	0.155	0.055 ± 0.1	0.002 ± 0.006	0.100
Cytophagia	0.030 ± 0.10	0.001 ± 0.002	0.208	0.0004 ± 0.001	0.063 ± 0.268	0.320
Actinobacteria	2.270 ± 2.0	4.519 ± 9	0.261	1.396 ± 2	3.520 ± 10	0.398
Bacilli	14.975 ± 17	17.794 ± 17	0.539	19.112 ± 29	20.131 ± 26	0.875
Clostridia	1.664 ± 30	3.525 ± 6	0.177	1.517 ± 2	5.084 ± 15	0.326
Mollicutes	4.334 ± 17	0.054 ± 0.076	0.274	4.424 ± 19	4.607 ± 20	0.324
Erysipelotrichia	0.0001 ± 0.0005	0.0006 ± 0.001	0.253	0.028 ± 0.06	0.0002 ± 0.0008	0.063
Negativicutes	1.038 ± 3.0	2.440 ± 5	0.138	0.387 ± 1	0.337 ± 0.813	0.857
Alphaproteobacteria	0.989 ± 1.0	0.017 ± 0.011	0.005	0.570 ± 1	0.237 ± 0.623	0.103
Betaproteobacteria	23.550 ± 27.0	0.643 ± 0.290	0.001	18.517 ± 28	12.789 ± 28	0.509
Deltaproteobacteria	1.585 ± 4.0	0.200 ± 0.593	0.081	0.163 ± 0.4	0.103 ± 0.168	0.582
Epsilonproteobacteria	0.130 ± 0.4	0.001 ± 0.002	0.179	0.009 ± 0.02	0.0009 ± 0.002	0.218
Gammaproteobacteria	40.831 ± 30.0	64.381 ± 24	0.004	51.063 ± 36	50.182 ± 34	0.921
Phylum						
Bacteroidetes	7.775 ± 15	6.786 ± 14	0.667	2.350 ± 3	2.943 ± 11	0.762
Actinobacteria	2.282 ± 2	4.131 ± 9	0.373	1.396 ± 2	3.520 ± 10	0.391
Firmicutes	17.678 ± 19	23.761 ± 17	0.219	21.046 ± 29	25.553 ± 28	0.558
Proteobacteria	67.087 ± 24	65.243 ± 24	0.772	70.324 ± 31	63.314 ± 31	0.403

There was no significant difference in the structure of the gut microbiota between the two groups after feeding.

DISCUSSION

Regarding clinical characteristics, even though it is well known that breast milk lowers the rate of late-onset sepsis and necrotizing enterocolitis[6], and we could have expected that the BM group would have this clinical significance compared to the MF group, these differences were not observed in our study possibly due to a no longer-term follow-up.

We analyzed the gut microbiota composition using 16S rRNA gene sequencing in preterm infants, comparing those exclusively fed with BM with those fed with MF. In the BM group, class comparison indicated higher counts of *Alphaproteobacteria* and *Betaproteobacteria* in the initial compared to the final sample and higher counts of *Gammaproteobacteria* in the final than in the initial sample. All three classes (*Alphaproteobacteria, Betaproteobacteria,* and *Gammaproteobacteria)* belong to the phylum *Proteobacteria*.

The gut microbial community of healthy newborns typically starts with the colonization of facultative anaerobes, followed by the establishment of obligate anaerobic organisms, such as *bifidobacteria* and *Bacteroidetes*[7,21]. However, intestinal colonization is delayed in preterm infants, and the microbiome is less diverse, with a lower abundance of *bifidobacteria* and *Bacteroidetes* and a greater abundance of *Proteobacteria*. Multiple factors can influence the immediate colonization of the gastrointestinal tract, especially the type of delivery (vaginal or cesarean section), and maybe gestational weeks, which could explain the differences observed in the relative abundances of classes and phyla in neonates depending on the type of feeding even before the intervention[7,22,23]. Vertical transmission of bacteria from mother to child is one of the most important factors that influence the maturation of microbiota. Different studies describe the impact caused by delivery mode. Cesarean delivery has a great influence on the early colonization of the gut, which is characterized by depletion of *Bacteroidetes*, unlike vaginally born infants[24].

Most of the patients in our study were born by cesarean section at the decision of the gynecologist. We observed similar results to those described in the literature, with fewer *Bacteroidetes* in subjects delivered by cesarean section.

Table 3 Statistics of alpha diversity analyzing phylum

Index	Type of feeding					
	Breast milk (*n* = 21)			Mixed (*n* = 19)		
	Initial, mean ± SD or median (range)	Final, mean ± SD or median (range)	*P* value	Initial, mean ± SD or median (range)	Final, mean ± SD or median (range)	*P* value
Species richness						
Margalef's index	1.60 ± 0.43	1.37 ± 0.27	0.042	1.53	1.31	0.006[1]
Fisher's alpha	2.20 ± 0.68	1.82 ± 0.41	0.037	2.05	1.71	0.007[1]
Chao-1 index	8.33 ± 1.96	7.29 ± 1.23	0.044	8	7	0.007[1]
Menhinick's index	0.9	0.7	0.079	0.8	0.7	0.007[1]
Species evenness or dominance						
Dominance	0.63 ± 0.18	0.60 ± 0.22	0.617	0.747 ± 0.153	0.699 ± 0.189	0.396
Evenness	0.26 ± 0.09	0.29 ± 0.13	0.363	0.194 ± 0.046	0.246 ± 0.080	0.020
Brillouin's index	0.51 ± 0.26	0.53 ± 0.33	0.807	0.344 ± 0.226	0.392 ± 0.265	0.551
Simpson's index	98.00	0.4600	0.589[1]	0.253 ± 0.153	0.301 ± 0.189	0.396
Shannon's diversity index	0.68 ± 0.29	0.65 ± 0.34	0.755	0.49 ± 0.250	0.51 ± 0.26	0.819
Equitability	0.33 ± 0.15	0.34 ± 0.19	0.873	0.226 ± 0.109	0.264 ± 0.141	0.355
Berger–Parker index	0.73 ± 0.16	0.67 ± 0.20	0.284	0.85	0.81	0.418[1]

[1]Mann–Whitney *U* test for different medians.

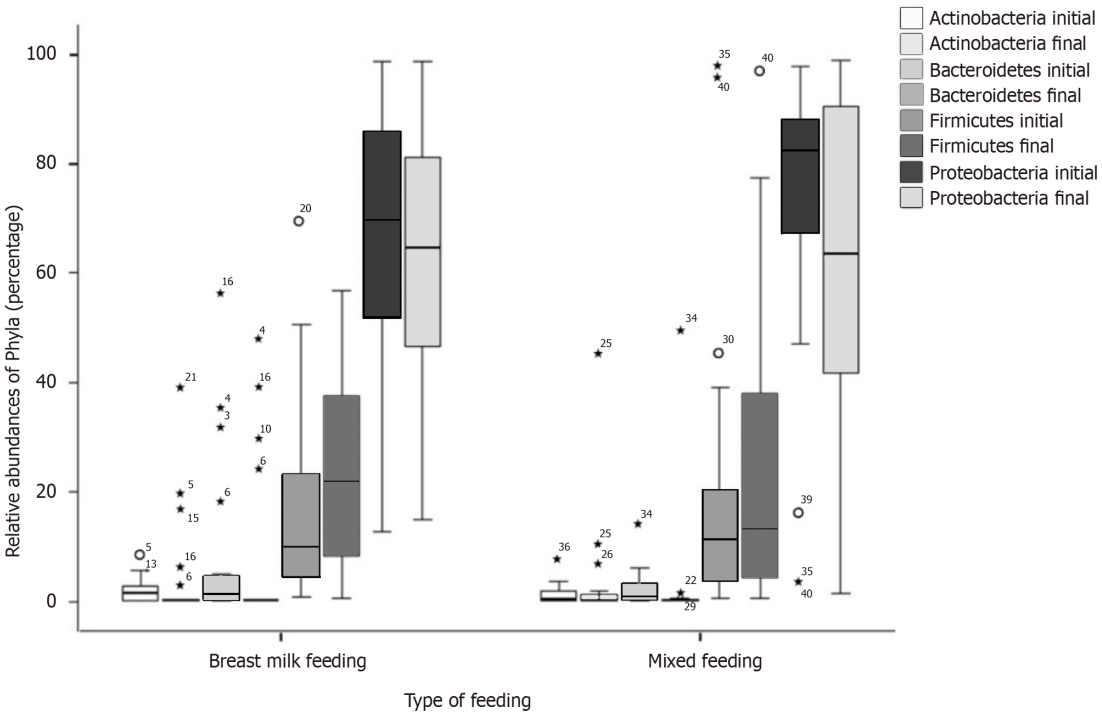

Figure 2 Relative abundance of phyla between the initial (before any feeding) and final (when full enteral feeding was achieved) samples of 40 preterm infants fed with breast milk or mixed feeding.

In our study, the four predominant phyla were *Bacteroidetes, Actinobacteria, Firmicutes,* and *Proteobacteria,* with *Proteobacteria* being the most abundant. No significant differences were observed between the initial and final samples at the phylum level and none of them suggested a tendency. However, a higher sample size or a long-term study may reflect a significant difference.

Our results indicated no significant differences between groups within the phylum *Proteobacteria*. However, analysis of specific classes from this phylum revealed a decrease in the counts of *Alphaproteobacteria* and *Betaproteobacteria* in preterm infants receiving breast milk. This result is significant because it has been described that the delayed gut microbiota maturation coupled with a *Proteobacteria*-dominated bacterial composition correlates with necrotizing enterocolitis and late-onset sepsis in preterm infants[25,26].

Higher counts of *bifidobacteria, Bacteroidetes*, and *Clostridium* were observed in the BM group compared to those in the MF group. However, the differences observed were not statistically significant. It has been described that *bifidobacteria* are fortified in the gut microbiome of term infants receiving breast milk compared with formula, thought to be due to the provision of human milk oligosaccharides. Embleton *et al*[27] also described a higher relative abundance of *Bifidobacterium* in human milk-fed preterms, although it was described in NICUs who were also using probiotics.

The presence of higher amounts of *Bifidobacteria* has been described to have benefits in the gut of preterm and is associated with protection from late-onset sepsis[28]. Also, it has been proposed that *bifidobacteria* produce acetate, which lowers luminal pH and favors the gut barrier function[29,30]. Additionally, this acetate may be converted into butyrate, which is considered an anti-inflammatory molecule[31].

Furthermore, the combination of *Bifidobacteria* with other probiotics has been associated with benefits for preterm infants. A systematic review that included 63 trials (15712 preterm infants) showed that the combination of one or more *Lactobacillus* species and 1 or more *Bifidobacterium spp* had moderate- or high-quality evidence of reduced all cause mortality (OR: 0.56; 95%CI: 0.39-0.80[32].

In our study, no human donor milk or milk fortifiers were used. Furthermore, our findings differ from a similar trial (Asbury *et al*[33]) that reported lower microbial diversity and lower abundances of *Clostridium* in preterm infants also receiving an exclusive human milk diet. However, these preterm infants were receiving newly available human-milk-based fortifiers. Clinical practice varies with some NICUs routinely using breast milk fortifiers whereas others never use fortifiers. Studies of the benefits of using fortifiers show there are no high-quality data on longer-term functional outcomes and most used fortifiers are of bovine origin[27].

Alpha diversity is the mean diversity of species in different habitats. It is considered a ubiquitous approach and has several definitions depending on the assumption of species diversity[34]. This means that more than one index of diversity can be used. In this study, we used 11 indices of diversity. Diversity indices are broadly divided into two types: indices that assess species richness (how many types there are) (*e.g.* Margalef's index, Menhinick's index, Chao-1 index, and Fisher's alpha[35]) and indices that assess species evenness or dominance (how individual organisms are distributed among species) (*e.g.*, Shannon's diversity index, Brillouin's index, Simpson's index, and evenness)[36]. Here, we compared the alpha diversity among the initial and final samples and observed a decrease in species richness from the initial to the final sample regardless of the feeding type (according to the results of Margalef's index, Fisher's alpha, and Chao-1 index) (Table 3). Although we expected an increase in species richness, we observed opposite results. Ma *et al*[37] found that alpha diversity can increase significantly up to six months of age, but in our study neonates were not followed up until that period. Similarly, previous studies have reported that microbial diversity increases with age, indicating a more complex microbiota over time, which could explain why such diversity is not yet observed in our samples[37-40].

The main limitation of this study was that we did not include an exclusively formula-fed group for comparison purposes. However, our NICU protocol was to promote breast milk administration in all premature infants. This means that finding exclusively formula-fed preterm infants would have been challenging, and randomization would have been unethical. Also, the impact of antibiotics administration that all neonates received for the initial week as part of their NICU treatment on the gut represents another limitation. The antibiotic treatment used in our NICU could have especially affected gram-negative bacilli such as *Haemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Pseudomonas aeruginosa, E. coli, Proteus mirabilis, Helicobacter pylori, Salmonella enteritidis, Salmonella typhi* among others. It is known that antibiotic exposure decreases gut microbiota diversity and changes its composition, allowing a much larger amount of *Proteobacteria*, which was observed in our study, and impairing quantities of *Clostridia* and *Bifidobacterium*[24].

CONCLUSION

In conclusion, this study contributes to the literature on the structure of the gut microbial community of breastfed, very low-birth-weight, preterm infants. Breastfeeding is associated with a decrease in counts of *Alphaproteobacteria* and *Betaproteobacteria* and an increase in *Gammaproteobacteria* in preterm infants. In addition, regardless of the feeding type, the species richness decreases from the initial to the final sample. Growing evidence in this field suggests that there is potential to create strategies that could help reduce morbidities associated with very low-birth-weight preterms through early-age manipulation of their microbiota[9]. Further studies are required to better understand the factors that may affect the development of newborn microbiota.

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FOOTNOTES

Author contributions: Sánchez-González SG designed the study, participated in the acquisition, analysis, and interpretation of the data, drafted the initial manuscript and gave final approval of the version of the article; Cárdenas-del-Castillo BG and Garza-González E made substantial contributions to conception and design of the study, analysis and interpretation of data, drafted the article and made critical revisions; Padilla-Rivas GR and Palacios-Saucedo GC participated in the analysis and interpretation of the data, drafted the initial manuscript and revised the article critically; Rodríguez- Balderrama I, Treviño-Garza C, Montes-Tapia FF and Gutiérrez-Rodríguez A made substantial contributions to conception of the study, interpretation of the data, revised the article critically for important intellectual content and gave final approval of the version of the article; de-la-O-Cavazos ME was the guarantor and designed the study, revised the article critically for important intellectual content and gave final approval of the version of the article.

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ORIGINAL ARTICLE

Observational Study

Assessing Moroccan physician knowledge and practices regarding maternal obesity's impact on childhood obesity: Implications for prevention and intervention

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Abstract

BACKGROUND

Childhood obesity is a growing global concern with far-reaching health implications. This study focuses on evaluating the knowledge and practices of physicians in Morocco regarding the link between maternal obesity and childhood obesity. Despite the increasing prevalence of childhood obesity worldwide, this issue remains inadequately addressed in the Moroccan context.

AIM

To assess the awareness and practices of physicians in Morocco concerning the connection between maternal obesity and childhood obesity.

METHODS

The research encompasses a comprehensive survey of practicing physicians, revealing significant gaps in awareness and practices related to maternal obesity.

RESULTS

Notably, a significant portion of doctors do not provide adequate guidance to

overweight pregnant women, highlighting the urgency for targeted educational programs.

CONCLUSION

In conclusion, this research illuminates critical areas for improvement in tackling childhood obesity in Morocco. By addressing these gaps, fostering awareness, and enhancing medical practices, the healthcare system can contribute significantly to preventing childhood obesity and improving the overall health of future generations.

Key Words: Childhood obesity; Maternal obesity; Management; Prevention; Screening

Core Tip: In this groundbreaking first study, we focused on an in-depth assessment of the knowledge and practices of physicians in Morocco regarding the link between maternal and childhood obesity, comprehensively investigating from the first months of life through childhood. The growing global scale of childhood obesity makes this research an essential milestone, highlighting significant gaps in medical awareness and practice in Morocco. Our findings highlight the urgency of targeted educational programs, underscoring that this study offers unique insights and crucial implications for the prevention of childhood obesity.

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INTRODUCTION

The World Health Organization (WHO) has defined obesity as excess body fat with adverse health consequences. In accordance with the International Classification of Diseases, overweight and obesity are assessed using the body mass index (BMI), which represents the ratio between weight (in kilograms) and the square of height (in meters)[1].

The WHO has established thresholds for adults to define corpulence in terms of BMI, irrespective of gender. However, this approach proves tricky when it comes to assessing corpulence in children.

Indeed, it is impractical to refer to a single BMI value, whatever the age and sex of the child, due to the physiological variations in corpulence during growth and development. In practice, doctors use child-specific BMI reference curves, as well as weight and height reference curves. This more adapted approach takes account of physiological differences linked to age and gender, offering a more accurate assessment of children's corpulence throughout their growth[2].

Certainly, childhood obesity emerges as a critical public health issue with far-reaching implications. The WHO underscores the global scale of this concern, revealing that in 2020, over 39 million children under the age of 5 were identified as overweight or obese[2]. Projections derived from predictive models paint a concerning picture, suggesting that the prevalence of this metabolic disorder is poised to affect more than 70 million children worldwide by 2025[1].

Turning attention to Africa, the WHO's data for 2016 indicated a substantial challenge, with approximately 38.2 million children under the age of 5 being reported as overweight or obese in the continent[2].

Childhood obesity is recognized as an important precursor to adult obesity. The interconnection between obesity in childhood and adulthood is clear, as around 80% of obese children are likely to retain this condition into adulthood[3]. The persistence of obesity from childhood to adulthood can be attributed to the expansion of adipose tissue, intensified during childhood by adipocyte hyperplasia and hypertrophy[4].

The global prevalence of obesity represents a significant issue with far-reaching implications, particularly concerning the health of mothers, fetuses, and children. The escalation of obesity during pregnancy is a parallel concern accompanying the global surge in obesity across various populations. Children born to obese mothers face potential disturbances in their growth trajectories, manifesting as either growth restriction or overgrowth. Elevated fetal weight and adiposity at birth contribute to the heightened risks of macrosomia, complicating the delivery process for larger infants. Furthermore, the ramifications extend beyond birth, impacting the long-term health outcomes of newborns, infants, and eventually, adults. The investigation by Whitaker and his team indicated that maternal obesity during the first trimester of pregnancy is associated with an increased risk. This risk is estimated at 2.0 (95%CI: 1.7 to 2.3%) at age 2, 2.3 (95%CI: 2.0 to 2.6%) at age 3, and 2.3 (95%CI: 2.0 to 2.6%) at age 4[5].

Given the potential and psychological health problems associated with childhood obesity[6,7], addressing this issue becomes paramount through education, prevention, and intervention strategies.

Treatment of childhood obesity involves a combination of lifestyle modifications, behavior therapy, and, in some cases, medication or surgery. Physicians play a pivotal role in both preventing and treating childhood obesity. As primary healthcare providers, they can identify children who are at risk of becoming overweight or obese and intervene early to prevent the development of obesity-related health problems. By providing education and guidance on healthy habits,

physical activity, and reducing sedentary behavior, physicians can also refer patients to other healthcare professionals for additional support.

The primary objective of this survey is to pinpoint the challenges and facilitators in primary care as perceived by physicians, proposing strategies to enhance overall practice. In the broader context, the aim is to enhance the likelihood of formulating national guidelines for childhood obesity care, ultimately elevating the quality of healthcare in this domain.

MATERIALS AND METHODS

In order to collect the necessary data for assessing the current state of childhood and maternal obesity care in Morocco, we collaborated through a partnership with the Ain CHock ASsociation of Moroccan doctors (ACHAS).

In the initial phase, we dispatched explanatory emails to practitioners engaged in the fields of maternal and childhood obesity, encompassing generalists, gynecologists, diabetologists, and pediatricians. These emails detailed the survey's objectives and acknowledged the main collaborators contributing to the study, namely the ACHAS Association and Hassan II University. The questionnaire was made accessible through a free survey website, and responses were gathered online from September 2021 to July 2022. Additionally, we endeavored to inform select physicians about the study through telephone outreach and during conferences. Unfortunately, the utilization of the free survey website yielded suboptimal results, potentially attributed to practitioners' skepticism regarding the link, as they exhibited a preference for printed questionnaires. Consequently, in alignment with Moroccan cultural preferences, we adopted an alternative strategy employing printed questionnaires and direct engagement with practitioners across various settings, including private clinics, private practices, public hospitals, universities, and military hospitals. This revised approach, however, demonstrated more favorable outcomes.

Survey participation was restricted, with a significant number of approached doctors declining due to time constraints and the questionnaire's length. Only 93 doctors ultimately consented to partake in the study. The questionnaire, comprising 30 distinct questions, necessitated approximately 30 minutes for completion. Respondents were queried on demographic characteristics, maternal obesity care, macrosomia, predictive maternal risk factors for childhood obesity, childhood obesity management, and training in the subject. All collected data were treated as confidential, with the identities of the participants omitted and preserved anonymously.

RESULTS

Demographic

Upon scrutinizing the data, a discernible gender disparity emerged, with 21% female and 78% male participants. This divergence primarily stems from discrepancies in questionnaire engagement and completion rates between the genders. Notably, male participation exhibited a more pronounced presence, marked by a higher rate of questionnaire completion. Conversely, among female participants, a relatively diminished level of interest was observed, with the majority of those who initially consented to participate failing to complete the questionnaire.

The preponderance of participants received their medical education in Casablanca (56.52%) and Rabat (21.73%). Concerning specialization, 52.17% of participants identified as generalists, while 41.93% were specialists, comprising 19.35% gynecologists and 8.7% pediatricians.

The survey revealed a significant disparity in professional activity between Casablanca and other centers. A notable proportion of survey participants practice in Casablanca (51.61%), the principal metropolitan area in Morocco.

Regarding professional experience, 52.78% of respondents have practiced their specialty for over ten years, whereas 47.12% have practiced for less than ten years. The distribution of respondents across practice settings is as follows: Private practice (46.7%), public hospital (42.2%), university teaching (3.3%), retired (1.1%), and military hospital (1.1%; Table 1).

Maternal obesity

The second section of the inquiry primarily focused on maternal obesity. According to participant responses, 77.9% of the interviewed doctors attend to less than ten overweight pregnant women per week, while 14% manage 11 to 20 cases, and 5.9% handle more than 21 overweight pregnant women weekly. A notable 76.8% of doctors recommend that overweight pregnant women undergo follow-up with a dietitian, while 19% do not. Additionally, 93.1% of doctors educate women about the diverse risks associated with excessive weight gain during pregnancy.

The findings indicate that 24.4% of overweight pregnant women are unaware of the health risks posed by their condition, both for themselves and their child. Furthermore, the results highlight that 52.3% of doctors lack sufficient information about the specific care required for overweight pregnant women (Table 2).

Concerning the incidence of obstetric complications in pregnant women, participating doctors in our survey observed that complications are infrequently noted in early pregnancy and during the peripartum period. However, they are more prevalent and frequent in later stages of pregnancy and during the fetal/neonatal period (Figure 1A).

Complications commonly observed in late pregnancy encompass pre-eclampsia, hypertension, and gestational diabetes, as illustrated in Figure 1B. On the other hand, regarding complications observed in the fetal and neonatal stages, doctors predominantly reported cases of infantile obesity, closely followed by macrosomia and shoulder dystocia, as depicted in Figure 1C.

Table 1 Demographic data results

Parameter	Effective, *n*	Percentage (%)
Gender		
Women	20	21.5
Men	73	78.5
Age range		
30 yr or less	32	34.4
31-40 yr	29	31.2
41-50 yr	18	19.4
51-65 yr	32	34.4
66-75 yr	2	12.9
City of education medicine		
Rabat	20	21.73
Casablanca	52	56.52
Fès	4	4.34
Marrakech	2	2.17
Oujda	1	1.08
Other	13	14.13
Status of participant		
Resident/internal	4	4.30
Generalist	48	51.6
Specialist	39	41.93
Researcher/teacher	2	2
City of medical practice		
Casablanca	49	51.61
Rabat	6	6.45
Other	38	40.86
Current specialty of participants		
Generalist	48	52.17
Gynecology	18	19.35
Pediatrics	8	8.7
Labour doctor	7	7.60
Endocrinologist	2	2.15
Diabetology	1	1.07
Sports medicine	2	2.15
Resident	2	2.15
Nutritionist	2	2.15
Surgery	1	1.07
Length of practice		
Less than 5 yr	28	30.10
5-10 yr	13	13.97
11-15 yr	9	9.67
16-20 yr	10	10.75

21-30 yr	23	24.73
31-40 yr	3	3.22
Over 40 yr	1	1075
Type of practice		
University teaching	3	3.3
Private practice	42	46.7
Public hospital	38	42.2
Military hospital	1	1.1
Retired	1	1.1
Other	6	6.45

Table 2 Analysis of healthcare practices in the management of overweight pregnant women

Parameter	*n*	Percentage (%)
Number of pregnant women consulting overweight		
Less than 10 per week	67	77.9
11 to 20 per week	14	16.3
21 to 50 per week	4	4.7
Over 50 per week	1	1.2
Do you suggest overweight pregnant women see a dietitian		
Yes	63	76.8
No	19	23.2
Do you educate women about the risks of excessive weight gain during pregnancy		
Yes	81	93.1
No	6	6.9
Are overweight women aware of the risks of overweight in their health and the health of their child		
Yes	65	75.6
No	21	24.4
Are you sufficiently aware of the care of obese pregnant women		
Yes	46	47.7
No	42	52.3

Macrosomia

The third section of the survey, dedicated to macrosomia, reveals that 81.2% of the interviewed doctors encounter fewer than ten macrosomic newborns per week, with 16.5% managing 11 to 20 macrosomic newborns and 2.4% handling more than 21 macrosomic newborns weekly. Participants identified five high-risk factors scientifically associated with macrosomia. The majority noted gestational diabetes (71.7%), followed by type 2 diabetes (57.6%), maternal obesity (51.1%), a history of macrosomic newborns (51.1%), and obesity acquired during pregnancy (40.2%; Table 3 and Figure 2A).

Maternal risk factors predictive of childhood obesity

An analysis of the participants' responses reveals that 76.7% of them consider diabetes during pregnancy to be closely linked to an increased risk of obesity in children, followed by excessive weight gain during pregnancy and maternal overweight at 56.7% each (Table 4).

Figure 2B, presented in our results, clearly shows participants' lack of interest in maternal weight during consultations. On the other hand, 51.7% of participants stated that they did not feel sufficiently informed about the impact of maternal overweight on paediatric obesity.

Table 3 Analysis of macrosomia rates and associated risk factors

Parameter	*n*	Percentage (%)
On average in a normal week, how many babies are born with macrosomia		
Less than 10 per week	69	81.2
11 to 20 per week	14	16.5
21 to 50 per week	1	1.2
Over 50 per week	1	1.2
Select the risks factors that you think are scientifically associated with a risk of macrosomia		
Maternal age	32	34.8
Parity	18	19.6
History of new born macrosomic	47	51,1
DT1	33	35.9
DT2	53	57.6
Gestational diabetes	66	71.7
Medical treatment	12	13
Maternal obesity	47	51.1
Obesity acquired during pregnancy	37	40.2
Fundal height	24	26.1
HTA	13	14.1
Term of pregnancy	15	16.3
Representation of the fetus	4	4.3
Newborn gender	4	4.3

HTA: Hypertension arterial; DT1: Type 1 diabetes; DT2: Type 2 diabetes.

Childhood obesity management practices among healthcare professionals

The final segment of the survey focused on the area of childhood obesity. Participants' responses indicate that 93.6% of surveyed physicians reported encountering fewer than ten obese children weekly. In contrast, 6.4% of surveyed physicians grapple with 11 to 20 cases, while 12.9%contend with more than 50 cases of childhood obesity per week.

Regarding the tools and criteria employed by healthcare professionals to identify childhood obesity, participants underscored several critical approaches. The most prevalent methods included the utilization of weight and height curves, favored by 49.4% of respondents, closely followed by the use of a BMI over 30, selected by 48.1% of participants. Moreover, an equal percentage of 48.1% emphasized the importance of traditional weight and height measurements as crucial tools for detecting childhood obesity.

An examination of participants' responses highlights that the most pivotal areas for addressing childhood obesity include nutrition and parental education, garnering a significant 61.5%. Additionally, children's lifestyle was identified as a crucial element, receiving a response of 60.3%. The significance of children's diet was also underscored, with a percentage of 51.3%.

Furthermore, participants accentuated the value of collaboration in managing childhood obesity, particularly with nutritionists (58.8%) and dietitians (53.8%). This underscores the acknowledgment among participants of the importance of involving specialized professionals in the interdisciplinary approach to addressing the multifaceted aspects of childhood obesity (Table 5).

Ensuring the well-being of these children presents significant challenges, with primary impediments encountered by healthcare professionals arising from families displaying reluctance to scrutinize their eating habits, inadequately adapted meals in the canteen, inconsistent monitoring practices, communication barriers between children and their families, and a lack of parental responsibility (Figure 2C). Approximately 87% of the surveyed physicians disclosed a lack of specific training in childhood obesity, indicating a substantial deficiency in their professional preparation to address this urgent health concern. This underscores a critical gap in the healthcare system's capacity to adeptly navigate the intricacies associated with pediatric obesity. Additionally, it is noteworthy that 56% of participants expressed a keen interest in receiving training specifically focused on nutrition.

Table 4 Maternal risk factors predictive of childhood obesity

Parameter	*n*	Percentage (%)
Which of the following risk factors do you think are scientifically associated with a risk of childhood obesity for the unborn child		
Smoking during pregnancy	8	8.9
Excessive weight gain during pregnancy	51	56.7
Diabetes during pregnancy	69	76.7
Maternal overweight	51	56.7
Excess or defective foetal growth	20	22.2
Socio economic level of siblings	7	7.8
Birth rank	3	3.3
Gender	4	4.4
Maternal depression	28	31.1
Do you feel sufficiently aware of the management of pediatric obesity		
Yes	54	62.8
No	32	37.2
Do you feel sufficiently aware of the effect of maternal overweight on pediatric obesity		
Yes	43	48.3
No	46	51.7

DISCUSSION

The study identifies critical findings and offers recommendations to address childhood obesity effectively. It underscores a lack of systematic risk assessment and awareness among healthcare providers regarding childhood obesity, emphasizing the need for standardized protocols and enhanced training programs. Additionally, the study emphasizes the importance of multifaceted approaches, including nutrition education, lifestyle modifications, and early screening initiatives, to combat childhood obesity. It also advocates for the establishment of pediatric obesity medicine centers to provide resources and research opportunities. Furthermore, integrating maternal health into obesity prevention strategies is proposed for a holistic approach to family health. The study aims to fill existing gaps in understanding how healthcare professionals in Morocco approach maternal obesity, providing insights that can inform targeted interventions and contribute to the broader goal of mitigating the childhood obesity epidemic. The implications of this study extend to improved healthcare practices, enhanced medical education, and the overall well-being of future generations.

According to United Nations International Children's Emergency Fund, Asia and Africa collectively harbor 42 million children under the age of five who are overweight or obese[8]. Despite the escalating prevalence of childhood obesity on a global scale, Morocco has not fully grasped the magnitude and repercussions of this phenomenon.

Ien terms of study limitations, data collection was a major challenge. Physicians' demanding workloads made it difficult to obtain respondents available to complete the questionnaire.

A 2011 survey conducted on the population and health of the Moroccan family revealed that 12.5% of children under the age of 5 are overweight, of whom 2.6% suffer from obesity. In 2003-2004, this proportion of affected children was 10.4%. Adolescents aged 13 to 15 are also affected, with overweight and obesity rates of 14.6% and 2.8%, respectively, according to the 2010 Moroccan School Student Health Survey[9].

Recent research indicates that the first three years of a child's life can predict the development of obesity. During this initial phase, there is a critical involvement in molding eating habits and lifestyle, exerting a substantial impact on the child's subsequent weight trajectory. Family environments and behaviors during these early years are crucial factors associated with predisposition to obesity in children. These findings highlight the importance of implementing preventive interventions and health promotion programs from the earliest years of life to reduce the risk of childhood obesity[10].

The WHO recognizes that the battle against the epidemic of childhood obesity cannot rely solely on isolated interventions. Indeed, it is crucial to consider the three critical stages of life: preconception and pregnancy, infancy, and childhood and adolescence[11].

Concerning preconception and pregnancy, the WHO strongly advocates for screening and management of hyperglycemia and gestational hypertension. It is also essential to ensure appropriate follow-up and management to control weight gain during pregnancy. Additionally, guidance and counseling for future parents before conception and during pregnancy must emphasize the importance of suitable nutrition[11,12].

Various studies have investigated the implications of fetal macrosomia, highlighting its significant association with overweight and obesity[13,14]. A systematic review, incorporating the results of 20 separate studies, corroborated this

Table 5 Childhood obesity management practices among healthcare professionals

Parameter	*n*	Percentage (%)
On an average week, how many obese children come to you		
Less than 10 per week	73	93.6
11 to 20 per week	5	6.4
21 to 50 per week	0	0
Over 50 per week	2	12.9
What criteria and tools do you use to detect obesity in children		
Clinical examination	37	45.7
Physical appearance	26	32.1
Weight and height	39	48.1
Balance	24	29.6
BMI > 30	39	48.1
Study of growth curve/chart	31	38.3
Weight and height curves	40	49.4
Weight/height ratio	22	27.2
What areas in the overall management of this child's obesity seem essential to you		
Nutrition and parenting	48	61.5
Family history, environment	23	29.5
Parent's motivations	29	37.2
Child education	38	48.7
Children's diet	40	51.3
Children's physical activity	33	42.3
Psychological evaluation of	28	35.9
Child's lifestyle	47	60.3
Children's habits	25	32.1
History of childhood obesity	23	29.5
Child's motivation	25	32.1
What network of partners would you be likely to mobilize in this care		
Dietician	43	53.8
Nutritionist	47	58.8
Psychiatrist	21	26.3
Pediatrician	33	41.3
Endocrinologist	33	41.3
Sport educator	24	24
Physical education teacher	14	17.4
Multidisciplinary team	1	1.3
Parents and child	1	1.3
Prent first	1	1.3
Child psychiatrist	1	1.3
Have you received training in childhood obesity		
Yes	7	9.1
No	67	87

If you were offered training in the management of childhood obesity, what topics would you like to see covered		
Behavioral approaches/therapies	29	38.7
Psychotherapy	15	20
Psychological approach	16	16
Low calories diets	20	26.7
Nutrition	42	56
Ways to motivate children and parents	23	30.7
Preventive and educational methods	21	28
Request for treatment in specialized centers	9	12
Follow-up of childhood obesity in clinical cases	26	34.7
Clinical and metabolic consequences of obesity	17	22.7
Complications in obese children	19	25.3

relationship, demonstrating that macrosomia is a significant risk factor for obesity in both children and adults[15].

In particular, the development of macrosomia is intimately linked to several factors, including maternal obesity and gestational diabetes. The mechanisms underlying this complex relationship highlight the impact of maternal health conditions on the child's birth weight and, by extension, on his or her subsequent risk of developing obesity problems[16].

From the results of the responses obtained from physicians (Figure 2A), it is clear that the search for risk factors associated with macrosomia is not a systematic practice within the medical community. This finding suggests variability in clinical approach, underlining the need for greater awareness of macrosomia risk assessment during medical consultations.

On the other hand, according to our findings, 19% of doctors do not recommend that overweight pregnant women consult a dietician, and 6.9% do not even educate women about the risks associated with excessive weight gain during pregnancy. Worryingly, 24.4% of overweight pregnant women are unaware of the repercussions of excess weight on their health and that of their child. In addition, more than half of doctors (52.3%) do not feel sufficiently informed about the care of obese pregnant women, which could explain the lack of advice given to their patients (Table 2).

WHO guidelines encompass all manifestations of malnutrition, whether nutritional deficiency or nutritional excess[11, 12], the latter being of particular concern in Morocco. Cultural influence, in particular the misconception widespread among Moroccan mothers and society, encourages pregnant women to adopt the erroneous idea that they need to eat for two, leading to maternal overweight. This misperception contributes to nutrition-related health problems, and underlines the importance of increased education and awareness to promote healthy eating practices during pregnancy. In addition, a study carried out at the maternity hospital in Morocco revealed that maternal overweight and obesity are a problem in the population studied, with prevalence of 34.9% and 41% respectively[17].

These data underline the urgent need for training programs to raise awareness and encourage doctors to provide appropriate advice to overweight pregnant women. Filling these knowledge gaps is essential to ensure proper management. Previous studies have clearly demonstrated that dietary advice can have very positive effects, not only on gestational weight gain, but also on glucose metabolism, underlining the importance of strengthening the training of healthcare professionals in this specific area[18,19].

According to our study, 76.7% of participants consider diabetes during pregnancy to be a risk factor for childhood obesity, followed closely by maternal weight gain and maternal obesity (56.7%; Table 4). These findings align with doctors' observations, which are in complete accordance with the extensive scientific research in this field. Various studies have conclusively established that gestational diabetes is a significant risk factor for the subsequent development of obesity in young children[20,21]. In addition, a review by Adriana Mannino and colleagues concluded that the impact of maternal overweight on childhood obesity is no longer open to dispute, underlining the need for targeted interventions to prevent this growing scourge[22].

However, it is interesting to note that, in practice, little attention is given to maternal weight during medical consultations (Figure 2B). This neglect may stem from the fact that 51.7% of participants did not feel sufficiently informed about the impact of maternal obesity on pediatric obesity. This finding raises further important questions about the awareness and importance given to this specific aspect of maternal health during medical interactions. This underscores the need for increased awareness and also highlights potential gaps in participants' knowledge and understanding of the crucial link between maternal weight and its impact on pediatric health. These observations further emphasize the imperative of targeted educational initiatives and reinforced communication strategies to bridge this awareness gap among individuals participating in medical consultations[11].

It is crucial to provide clear guidance and support to promote good nutrition, healthy diets, physical activity and to discourage the use of, and exposure to, tobacco, alcohol, drugs and other toxins[11].

As far as early childhood is concerned, the WHO has drawn up a number of guidelines to promote healthy eating, adequate sleep and physical activity, with the aim of instilling good habits in children from an early age. Among these guidelines, some are of particular importance. Firstly, it is crucial to raise parents' awareness of the benefits of breast-feeding for both mother and child, through appropriate training. It is also essential to actively encourage mothers to opt

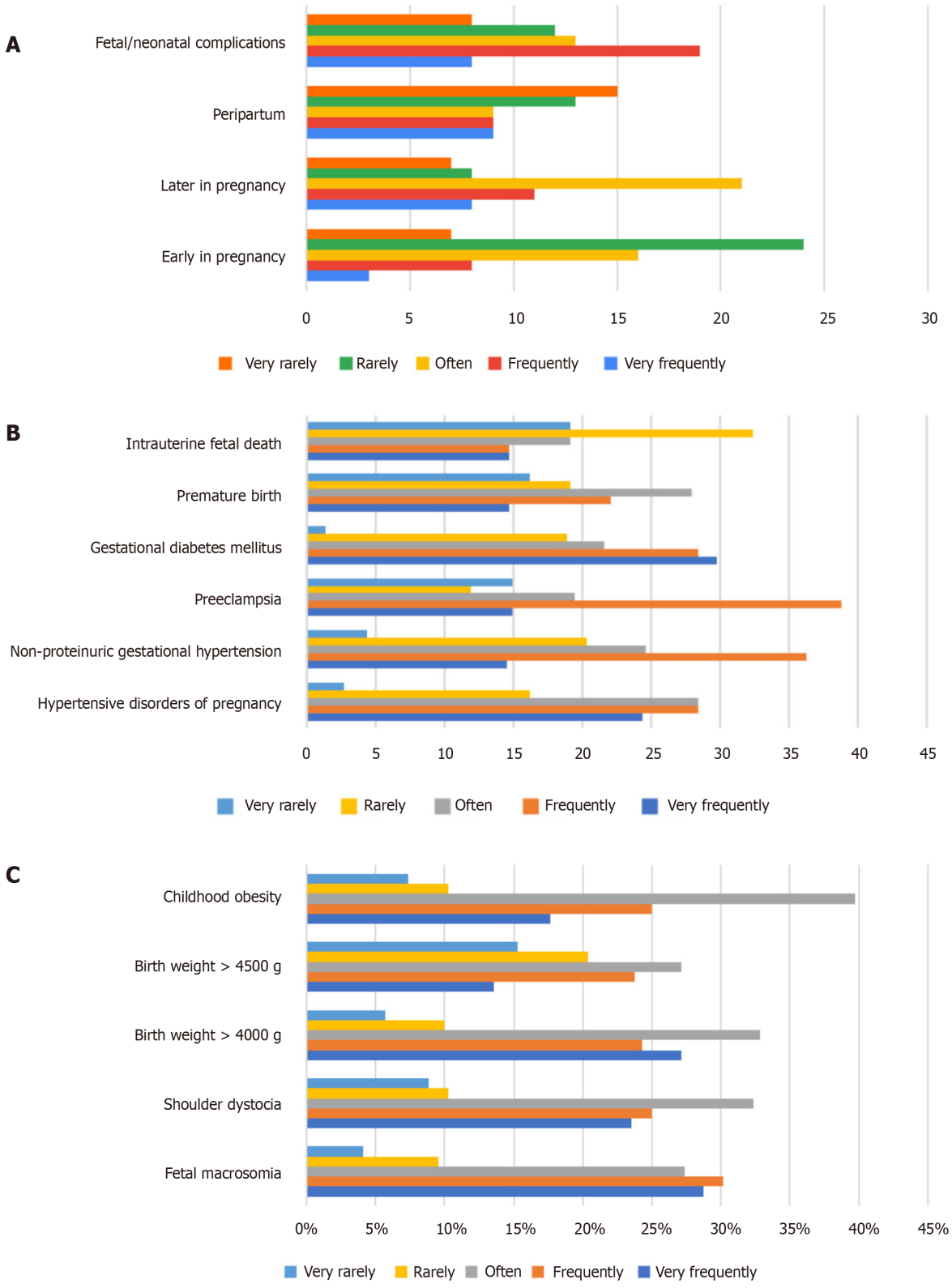

Figure 1 Frequency of obstetrical complications. A: Frequency of obstetrical complications in obese pregnant women; B: Frequency of obstetrical complications during pregnancy in obese women; C: Frequency of fetal/neonatal complications obstetrical complications in obese women.

for breastfeeding. In addition, clear guidance needs to be provided on recommended sleep duration and the management of time spent on sedentary activities, helping to foster healthy development in children and prevent potential health problems[11].

A recent study in Norway, involving 170 children, has deepened our understanding of the links between childhood obesity and sleep patterns. The results of this research highlight an important nuance beyond simple sleep duration, emphasizing that the precise timing of sleep may represent a risk factor of more substantial concern than total sleep duration. In particular, the study significantly observed that overweight children have later sleep schedules compared

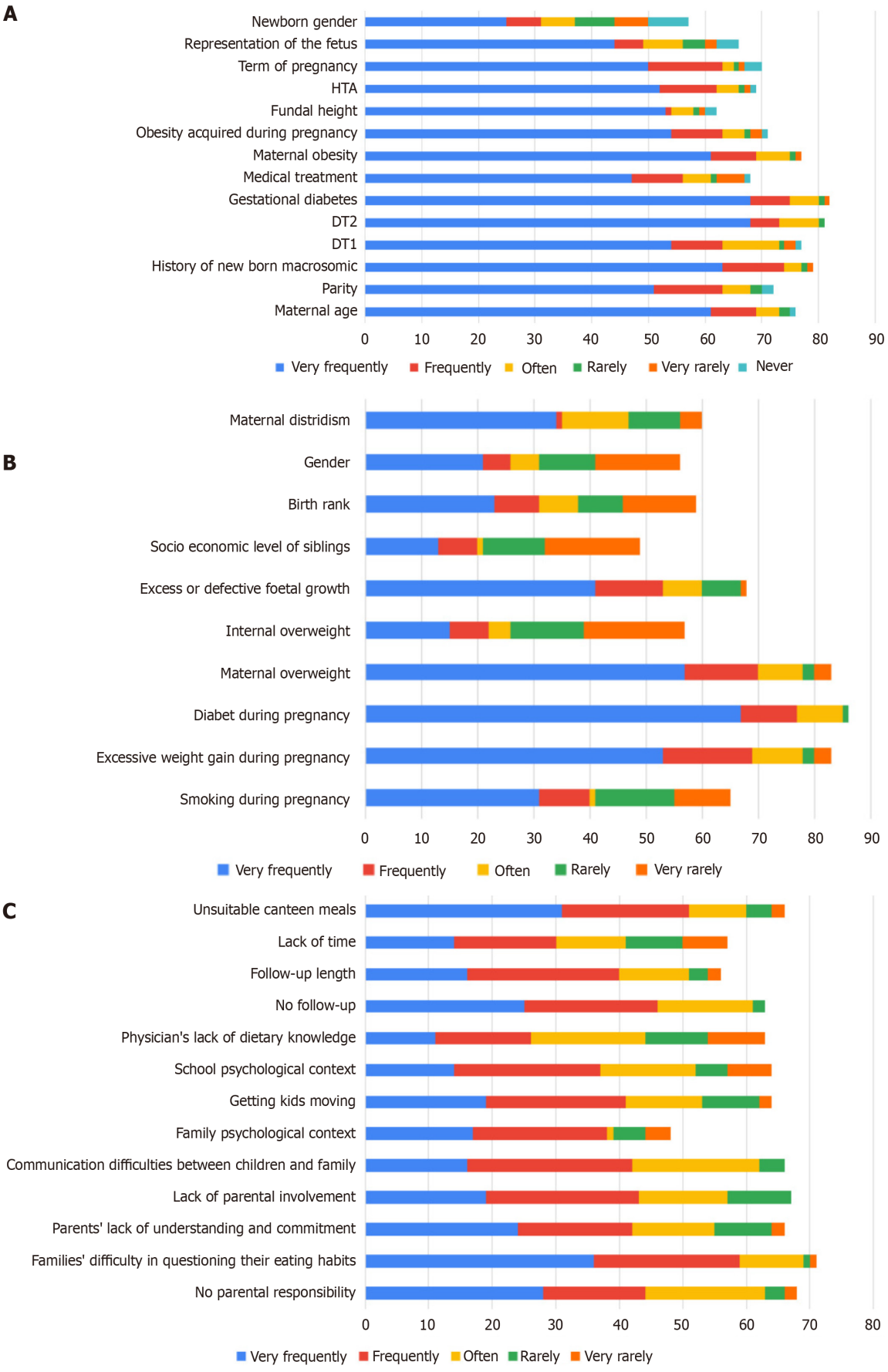

Figure 2 Frequency of risk factor and obstacles. A: Frequency of risk factor screening during consultations; B: Prenatal consultations: frequency of maternal risk factor testing; C: Obstacles facing healthcare professionals to monitoring obese children. HTA: Hypertension arterial; DT1: Type 1 diabetes; DT2: Type 2

diabetes.

with their normal-weight peers. This finding suggests a correlation between late sleep schedules and the risk of obesity in children. These findings could inform health recommendations to promote healthy sleep habits from childhood onwards, aiding in averting childhood obesity and enhancing the general well-being of young people[23].

According to the conclusions drawn from our study, a significant proportion - 61.5% of participants - consider nutrition and parental education to be crucial areas in the management of childhood obesity. In a more immediate context, the child's lifestyle was also considered essential by 60.3% of participants, closely followed by diet-related concerns, mentioned by 51.3% (Table 4).

With regard to obesity screening, there is no comprehensive guide to optimal screening for childhood obesity. This is in line with the results of our survey: participants used different strategies to identify childhood obesity (Table 4).

Early detection of childhood obesity represents a crucial issue in promoting children's health, but it proves to be a delicate task due to the various factors involved. Identifying the early signs of this condition in its initial stages is, of course, complex. Indeed, it's becoming imperative for doctors to systematically incorporate the calculation of BMI into every pediatric consultation.

Regular monitoring of BMI and its recording on weight curves creates a visual history of a child's weight growth over time. According to the latest research, doctors should systematically examine weight/length curves for children under two and BMI for children over 2, in addition to standard weight and length curves. As BMI increases with age, percentiles specific to age and sex are used rather than BMI[24].

This procedure provides health practitioners with crucial data for assessing a child's physical evolution and detecting any propensity towards obesity in the early stages. It is also relevant to note that 60% of children who are overweight at the age of one will not maintain this condition into adulthood. These curves are particularly useful around the age of 6, when physiological variations in weight can make clinical assessment misleading. At this age, children of normal build who may appear slim could be masking overweight in those whose BMI is around the 97th percentile, which may not be clinically evident[25].

Emphasizing the importance of this approach highlights the need for constant vigilance on the part of practitioners to anticipate and treat any risk of obesity in children at an early stage. This proactive approach not only contributes to preventive health management, but also offers the opportunity to initiate early interventions, thus promoting more positive outcomes for children's long-term well-being.

Participants also recognized the importance of collaboration with nutritionists and dietitians in the management of childhood obesity. Participants acknowledged several obstacles in the management of obesity (Figure 2C), arising from families reluctant to examine their eating habits, insufficiently adapted meals in the canteen, inconsistent monitoring practices, communication barriers between children and their families, and a lack of parental responsibility (Figure 2C). These challenges collectively underscore the complex nature of effectively monitoring the health of these children. Tackling these obstacles requires a comprehensive, multidimensional approach, encompassing not only dietary and lifestyle considerations but also a nuanced understanding of the interpersonal dynamics within families. Effective monitoring demands a holistic approach to these issues, recognizing the interdependence of the various elements that contribute to the overall health and well-being of these children.

A significant portion of participants indicated that they had never received training in childhood obesity and expressed an interest in undergoing nutrition training to enhance their ability in dealing with obesity cases (Figure 2C). This desire for additional training underscores the recognition among healthcare professionals of the central role nutrition plays in the management of childhood obesity. The call for nutrition training reflects a proactive stance on the part of these professionals, signaling a commitment to enhancing their expertise and, ultimately, improving the quality of care provided to children facing obesity problems.

These findings collectively underscore the imperative of targeted educational programs and initiatives within the medical community. Bridging the knowledge gap through comprehensive training not only meets the immediate needs of healthcare professionals, but also contributes to the broader goal of fostering a more informed and competent healthcare system in the face of the growing concern about childhood obesity.

In 2014, a collaborative team of researchers from Tunisia, Morocco, France and England formulated a strategy to mitigate the escalation of obesity as part of the Cooperation in university and scientific research obe-Maghreb research project. This initiative, active from 2013 to 2017, comprises five distinct strands: encouraging the production of health-promoting foods, shaping the environment to encourage healthy physical activity, educating the population on the adoption of good eating behaviors, improving obesity screening and management services, and implementing an information, communication and education plan to prevent and combat obesity. The fourth component is dedicated to strengthening the basic training program for medical and paramedical students in the detection and management of obesity. This involves developing teaching modules, integrating and implementing training in screening and management, and establishing systematic screening for excess weight before and during the university career. In addition, this component aims to reinforce the basic training program for students in the medical and paramedical fields by systematically screening for obesity in pre-school, school, professional (public and private) and health environments [26].

Morocco's commitment to tackling childhood obesity and aligning itself with the recommendations of WHO and the guidelines of the 2011-2019 national nutrition strategy cannot be overlooked. Indeed, Morocco launched a promising strategy in 2016 to develop a plan to prevent and control obesity and overweight among children. This strategy is structured around several stages: first, a literature review of international recommendations and other countries'

experiences in preventing and controlling childhood obesity. Next, a summary of the current nutritional and epidemiological situation of children, identifying the gains to be consolidated and the opportunities to be seized. In addition, a review of programs and interventions carried out by the Ministry of Health and its partners, aimed at contributing to the prevention and control of overweight and obesity, identifying strengths and areas for improvement. Secondly, the establishment of an operational plan adopting a logical framework approach, followed by the presentation and discussion of the operational plan. Finally, the facilitation of national consensus workshops and the final validation of the above-mentioned plan. This approach demonstrates Morocco's commitment to the fight against childhood obesity by adopting a strategic and coordinated approach in line with international best practices[9]. However, to date, there has been no feedback on the implementation of the above-mentioned steps. This observation underlines the importance of implementing transparent and regular monitoring to assess the effectiveness of the measures undertaken and guarantee the success of this national strategy.

Establishing dedicated pediatric obesity medicine centers would be highly advantageous in addressing this significant health concern. Such centers are pivotal in the comprehensive management of childhood obesity, offering essential resources and infrastructure to foster new knowledge generation and innovation in the field. Additionally, these centers hold the potential to provide multidisciplinary training, equipping the next generation of scientists with the skills necessary to effectively address this intricate issue.

Specialized training programs conducted by these centers can play a crucial role in increasing awareness and educating healthcare professionals, researchers, and practitioners involved in childhood obesity management. By disseminating in-depth knowledge and promoting a collaborative approach, these centers contribute to the development of expertise in the field.

Moreover, these specialized centers possess the capability to actively contribute to the formulation of guidelines and treatment protocols aimed at enhancing childhood obesity management. Through collaboration with multidisciplinary experts, they can develop evidence-based guidelines that incorporate the latest scientific advancements, guiding practitioners toward effective and appropriate interventions.

Furthermore, it would be pertinent to integrate the monitoring of overweight pregnant women into initiatives aimed at preventing obesity associated with maternal obesity. In particular, centers dedicated to pediatric obesity medicine could expand their focus to encompass the monitoring and management of pregnant women's weight.

This integrated approach, addressing both maternal and child health, holds significant preventive potential in the battle against obesity from the earliest life stages. It underscores the importance of a holistic strategy, wherein the management of childhood obesity is intricately linked to the monitoring and management of maternal weight, fostering more positive outcomes for the overall health of the family.

Finally, nations should implement or establish effective mechanisms for disseminating research findings to both the general public and relevant healthcare professionals. This dissemination can be achieved through existing national, regional, and international journals, as well as through websites and databases that may have regional relevance.

In Morocco, there is a deficiency in scientific publications, newspapers, and magazines dedicated to the subject of obesity. The accessibility of scientific information plays a crucial role in keeping knowledge up-to-date and conveying new expertise and treatments to physicians. Nevertheless, there is a genuine necessity to facilitate the transmission of information to physicians by establishing Moroccan publications specifically focused on obesity.

CONCLUSION

If the primary objective of medicine is to prioritize the preservation of life, it becomes essential to articulate the conditions of survival and define the quality of that survival. Through adequate education and comprehensive training, we can collaboratively enhance the quality of life for our patients. Empowering local doctors with the necessary expertise to deliver specialized care for childhood and maternal obesity is the only sustainable approach to expanding access to such care. However, achieving this goal requires the establishment of a robust infrastructure, backed by local authorities, and collaboration with countries where the management of childhood and maternal obesity is a common practice. If the primary objective of medicine is to prioritize the preservation of life, it becomes essential to articulate the conditions of survival and define the quality of that survival. Through adequate education and comprehensive training, we can collaboratively enhance the quality of life for our patients. Empowering local doctors with the necessary expertise to deliver specialized care for childhood and maternal obesity is the only sustainable approach to expanding access to such care. However, achieving this goal requires the establishment of a robust infrastructure, backed by local authorities, and collaboration with countries where the management of childhood and maternal obesity is a common practice.

FOOTNOTES

Author contributions: Douiyeh I performed the research and analyzed the data; Khamlich J contributed to analyze the data; Nabih N and Saih A contributed to data collection; Boumendil I and Regragui A contributed to article format; Kettani A and Safi A contributed to manuscript editing; All authors have read and approve the final manuscript.

Institutional review board statement: We surveyed physicians who were members of the association with which we collaborated on our study. This collaborative initiative was established to uphold rigorous ethical and professional standards in the research process. It is imperative to acknowledge that this research has secured requisite ethical approval from the Association of Physicians (ACHAS), which

functions as the designated ethical review board overseeing this investigative inquiry. All procedural aspects were meticulously executed in alignment with the ethical principles set forth by the association, thereby safeguarding the research's integrity and upholding the rights of participating individuals.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Transcranial direct current stimulation as early augmentation in adolescent obsessive compulsive disorder: A pilot proof-of-concept randomized control trial

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Abstract

BACKGROUND

Transcranial direct current stimulation (tDCS) is proven to be safe in treating various neurological conditions in children and adolescents. It is also an effective method in the treatment of OCD in adults.

AIM

To assess the safety and efficacy of tDCS as an add-on therapy in drug-naive adolescents with OCD.

METHODS

We studied drug-naïve adolescents with OCD, using a Children's Yale-Brown obsessive-compulsive scale (CY-BOCS) scale to assess their condition. Both active and sham groups were given fluoxetine, and we applied cathode and anode over the supplementary motor area and deltoid for 20 min in 10 sessions. Reassessment occurred at 2, 6, and 12 wk using CY-BOCS.

RESULTS

Eighteen adolescents completed the study (10-active, 8-sham group). CY-BOCS scores from baseline to 12 wk reduced significantly in both groups but change at baseline to 2 wk was significant in the active group only. The mean change at 2 wk was more in the active group (11.8 ± 7.77 *vs* 5.25 ± 2.22, $P = 0.056$). Adverse effects between the groups were comparable.

CONCLUSION

tDCS is safe and well tolerated for the treatment of OCD in adolescents. However, there is a need for further studies with a larger sample population to confirm the effectiveness of tDCS as early augmentation in OCD in this population.

Key Words: Adolescents; Early augmentation; Obsessive compulsive disorder; Safety; Transcranial direct current stimulation

Core Tip: Transcranial direct current stimulation (tDCS) is a safe treatment modality in the management of obsessive compulsive disorder (OCD) in adolescents. Cathodal stimulation over the supplementary motor area produces insignificant improvement in severity of OCD than sham controls. The improvement following 10 sessions of tDCS is mostly short-lasting and usually goes off by 6 wk.

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INTRODUCTION

Obsessive compulsive disorder (OCD) is among the most common psychiatric disorders, with a mean age of onset of 19 years and almost a quarter of patients developing the disorder by the age of 10 years[1]. Transcranial direct current stimulation (tDCS) stimulates the brain with electrodes on the scalp. It's safe, affordable, and can activate or suppress cortical activity. Research on its effectiveness in treating OCD is limited to a few studies on adults who are treatment-resistant[2,3]. Even in such patients, it has shown promising results. There is significant heterogeneity between the studies. The electrode placement sites include the supplementary motor area (SMA), orbitofrontal cortex (OFC), and right and left dorsolateral prefrontal cortex (DLPFC)[2,4]. A systemic review of three meta-analyses of tDCS in OCD suggested cathode placement at pre-SMA and anode placement at the extra-cephalic sites as the site with better results. Pre-SMA has been shown to be an effective site for neuromodulation in OCD in repetitive transcranial magnetic stimulation (rTMS) studies as well[4]. By keeping the anode at extracephalic sites the chance of short-circuiting the current flow is reduced. Extra-cephalic sites that have previously been studied include the occipital region, neck, and deltoid. Theoretically, when using deltoid as the extracephalic site, the current will traverse through a pathway involving the striatum, which is a major brain area involved in OCD. The choice of right deltoid was made to save the cardiac electrical conduction from the ill effects of tDCS current. This electrode placement results in modulation of the majority of areas (cortical and sub-cortical) implicated in OCD[5].

tDCS use as early augmentation can lead to an earlier response in OCD with functional recovery. The aim of this randomized controlled trial (RCT) was to assess the efficacy of tDCS as an add-on therapy in drug-naive adolescents with OCD. We hypothesized that utilizing tDCS as an additional therapy with fluoxetine would result in an earlier reduction in obsessive-compulsive symptoms than using fluoxetine with sham tDCS.

MATERIALS AND METHODS

Study design

This was a randomized, single-blind, sham-controlled trial conducted at a tertiary care centre in India. Adolescents between 10 and 18 years of age attending Outpatient services at King George's Medical University, Lucknow and diagnosed with moderate OCD based on DSM-5 criteria who were drug naïve (for at least 1 month) were included[6,7]. The diagnosis of OCD and comorbidities was made using the Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version. The Children's Yale-Brown obsessive-compulsive scale (CY-BOCS) was used to assess the severity of OCD, and cases with a score ≥ 13 were included. Individuals with medical comorbidities requiring priority management, any other psychiatric conditions except major depressive disorder (MDD), intellectual disability, or any contraindication to tDCS were excluded. Revised Child Anxiety and Depression Scale-Hindi adaptation (RCADS-H) was applied to assess anxiety and depressive symptoms. Ethical approval was obtained from the Institutional Ethics Committee (107th ECM II B-Thesis/P16). Written informed consent from the parent/guardian and assent of the adolescent was obtained. The study was registered prospectively with the Clinical Trials Registry of India (CTRI/2021/12/039002). After which recruitment was done between January 2022 and January 2023 with follow up completed by April 2023.

Randomization and blinding

The sample size was calculated through Priori analysis using "G Power: Statistical Power analyses 3.1.9.7" application[8]. Considering an effect size of 0.25, power of 80%, and drop out rate of 20%, the sample size calculated was 30. Participants attending child and adolescent psychiatry outpatient department on specified days of the week were enrolled in the study by investigators (Agarwal V, Kar SK, Arya A). Adolescents who met the inclusion criteria were enrolled in the study by the principal investigator (Agrawal A). Randomization was done using a computer-generated random number

table which was generated by the co-investigator (Agarwal V) method in a 1:1 manner in true and sham groups. Blinding of allocation was ensured by giving a sham stimulation as described below. The investigator was aware of the allocation.

tDCS protocol

Stimulation was provided with tDCS device-Neurostim by Neurosoft, Russia. Two electrodes (size: 5 cm × 5 cm) were placed in the sponge (7 cm × 5 cm) to deliver current after being secured with rubber bands. The cathode was placed on the left SMA, corresponding to FC1 as per the international 10-20 EEG system[9]. During the tDCS session, a current of 2 mA was applied for 20 min with an anode on the right deltoid. The procedure was performed once daily until 10 sessions were completed, with no more than two consecutive days skipped. Adolescents were started on 20 mg of fluoxetine daily, with the dose increased to 40 mg/d after 1 wk. The treating psychiatrist adjusted the dose of fluoxetine during the follow-up period. Adolescents in the sham group were also provided with a brief stimulation after applying electrodes at the same site. Sham stimulation consisted of ramp-up and ramp-down periods of 20 s each during the start and end of the session. During the intervening 20 min, no current was passed. Such stimulation provides somatosensory effects and is recognized as the 'gold standard' sham tDCS method[10,11].

Clinical assessments of study variables

We gathered information from adolescents on their demographics and illness. We measured clinical symptoms, CY-BOCS, and RCADS-H scores at different intervals. CY-BOCS is an adapted version of the Yale-Brown Obsessive compulsive scale (Y-BOCS) for use in children and adolescents. Y-BOCS is the gold standard assessment scale for obsessive compulsive symptoms and is widely used in research as well as clinical practice. It has high reliability (α = 0.87) and good validity (r = 0.62)[12]. RCADS-H is the Hindi adaptation of RCADS done by Mishra *et al*[13]. The original scale has high reliability and moderate validity scores in all subscales except for OCD subscale[14]. To check for side effects, we used a checklist developed from a study done by Eryilmaz *et al*[15]. Due to coronavirus disease 2019 (COVID-19), assessments were in-person or over the phone. Any minor adverse effects were treated conservatively. We took all COVID-19 precautions.

Statistical data analysis

We used IBM SPSS version 25.0 to analyze data. Descriptive statistics were performed on demographic and clinical variables. We checked for normalcy with the Shapiro-Wilk test. We compared baseline characteristics using the Fisher's exact test for categorical data and the Mann-Whitney *U* test for numeric variables. Repeated measures ANOVA assessed outcome measure changes. We used Hedges' g for effect size and considered *P*-values < 0.05 statistically significant.

RESULTS

The trial was conducted in 2022 after the clinical trial registry was completed. A total of 72 adolescents with OCD were screened, out of which 51 were excluded. Finally, 21 adolescents were enrolled and randomized; 18 completed the intervention, and 9 followed up till 12 wk (Figure 1). Baseline clinical and demographic characteristics of the groups were comparable (Table 1). Among the active group, the majority had contamination-related obsessions (n = 7, 70%), washing/cleaning compulsions (n = 6, 60%). In the sham group, the majority had aggression-related obsessions (n = 6, 75%) and washing/cleaning compulsions (n = 5, 62.5%).

Outcome measures

Patients who completed the entire protocol till 12 wk were included in the per protocol (PP) analysis (n = 9, active-6, sham-3). Intention to treat (ITT) analysis was done, with missing data handled using the Last Observation Carried Forward method (n = 18, active-10, sham-8). Total CY-BOCS score in ITT analyses is shown in Figure 2. The total CY-BOCS score was higher in active tDCS (M = 26.83, SD = 6.01) as compared to sham tDCS (M = 22.67, SD = 4.51) but the difference was not statistically significant. (U = 18, P = 0.056). Intergroup differences were not observed in CY-BOCS or RCADS-H scores at any point of assessment. The repeated-measures ANOVA for the CY-BOCS obsession subscale scores and total scores revealed a significant reduction with time in both groups in ITT but only in the active group in PP analysis (Table 2). Significant reduction in CY-BOCS compulsions subscale over time was present in both groups (in ITT and PP analyses).

RCADS-H subscale and total scores showed group time interaction to be significant in the MDD subscale [F (3) = 3.55, P = 0.50]. The mean reduction in CY-BOCS subscales and total scores was compared between both groups during the initial 2 wk (Table 3). Response rates at 2 wk were not significantly different [8 out of 10 in the active *vs* 3 out of 8 in the sham group, Fischer's exact test P = 0.145, number needed to treat = 2.35, 95%CI: (1.2, 127.2)].

Safety measures

No major adverse effects were recorded, and tDCS stimulation was well tolerated. 8 of 10 adolescents in active and 7 of 9 in sham group reported at least one side effect (relative risk = 1.03, number needed to harm = 45.0). For an individual session, 53.3% of active stimulations and 51.4% of sham stimulations resulted in a side effect. The maximum relative risk was for headache (2.14 for per session incidence and 1.56 for per individual incidence). Other side effects commonly reported were numbness, itching, pain at the stimulation site and sedation.

Table 1 Sociodemographic and clinical variables among the groups, mean ± SD, *n* (%)

	Active tDCS (*n* = 10)	Sham tDCS (*n* = 8)	*P* value
Age, yr	14.4 ± 2.4	14.3 ± 2.5	0.96
Age at onset, yr	12.7 ± 3.6	12.0 ± 1.9	0.69
Duration of symptoms, months	19.8 ± 19.5	28.3 ± 29.2	0.68
Gender, male	5 (50)	6 (75)	0.28
Religion, Hindu	8 (80)	7 (87.5)	0.67
Education			
Up to high school	8 (80)	6 (75)	0.8
Up to intermediate	2 (20)	2 (25)	
Domicile, urban	6 (60)	4 (50)	0.67
Type of family, nuclear	5 (50)	5 (62.5)	0.59
Number of family members			
≤ 5	4 (40)	5 (62.5)	0.34
> 5	6 (60)	3 (37.5)	
Family income in Rs/month			
≤ 20000	4 (40)	3 (37.5)	0.91
> 20000	6 (60)	5 (62.5)	
Developmental delay	2 (20)	-	
Past history			
Hypothyroidism	1 (10)	-	
OCD	2 (20)	-	
Depressive episode	-	1 (12.5)	
Family history of psychiatric illness, present	2 (20)	-	
Co-morbidities			
Excoriation	1 (10)	-	
Subnormal intelligence	1 (10)	-	
Major depressive disorder	-	1 (12.5)	
Hospitalization	2 (20)	-	

tDCS: Transcranial direct current stimulation; OCD: Obsessive compulsive disorder.

Medications

The average dose of fluoxetine and clonazepam was similar in both groups across all assessments. Adjunct medications were Risperidone 0.5 mg/d (2 in the active group, started after completion of tDCS sessions), Melatonin 3 mg (1 in the active group), and Propranolol 20 mg/d (1 in the sham group).

DISCUSSION

To the best of our knowledge, this is the first study worldwide that: (1) Has utilized tDCS as an early intervention strategy and (2) has studied tDCS in adolescents with OCD[16]. There was a significant reduction in CY-BOCS scores in both groups across the time frame assessed. Despite having a small sample size and single blinding, we did not find any significant intergroup differences in various statistical methods. A number of reasons could explain these findings, including early onset is a known risk factor for poor prognosis, biological differences in early onset OCD could be one of the key reasons. Stimulation parameters that showed positive results had tried twice-daily sessions and longer sessions of up to 30 min. Whether such changes in stimulation parameters could affect response in OCD is yet to be studied. An RCT targeting left OFC with cathodal stimulation also found non-significant changes between active and sham groups, which were controlled for single drug[17]. Another RCT with left DLPFC anodal and right OFC cathodal stimulation along with

Table 2 Repeated measures ANOVA test for within group and between groups in both intention to treat and per protocol analysis, mean ± SD

Clinical variables	Active tDCS group	Sham tDCS group	Time group interaction (*F*; *P* value)
CY-BOCS obsession score (0-20)			
Baseline			
ITT	13.60 (3.06)	11.38 (3.20)	
PP	15.16 (2.92)	11.67 (3.51)	
2 wk			
ITT	7.40 (3.20)	9.0 (3.21)	
PP	6.17 (3.54)	9.00 (3.60)	
6 wk			
ITT	7.60 (2.84)	6.50 (2.39)	
PP	6.33 (2.73)	7.67 (2.08)	
12 wk			
ITT	6.20 (3.29)	5.75 (2.19)	
PP	4.00 (1.67)	5.67 (2.08)	
Within the group (*F*; *P* value)			
ITT	13.42; < 0.001[1]	39.34; < 0.001	2.20; 0.129[1]
PP	29.52; 0.001[1]	9.12; 0.09[1]	3.77; 0.051[1]
CY-BOCS compulsion score (0-20)			
Baseline			
ITT	11.50 (2.99)	9.0 (3.82)	
PP	11.67 (3.93)	11.0 (1.00)	
2 wk			
ITT	5.90 (4.09)	6.12 (3.00)	
PP	4.0 (4.19)	7.0 (2.64)	
6 wk			
ITT	5.90 (3.63)	4.50 (2.92)	
PP	3.83 (2.71)	6.67 (2.51)	
12 wk			
ITT	5.10 (4.07)	3.38 (2.06)	
PP	2.50 (2.42)	3.67 (1.52)	
Within the group (*F*; *P* value)			
ITT	17.10; < 0.001[1]	17.83; < 0.001	1.44; 0.247[1]
PP	34.58; < 0.001	16.05; 0.015[1]	2.22; 0.115
CY-BOCS total score (0-40)			
Baseline			
ITT	25.10 (5.06)	20.38 (4.21)	
PP	26.83 (6.01)	22.67 (4.51)	
2 wk			
ITT	13.30 (6.92)	15.12 (4.39)	
PP	10.17 (7.19)	16.00 (6.00)	
6 wk			

ITT	13.50 (5.87)	11.0 (4.57)	
PP	10.17 (4.26)	14.33 (4.16)	
12 wk			
ITT	11.30 (7.18)	9.12 (3.44)	
PP	6.50 (3.62)	9.33 (3.51)	
Within the group (*F*; *P* value)			
ITT	16.55; < 0.001[1]	40.91; < 0.001	2.13; 0.137[1]
PP	43.76; < 0.001	14.82; 0.051[1]	3.91; 0.06[1]

[1]Greenhouse-Geisser correction. CY-BOCS: Children's Yale-Brown obsessive-compulsive scale; ITT: Intention to treat; PP: Per protocol.

Table 3 Comparison of mean reduction in Children's Yale-Brown obsessive-compulsive scale total score between 2 groups across different follow-up periods, mean ± SD

Change in C-YBOCS scores	Active tDCS (*n* = 10)	Sham tDCS (*n* = 8)	*U* value	*P* value
Baseline *vs* 2 wk				
Obsessions	6.2 ± 4.26	2.37 ± 0.99	18	0.056
Compulsions	5.6 ± 3.75	2.87 ± 1.76	22	0.121
Total	11.8 ± 7.77	5.25 ± 2.22	18	0.056
Baseline *vs* 6 wk				
Obsessions	7.0 ± 3.90	4.87 ± 1.53	22.5	0.131
Compulsions	6.5 ± 3.75	4.5 ± 2.64	25	0.312
Total	11.6 ± 6.93	9.37 ± 2.87	28	0.307
Baseline *vs* 12 wk				
Obsessions	11.2 ± 1.66	9.0 ± 4.0	27.5	0.285
Compulsions	10.0 ± 2.0	7.5 ± 3.2	19	0.069
Total	13.8 ± 8.68	11.25 ± 3.34	29.5	0.373

tDCS: Transcranial direct current stimulation; CY-BOCS: Children's Yale-Brown obsessive-compulsive scale.

fluoxetine found non-significant differences with active and sham tDCS[18].

There were no comparator studies available for the adolescent age group. Among studies in adults with OCD, a trial by Silva *et al*[19] using cathodal SMA stimulation and anodal stimulation over the right supraorbital area showed a significant difference on repeated measures-ANOVA, group time interaction. However, the change in YBOCS scores was noticed in the period between 6 and 12 wk. The authors suggested a delayed effect of tDCS through neuroplasticity. Since we lost a number of patients during this period, we could have missed the delayed effects of tDCS. Multiple studies, including 3 RCTs, however, support the contrary hypothesis of acute effects of tDCS[3,17,20].

Stimulation sites on SMA have shown positive results in a number of studies other than one by Silva *et al*[19]. An open-label trial using cathodal tDCS over SMA and anodal stimulation over the right supraorbital area in treatment-resistant OCD reported a 26% decrease in Y-BOCS scores[20]. Even anodal stimulation at SMA has reported comparable findings in 25 adults with treatment-resistant OCD[3]. These differences arise from the use of medications, the level of resistance in patients included in the study, and other methodological issues.

A significant difference between groups was observed in the change in depressive symptoms. Comorbid depressive symptoms are common in OCD, and one RCT has reported such a change in depressive symptoms, while another one reported a non-significant reduction in depressive and anxiety symptoms[19,20].

Response in OCD is defined as ≥ 35% reduction in CY-BOCS scores and remission as CY-BOCS ≤ 12 score[21]. Our study found a high response rate of 80% at 2 wk, although the difference with the sham group was not significant. Larger trials have found lower response rates ranging between 15% and 19%[19,20]. The high response rate can be attributed to the selection of drug naïve adolescents and the exclusion of treatment-resistant cases and those with comorbidities. A trial by Yoosefee *et al*[18], which excluded treatment-resistant cases and used fluoxetine, reported similar response rates in active and sham groups. But they used a lower frequency of sessions (3/wk) and did not target SMA[5]. 5 of 10 adolescents in the active group attained remission, which was sustained till 12 wk. No other studies reported remission in study participants.

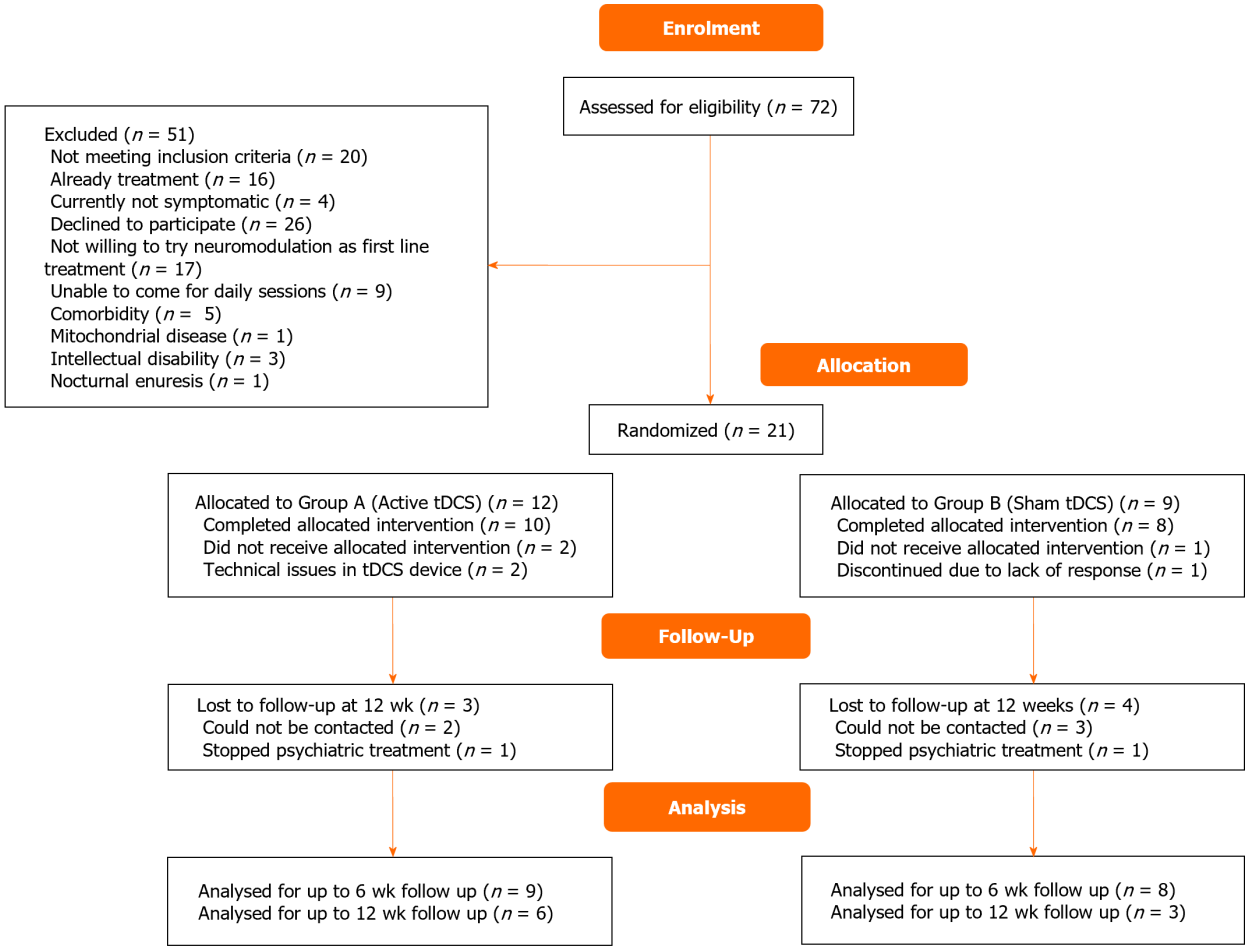

Figure 1 Consolidated Standards of Reporting Trials 2010 flow diagram for the study.

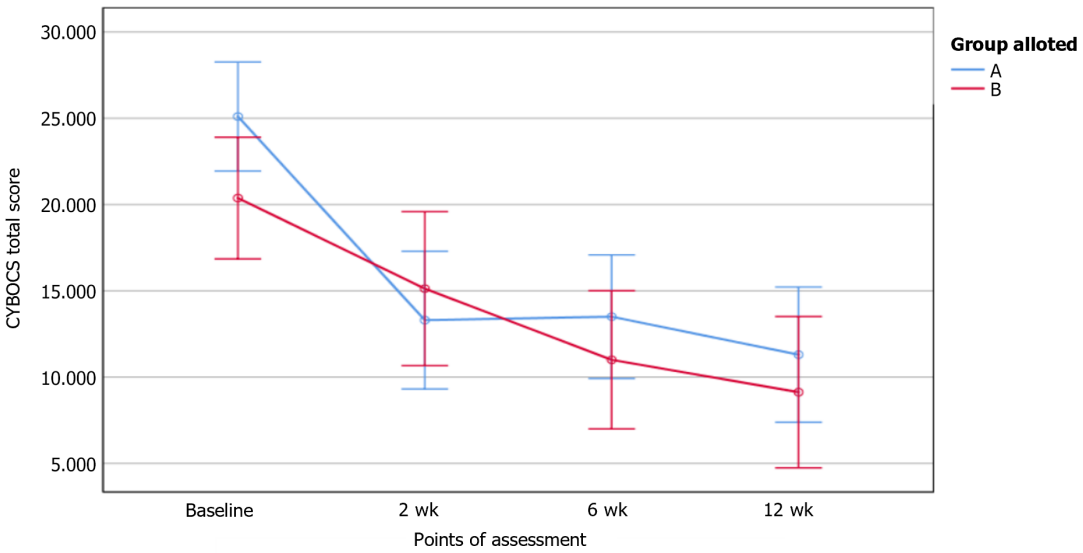

Figure 2 Children's Yale-Brown obsessive-compulsive scale total score in between the group across time. CY-BOCS: Children's Yale-Brown obsessive-compulsive scale.

One of the reasons for finding no significant difference between the groups could be due to the placebo effect, which has been observed highly in studies of rTMS and tDCS[22]. Participants receive considerable information during the recruitment phase and speculate about its effects[23]. The placebo effect through caregiving, change of environment, and its neurobiological component have been seen in OCD patients as well[24]. The placebo effect in tDCS has been less in treatment-resistant cases[25]. Since the majority of the previous studies have been in such patient populations, the

difference in response rates and comparable effects in sham and true groups can be explained by placebo response. Placebo and expectation effects can also change with the wording used to explain participants[26].

Another aspect is that tDCS affects long-term neuroplasticity, which takes several weeks to show its effects. This is particularly important in OCD, as a measurable response can take 8 to 12 wk[27,28]. Meanwhile, the long-term effects of tDCS, particularly on developing brains have not been studied thoroughly. Data from tDCS use in children with neurodevelopmental and motor disorders does not show any serious long-term adverse effects[29]. But long-term follow-up studies in mood and anxiety disorders in children are lacking.

Strengths and limitations

Our study found that tDCS is a safe modality of treatment in adolescents with OCD. tDCS was not found to be effective in this study. Limitations of our study include a small sample size, single blinding, and not conducting twice daily or extended protocols. This highlights the need for further research with robust methodology, controlled drug use, and a larger sample size. The study was also limited by a short follow-up period; therefore, we may have missed out on the long-term effects of tDCS on adolescents, particularly in terms of development. This is another area of research in future studies.

CONCLUSION

tDCS as a modality for the treatment of OCD in the adolescent age group is safe and well tolerated. However, early intervention with tDCS, may not be effective in adolescents with OCD.

FOOTNOTES

Author contributions: Agrawal A, Agarwal V, Kar SK, and Arya A designed the research study; Agrawal A conducted the research; Agarwal V, Kar SK, and Arya A have supervised the research; Agrawal A, Agarwal V, Kar SK did the analysis and wrote the manuscript; all authors have read and approve the final manuscript.

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SYSTEMATIC REVIEWS

Metabolomic changes in children with autism

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Abstract

BACKGROUND

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in social communication and repetitive behaviors. Metabolomic profiling has emerged as a valuable tool for understanding the underlying metabolic dysregulations associated with ASD.

AIM
To comprehensively explore metabolomic changes in children with ASD, integrating findings from various research articles, reviews, systematic reviews, meta-analyses, case reports, editorials, and a book chapter.

METHODS
A systematic search was conducted in electronic databases, including PubMed, PubMed Central, Cochrane Library, Embase, Web of Science, CINAHL, Scopus, LISA, and NLM catalog up until January 2024. Inclusion criteria encompassed research articles (83), review articles (145), meta-analyses (6), systematic reviews (6), case reports (2), editorials (2), and a book chapter (1) related to metabolomic changes in children with ASD. Exclusion criteria were applied to ensure the relevance and quality of included studies.

RESULTS
The systematic review identified specific metabolites and metabolic pathways showing consistent differences in children with ASD compared to typically developing individuals. These metabolic biomarkers may serve as objective measures to support clinical assessments, improve diagnostic accuracy, and inform personalized treatment approaches. Metabolomic profiling also offers insights into the metabolic alterations associated with comorbid conditions commonly observed in individuals with ASD.

CONCLUSION
Integration of metabolomic changes in children with ASD holds promise for enhancing diagnostic accuracy, guiding personalized treatment approaches, monitoring treatment response, and improving outcomes. Further research is needed to validate findings, establish standardized protocols, and overcome technical challenges in metabolomic analysis. By advancing our understanding of metabolic dysregulations in ASD, clinicians can improve the lives of affected individuals and their families.

Key Words: Autism spectrum disorder; Metabolic dysregulations; Metabolomic changes; Children; Mitochondrial dysfunction; Oxidative stress; Amino acids

Core Tip: This systematic review examines metabolic changes in individuals with autism spectrum disorder (ASD) by integrating various sources of evidence. Through an extensive search, the review explores factors influencing metabolomic changes in children with ASD, such as age, genetics, diet, gut microbiota, and medical interventions. The systematic review identifies common metabolic dysregulations, including abnormalities in energy metabolism, oxidative stress, mitochondrial dysfunction, and neurotransmitter metabolism. Despite limitations, integrating metabolomic changes in ASD holds promise for improving diagnosis and treatment approaches.

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INTRODUCTION

Metabolites are crucial molecules that are essential to the body's metabolism. These molecules are produced during the breakdown of substances and help create energy and other vital compounds necessary for various cellular functions. Metabolites come in different sizes and involve a variety of biochemical reactions that take place within the cells. Metabolites are also crucial in drug biotransformation and can result from microbial activity, which can have both beneficial and harmful effects on the human body[1]. The metabolome refers to the complete set of small molecules, or metabolites, present within a biological sample (such as a cell, tissue, organ, or organism) at a given time. These include sugars, amino acids, lipids, and other compounds that are fundamental to the body's functioning and can act as signaling molecules, energy sources, building blocks for larger molecules, or waste products that are subsequently eliminated[2]. Metabolomes can change dramatically based on external and internal factors like nutrient availability and medications. Metabolomics is a field of study that involves analyzing the various metabolites present in a biological sample using specialized techniques like mass spectrometry. For example, metabolomics can tell us about antioxidants, their types and quantities, and the factors influencing their trends to maintain good health. Advancements in metabolomics research hold great promise for improving medicine and enhancing human health[3].

Metabolomic changes refer to variations in the levels or patterns of small molecules or metabolites within a biological system, which can occur due to various factors[4]. Different diseases or health conditions can result in distinctive changes in metabolite levels, which can help diagnose or monitor diseases. External factors like diet, exercise, exposure to toxins,

or medications can alter metabolite concentrations or profiles[5]. Genetic differences can also influence the body's metabolism, leading to individual metabolomic profile differences. In addition, metabolomic profiles can change during different stages of life, such as during growth, aging, or in response to hormonal changes[6]. Analyzing these changes through metabolomics can provide valuable insights into the biochemical pathways affected by various conditions, potentially aiding in disease diagnosis, understanding biological mechanisms, and developing targeted treatments or interventions[7].

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that is characterized by difficulties in social interaction, communication, and repetitive behaviors[8]. Although the exact causes of autism are not fully understood, there is a growing interest in exploring its metabolic aspects to gain insights into its underlying mechanisms and enhance its management. Several studies have reported abnormalities in amino acid metabolism in children with autism. These abnormalities include imbalances in amino acid levels, particularly elevated levels of specific amino acids such as tryptophan and phenylalanine[9]. These changes may relate to disruptions in neurotransmitter synthesis and signaling pathways. Moreover, there are also differences in energy metabolism in children with autism, which may involve altered mitochondrial function. Mitochondria are critical for cell energy production; hence, any changes in their function can have significant consequences[10]. Some metabolomic studies have suggested a connection between the gut microbiome and autism[11].

Changes in metabolites produced by gut bacteria, such as short-chain fatty acids (SCFAs) and certain neurotransmitter-related compounds, have been observed in children with autism[12]. In addition, aberrations in purine and pyrimidine metabolism have been identified in metabolomic analyses of children with autism. These metabolic pathways involve DNA and RNA synthesis and various cellular processes[13].

Alterations in lipid metabolism, including changes in phospholipids and fatty acid profiles, have been reported. Lipids are essential components of cell membranes and play crucial roles in brain development and function[14]. Some studies have indicated increased oxidative stress and differences in antioxidant metabolism in children with autism. Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, potentially leading to cellular damage[15]. However, metabolomic findings in children with autism can vary between individuals and may be influenced by factors such as age, sex, genetics, and environmental factors. Additionally, interpreting these findings is complex, and more research is needed to fully understand the significance of these metabolic differences in the context of autism. Metabolomics is just one piece of the puzzle in understanding the intricate mechanisms underlying autism. Integrating metabolomic data with other omics data (genomics, transcriptomics, proteomics) and clinical information can provide a more comprehensive view of the disorder and potentially lead to identifying biomarkers for early diagnosis, subtyping, and personalized interventions[16]. Metabolic changes that occur early in life could help in the early detection of autism in a clinical-stage called pre-autism[17]. This systematic review aims to discuss the various metabolomic changes that can affect the degree of autism and help in diagnosis and management.

MATERIALS AND METHODS

We conducted a comprehensive systematic literature review until January 2024 to investigate the changes in metabolites found in children with ASD. Our search included research articles, review articles, meta-analyses, systematic reviews, case reports, editorials, and a book chapter published up until January 2024, written in English and focusing on metabolomic alterations in children with ASD. We searched across electronic databases such as PubMed, PubMed Central, Cochrane Library, Embase, Web of Science, CINAHL, Scopus, LISA, and NLM catalog using relevant keywords related to ASD, metabolomics, metabolic dysregulation, amino acids, lipids, oxidative stress, gut microbiota, and children. After screening based on titles, abstracts, and full-text reviews, we extracted data from selected articles, including study design, sample characteristics, metabolomic techniques, key findings, and implications. Exclusion criteria were applied to ensure the relevance and quality of included studies. We excluded articles not written in English, studies not focusing on metabolomic changes in children with ASD, studies not accessible through electronic databases, or not available in full-text format.

The retrieved data was synthesized and analyzed to identify potential biomarkers, underlying metabolic dysregulations, clinical implications, and patterns. We conducted a quality assessment of the included studies using appropriate tools and adhered to ethical guidelines and standards for systematic reviews. Findings were reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, while limitations, such as publication bias and study quality variations, were acknowledged. Please refer to Figure 1 for the flow chart of the study.

RESULTS

The comprehensive literature review identified 83 research articles, 145 review articles, 6 meta-analyses, 6 systematic reviews, 2 case reports, 2 editorials, and 1 book chapter that meet the inclusion criteria (Figure 1). The search of electronic databases found relevant publications until January 2024, focusing on the metabolomic changes in children diagnosed with ASD. The data synthesis revealed several key themes, including age-related differences in metabolomic profiles, sex-specific metabolic variations, genetic influences on metabolism, environmental factors that impact metabolomic alterations, dietary patterns affecting metabolic pathways, associations between the severity of ASD symptoms and metabolic dysregulations, and the role of gut microbiota in modulating metabolomic profiles. Moreover, metabolic

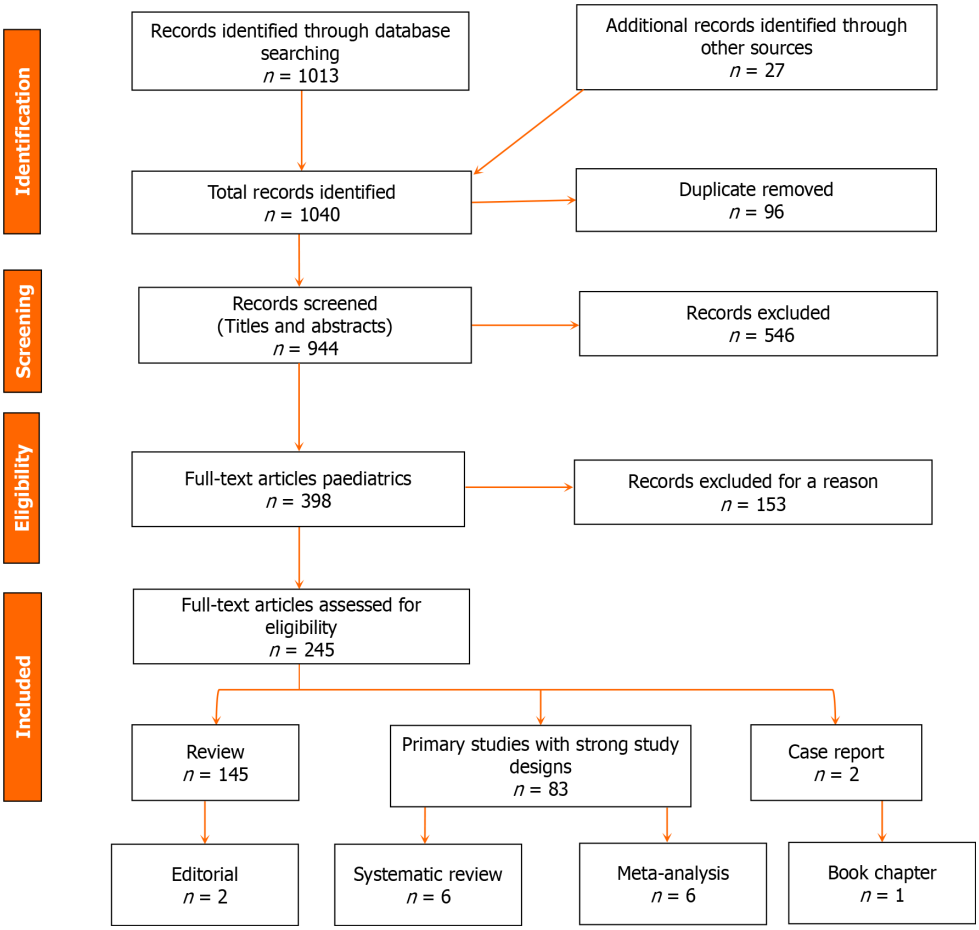

Figure 1 The PRISMA flow chart of the study.

dysregulations related to energy metabolism, oxidative stress, mitochondrial dysfunction, neurotransmitter metabolism, and comorbid medical conditions were identified. These findings provide insights into the complex metabolic landscape of ASD and highlight the potential for personalized diagnostic and therapeutic approaches targeting specific metabolic pathways. The limitations of the reviewed studies, such as variations in methodology, sample size, and study design, were noted, underscoring the need for further research to validate findings and establish standardized protocols in the field of ASD metabolomics.

DISCUSSION

Pathophysiology of autism

ASD is a complex condition that affects neurodevelopment. It is characterized by social communication and interaction challenges and restricted and repetitive behaviors[18]. While the exact causes of autism are not yet clear, research suggests that it is a multifactorial condition that involves genetic, environmental, and neurobiological factors. The pathophysiology of autism is complex and involves interactions between genetic predisposition, environmental influences, brain development, and neural functioning[19]. Genetics plays a significant role in autism, and studies have shown that certain genetic mutations or variations can increase the risk of developing ASD. Some cases of autism are associated with spontaneous mutations that occur in the sperm or egg cells or early in fetal development rather than being inherited from parents[20]. Certain genetic syndromes, such as Fragile X syndrome or Rett syndrome, have a higher prevalence of ASD. However, a straightforward genetic pattern is not always present, and multiple genes interacting with environmental factors might be involved. In addition, changes in gene expression without altering the DNA sequence, known as epigenetic modifications, are also being investigated concerning ASD. Environmental factors might influence these modifications and could contribute to ASD risk[21].

Environmental factors may have a significant effect on autism manifestations. Exposures during pregnancy, such as maternal infections, certain medications, or prenatal complications, have been suggested as potential environmental factors contributing to autism[22]. Postnatal environmental influences might also contribute, including exposure to toxins, pollutants, or certain medications during early development. Altered brain development is a key aspect of the pathophysiology of autism[23]. Studies using neuroimaging techniques have shown differences in brain structure and connectivity in individuals with ASD, particularly in areas involved in social cognition and communication. Imbalances

in neurotransmitters [such as serotonin, dopamine, and gamma-aminobutyric acid (GABA)] have been implicated in ASD, affecting neural signaling and communication between brain regions[24].

Several studies suggest that individuals with ASD may have immune system dysregulation and inflammation, which could play a role in the development of the condition[25]. Chronic inflammation in the brain has also been linked to ASD, which genetic factors, environmental triggers, or a combination of both may cause. Differences in the structure and function of the brain have been observed in individuals with ASD, including atypical neuronal pruning, abnormal connectivity between brain regions, and changes in the size and shape of certain brain structures[26]. Irregularities in synaptic function and signaling pathways have also been suggested as contributors to ASD. Developmental alteration of synapse formation, function, and pruning could affect neural circuitry. Furthermore, researchers are investigating disruptions in the balance between excitation and inhibition in neuronal networks and differences in long-range and local connectivity in the brain as potential factors in autism[27].

Many individuals with ASD experience atypical sensory processing, such as heightened or reduced sensitivity to sensory stimuli. They might be hypersensitive or hyposensitive to sensory stimuli like light, sound, touch, taste, or smell, which can influence their behavior and reactions to the environment[28]. Variability in cognitive abilities, including strengths in specific areas (such as visual thinking or pattern recognition) and challenges in others (such as social cognition), is a hallmark of ASD. Many individuals with ASD also experience gastrointestinal problems, such as constipation, diarrhea, and bloating. It is not yet clear whether these problems are a direct cause of ASD or a symptom of the underlying condition[29].

Metabolomic derangement in children with autism

Research into the metabolic profiles of children with ASD has revealed significant differences compared to those of neurotypical children. These differences can alter various metabolic pathways, including amino acid, energy, and neurotransmitter metabolism[30]. By understanding the underlying metabolic mechanisms, researchers may be able to pinpoint reliable biomarkers for early diagnosis of ASD. In addition, understanding these metabolic disturbances could open avenues for targeted interventions, highlighting the complex interplay between genetic predispositions and environmental factors in shaping metabolic profiles associated with ASD[31]. Solving the metabolomic derangement puzzles in patients with ASD could lead to the development of personalized and targeted interventions and treatments to correct metabolic imbalances and improve their outcomes. However, identifying specific metabolomic signatures as potential biomarkers for ASD remains a challenge due to heterogeneity in ASD presentations and variations among individuals[16].

Research into the metabolic profiles of children with ASD has found significant differences when compared to the metabolic profiles of neurotypical children. These differences can affect various metabolic pathways, including amino acid, energy, and neurotransmitter metabolism. By understanding the underlying metabolic mechanisms, researchers may be able to identify reliable biomarkers for early diagnosis of ASD, especially for at-risk populations[17]. Furthermore, understanding these metabolic discrepancies can open up avenues for highlighting how genetic predispositions and environmental factors interact to shape the metabolic profiles associated with ASD[32]. Solving the metabolomic derangement puzzles in patients with ASD could pave the way for developing personalized treatments and targeted interventions to correct specific metabolic imbalances, potentially leading to more effective treatments and improved outcomes[33]. However, pinpointing specific metabolomic signatures as potential biomarkers for ASD remains a challenge due to the heterogeneity in ASD presentations and the variations among individuals.

Amino acid imbalance in autism

Research has shown that there are differences in the amino acid profiles of children with ASD compared to those without ASD. Some studies have identified specific changes in amino acid metabolism in individuals with ASD, which could have implications for their neurological function and behavior. These changes may include increases, decreases, or disruptions to the metabolic pathways of certain amino acids. For instance, children with ASD may have elevated levels of phenylalanine and glutamate but decreased levels of glutathione and creatine. These alterations can cause neurotransmitter imbalances, which are important for brain communication. They can also contribute to oxidative stress, damage brain cells, and disrupt energy production, which can affect the proper functioning of the brain[34].

Tryptophan and serotonin pathway

Tryptophan, an essential aromatic amino acid, is a precursor for quinolinic and kynurenic acid serotonin, a neurotransmitter implicated in mood regulation and social behavior. We obtain tryptophan through protein-rich foods like dairy, oats, bananas, peanuts, dried prunes, bread, eggs, tuna, fish, cheese, poultry, and chocolate[35]. Once ingested, tryptophan is absorbed in the gut, transported to the liver, and then to various organs, including the brain. In the brain, tryptophan gets metabolized through two main pathways: The kynurenine pathway and the serotonin (5-hydroxytryptamine) pathway[36]. The Kynurenine pathway generates various metabolites, including kynurenic acid and quinolinic acid, which influence brain function and immunity. In contrast, the serotonin pathway produces serotonin, a vital neurotransmitter involved in mood, sleep, and social behavior[37].

Some research indicates disruptions in the tryptophan pathway in individuals with ASD. These disruptions might reduce blood and CSF tryptophan levels, inducing altered serotonin levels. Serotonin is crucial in social interaction and emotional regulation[38]. Therefore, the reduction of serotonin could potentially influence mood, social interactions, and other behaviors associated with ASD. Some studies have reported lower levels of tryptophan, the precursor for serotonin, in individuals with ASD compared to neurotypical individuals[39]. Boccuto *et al*[40] found that patients with ASD have lower levels of tryptophan, which is linked to the behavioral traits associated with autism, regardless of their genetic

background. Ormstad *et al*[41] showed elevated brain-derived neurotrophic factor levels and lower tryptophan and kynurenic acid levels in children with ASD and intellectual disability disorder. At the same time, they found elevated tryptophan and lower serotonin synthesis in patients with Asperger syndrome. This reduction might affect serotonin synthesis, potentially impacting mood regulation, social behavior, and emotional processing, all of which are areas often affected in ASD. However, a meta-analysis by Almulla *et al*[42] found no abnormalities in peripheral blood tryptophan metabolism, indoleamine 2,3-dioxygenase enzyme activity, or tryptophan catabolite production in ASD.

Tryptophan can also be metabolized through the kynurenine pathway, producing various metabolites, including kynurenic acid and quinolinic acid, which influence brain function and immunity. Kynurenic acid might have excitatory effects on brain cells, potentially contributing to repetitive behaviors[43]. Imbalances in the kynurenine pathway have been observed in individuals with ASD, with some studies reporting abnormalities in kynurenine pathway metabolites, such as increased levels of certain kynurenine pathway metabolites or imbalances in the ratios between these metabolites [44].

Serotonin is crucial in various developmental processes, including cell proliferation, migration, and differentiation. It affects various brain regions, including those involved in mood, social behavior, sensory processing, and repetitive behaviors, as it plays an important neurotrophic role during brain development. Disruptions in serotonin pathways during critical developmental periods may affect brain wiring, potentially contributing to the development of ASD traits [45]. Early serotonin system disturbance affects cortical development and thalamocortical innervation maturation and development, a potential mechanism common to autism and pediatric epilepsies associated with cortical dysplasia. Studies have shown variations in serotonin levels in the blood, brain, and gastrointestinal tract of individuals with ASD compared to those without ASD. However, these differences aren't consistent across all individuals with ASD, suggesting that serotonin dysregulation might be only one of many factors contributing to ASD[46,47]. In addition, serotonin interacts with various other neurotransmitter systems and affects multiple physiological processes, which might contribute to the diverse array of symptoms and traits seen in ASD[48].

A high level of serotonin in the blood, known as hyperserotonemia, is the first biomarker identified in ASD. This condition is found in more than 25% of children with ASD, and there is an inverse relationship between serotonin levels and patient intelligence[39]. Genetic linkage and association studies have shown that hyperserotonemia and ASD risk are linked to the chromosomal region containing the serotonin transporter gene in males but not females[49]. Studies in the Hutterite population have also revealed associations between genes like integrin β3 subunit (ITGB3) and the Vitamin D receptor gene with serotonin levels. Further investigations in males have shown that ITGB3 and serotonin transporter (SLC6A4) genes are linked to 5-HT levels in the blood. These genes were found to interact, influencing platelet serotonin uptake and potentially relating to autism susceptibility genes[50,51]. However, despite its strong and specific association with ASD, no prospective study has yet assessed whether hyperserotonemia may predict ASD risk in infants, including baby siblings of children with ASD[52].

While direct serotonin measurements in the brain are challenging, studies examining peripheral markers (like blood or platelet levels) of serotonin or its metabolites have shown mixed results. Some individuals with ASD have displayed alterations in these peripheral markers, suggesting potential dysregulation in serotonin signaling[53]. Additionally, medications that target serotonin pathways (like selective serotonin reuptake inhibitors - SSRIs) have been explored in managing certain symptoms associated with ASD, although their effectiveness varies among individuals[54].

Many factors can influence tryptophan levels in individuals with ASD. Deficiencies in certain vitamins and minerals, like vitamin B6 and zinc, can hinder tryptophan metabolism. Therefore, consuming adequate protein sources naturally containing tryptophan can support healthy levels. Dietary supplementation with vitamins B and magnesium influences tryptophan levels due to their impact on its metabolic homeostasis[38]. The gut microbiome plays a role in tryptophan metabolism, and imbalances in gut bacteria might impact its absorption and utilization. Gut dysbiosis is common in patients with autism. Gut dysbiosis, in turn, causes impaired tryptophan metabolism and consequently impairs cognitive functions in patients with ASD[55]. Certain genetic variations might influence genes related to tryptophan metabolism, contributing to potential differences in individuals with ASD. Higazi *et al*[56] found a significant decrease in the expression levels of monoamine oxygenase A, 3-hydroxy anthranilate oxygenase, and aminoadipate aminotransferase genes in Egyptian children with ASD compared to individuals without ASD. They were negatively correlated to ASD scoring. Solute carrier transporter 7a5 (SLC7A5), a large neutral amino acid transporter localized at the blood-brain barrier (BBB), is essential in maintaining normal levels of brain-branched-chain amino acids. Tărlungeanu *et al*[57] found that the deletion of Slc7a5 from the endothelial cells of the BBB leads to atypical brain amino acid profile, abnormal mRNA translation, and severe neurological abnormalities. Many patients with autistic traits and motor delay carry deleterious homozygous mutations in the *SLC7A5* gene. Most plasma tryptophane is bound to albumin and hence is unavailable for transport into the brain. Therefore, the plasma levels of free tryptophan depend on the albumin level and can be affected by the different causes of hypoalbuminemia. It should also be noted that a lack of tryptophan leads to a lack of albumin synthesis[58]. Chronic stress and anxiety can deplete serotonin levels, potentially impacting tryptophan utilization[59]. Disrupted sleep patterns, common in ASD, can affect the synthesis and breakdown of serotonin, influencing tryptophan availability[60]. Certain medications, like some antidepressants, might interact with tryptophan metabolism, requiring careful monitoring[61].

Phenylalanine and tyrosine metabolism

The metabolism of phenylalanine and tyrosine is a complex process that plays a vital role in human health. Phenylalanine is an essential amino acid obtained from our diet, which the body processes through several enzymatic steps. One crucial process is when phenylalanine hydroxylase (PAH), an enzyme, converts phenylalanine into tyrosine. This conversion is necessary to maintain appropriate levels of tyrosine in the body[62]. Tyrosine is another amino acid that is a precursor for many essential compounds. It is a critical building block for producing neurotransmitters such as dopamine, nor-

epinephrine, and epinephrine, which helps regulate mood, cognition, and stress response. Phenylalanine and tyrosine are building blocks for proteins involved in various cellular processes[63]. Tyrosine also contributes to melanin production, the pigment responsible for skin and hair color. Any disruptions in the phenylalanine-to-tyrosine conversion process, often resulting from enzyme deficiencies like phenylketonuria (PKU), can lead to significant health problems. PKU is a genetic disorder characterized by a deficiency in PAH activity, causing an accumulation of phenylalanine in the body. If left untreated, high levels of phenylalanine can cause intellectual disabilities, developmental delays, and other neurological problems[64].

The connection between phenylalanine and tyrosine metabolism and ASD has been an area of research interest, though it's complex and not fully understood. Some studies have explored metabolic pathways involving phenylalanine and tyrosine in individuals with ASD[32]. Alterations in these metabolic pathways, including abnormalities in the levels or ratios of these amino acids and their metabolites, have been observed in some individuals with ASD. Research has suggested that disruptions in the phenylalanine-to-tyrosine conversion pathway might affect neurotransmitter synthesis, particularly dopamine and serotonin. Dopamine and serotonin play essential roles in regulating mood, behavior, and social interactions, aspects often affected in individuals with ASD[65]. Naushad *et al*[66] showed a significant decrease in levels of essential amino acids tryptophan, phenylalanine, and methionine and reduced amino acids precursors of neurotransmitters such as tyrosine and tryptophan in children with autism than in neurotypical children. Moreover, some individuals with ASD reportedly have abnormalities in enzymes involved in phenylalanine and tyrosine metabolism, leading to variations in amino acid levels[67]. These variations may contribute to differences in neurotransmitter levels or function, potentially impacting behavior and cognitive functioning associated with ASD. Other studies claimed that gut microbiota's disturbed metabolic action on phenylalanine and tyrosine produced different metabolites that can be used as biomarkers for ASD[68].

However, the phenylalanine to tyrosine ratio is more predictive of cerebral glucose metabolism than the phenylalanine level alone. Some researchers have proposed that imbalances or abnormalities in the phenylalanine-to-tyrosine ratio might affect neurotransmitter levels, potentially contributing to certain aspects of ASD symptomatology. The high ratio observed in children with autism is due to the failure of phenylalanine to be converted to tyrosine and, consequently, to dopamine in the brain[69]. The high levels of phenylalanine and tyrosine were not observed only in children with ASD but also observed in their parents and siblings[70]. Increased phenylalanine levels in children with ASD could be related to increased intakes. Arum *et al*[71] observed high phenylalanine and tryptophan intake in children with autism and hyperactivity, and the level of intake is significantly associated with the severity of hyperactivity.

The relationship between phenylalanine, tyrosine metabolism, and ASD is not yet fully established and remains complex. Not all individuals with ASD show abnormalities in these metabolic pathways[72]. Further investigation is required to determine if these metabolic differences play a causative role in ASD development. It's important to note that ASD is a multifactorial condition influenced by a combination of genetic, environmental, and neurological factors[73]. Phenylalanine and tyrosine metabolism are just a part of the broader spectrum of factors that researchers are studying to understand the complexities of ASD[65]. More studies are necessary to determine the specific contributions, if any, of phenylalanine and tyrosine metabolism to the development or characteristics of ASD. Additionally, it is vital to determine whether targeted interventions in these pathways could be beneficial for individuals with ASD.

Methionine cycle and sulfur metabolism

The methionine cycle is an essential biochemical pathway that plays a vital role in various biological processes in the body. This pathway involves sulfur metabolism and synthesizes certain amino acids, proteins, and other molecules critical for cellular function[74]. Methionine, an essential amino acid needed by the body, is produced in cells through a series of reactions that start with the amino acid homocysteine and use molecules like adenosine triphosphate (ATP) and methyl donors like S-adenosylmethionine (SAM)[75]. The transsulfuration pathway connects the methionine cycle to the synthesis of cysteine, another important sulfur-containing amino acid and precursor of glutathione. Homocysteine, derived from the methionine cycle, can convert to cysteine through several enzymatic reactions[76]. It can also be remethylated into methionine by methionine-synthase using vitamin B12 as a cofactor. SAM is a critical molecule formed during the methionine cycle. It acts as a methyl donor in various biological methylation reactions, contributing to modifying DNA, RNA, proteins, and other molecules[77]. Enzymes involved in the methionine cycle and sulfur metabolism are regulated to maintain a balance of sulfur-containing molecules and prevent the accumulation of potentially harmful intermediates[78]. Disruptions or deficiencies in the methionine cycle and sulfur metabolism can lead to various health issues. For instance, deficiencies in enzymes involved in these pathways can cause homocystinuria, a genetic disorder characterized by the buildup of homocysteine in the body[79].

Disorders in the metabolic pathways of the methionine cycle might play a role in the development of ASD in a subset of individuals. The methionine cycle is involved in methylation reactions, which regulate gene expression through epigenetic modifications. Altered methylation patterns have been observed in some individuals with ASD[80]. Changes in DNA methylation could potentially influence brain development and function, including neurogenesis, neuronal differentiation, synaptogenesis, learning, and memory[81].

Disruptions or abnormalities in the methionine cycle and related metabolic pathways may cause imbalances in essential molecules like SAM (S-adenosylmethionine), affecting various cellular processes[82]. Studies have reported differences in methionine metabolism in individuals with ASD compared to neurotypical individuals. For instance, Indika *et al*[83] found reduced methionine and SAM levels in children with ASD, which may reflect the impaired remethylation pathway. Similarly, Geier *et al*[84] observed reduced levels of serum glutathione, cysteine, methionine, cystathionine, and homocysteine in pre-pubertal children with ASD compared to typically developed children. They also noted elevated levels of serum dehydroepiandrosterone and total testosterone relative to the sex and age-specific normal ranges, which could indicate a possible cyclical relationship between the androgen pathways and methionine cycle-

transsulfuration in some children with ASD. Additionally, the intracellular concentration of methionine and its metabolites is also reduced in children with ASD. Suh *et al*[85] found a significant reduction of intracellular cysteine, glutathione, and S-adenosylmethionine and elevated intracellular homocysteine levels in leukocytes of children with ASD than in children with normal neurodevelopment. These intracellular changes in leukocytes of children with ASD could explain the immune disorders that are common in children with ASD. A meta-analysis by Guo *et al*[86] found impaired methylation capacity with significantly decreased levels of Methionine, S-adenosylmethionine, and S-adenosylmethionine/S-adenosylhomocysteine ratio and significantly increased levels of S-adenosylhomocysteine in children with ASD.

Both genetic and environmental factors contribute to the development of ASD. Genetic variations in genes involved in the methionine cycle and related pathways concerning ASD have been studied. genetic mutations and epimutation of DNA methylation can be seen in children with ASD on multiple levels from fetal life throughout postnatal life, affecting both embryonic brain development and early childhood synaptogenesis[87,88]. Haghiri *et al*[89] found a significant increase in the incidence of genetic mutations of methionine synthase in children with ASD than in controls. Mutations in the methionine synthase reductase gene were also more common among Iranian children with ASD than in typically developed controls. The methylenetetrahydrofolate reductase (MTHFR) gene produces an enzyme involved in the methionine cycle[90]. Variations in the MTHFR gene, particularly a common polymorphism known as the MTHFR C677T variant, have been studied concerning ASD[91]. Some studies have suggested an association between the MTHFR C677T variant and an increased risk of ASD[92,93], while others have found no significant association[94]. The catechol-O-methyltransferase (COMT) gene regulates the production of the COMT enzyme that breaks neurotransmitters like dopamine. Variations in the COMT gene have been associated with altered dopamine levels and cognitive impairments. Some studies have found an association between COMT gene variants and ASD, particularly with social and communication difficulties[95,96]. Furthermore, genes involved in the folate pathway, such as the folate receptor alpha (FOLR1) gene, have also been extensively studied in patients with ASD. Folate is essential for DNA methylation and other methylation processes, and disruptions in folate metabolism have been linked to neurodevelopmental disorders, including autism. Variations in the FOLR1 gene have been implicated in ASD susceptibility in some studies[97].

Environmental factors, such as nutritional influences or exposure to certain substances, might also impact these metabolic pathways. Sulfate deficiency in both the mother and the child due to excess exposure to environmental toxins and inadequate skin exposure to sunlight leads to widespread hypomethylation in the fetal brain with devastating consequences, including increased incidence of ASD[98]. In addition, the methionine cycle is directly influenced by the availability of methionine in the diet. Methionine-rich foods like certain meats and dairy products contribute to the amino acid pool[99]. The methionine cycle requires essential cofactors such as folate and B vitamins (especially B6, B12, and folate) to function properly. Inadequate intake of these vitamins can disrupt the normal functioning of the pathway[100]. The composition of gut microbiota can also affect methionine metabolism. Interactions between the host and gut bacteria can impact the availability and utilization of methionine[101].

Physical activity can likewise affect methionine metabolism. Exercise-induced changes in metabolism may influence the demand for sulfur-containing amino acids like methionine[102]. Chronic stress or psychological factors, including the methionine cycle, can impact overall metabolism. Stress hormones may affect the regulation of enzymes involved in these pathways[103]. Certain medications can interfere with methionine metabolism. For example, drugs that affect folate or vitamin B12 absorption can indirectly impact the methionine cycle. Exposure to heavy metals such as lead, cadmium, or mercury can also interfere with enzymes involved in the methionine cycle and disrupt sulfur metabolism[104]. Certain environmental toxins or pollutants may also adversely affect the methionine metabolic pathways. This can include exposure to industrial chemicals or pollutants in the air or water[105]. Table 1 summarizes some studies concerned with the alteration of the amino acid metabolic pathway in individuals with ASD.

Purine and pyrimidine metabolism

Purine and pyrimidine nucleotides serve as crucial building blocks for nucleic acid synthesis, yet their roles extend beyond this primary function. Purines function as metabolic signals, provide energy, regulate cell growth, participate in coenzymes, contribute to sugar transport, and donate phosphate groups in phosphorylation reactions[106]. On the other hand, pyrimidines play roles in biosynthesizing polysaccharides and phospholipids, participating in detoxification processes, and contributing to protein and lipid glycosylation[107]. A substantial amount of ATP in the nervous tissue is produced, primarily supporting energy needs for membrane-active pumps like Na+/K+ ATPase. This energy is vital for sustaining synaptic transmission and facilitating collaboration between neurons and glial cells[108]. Perturbations in purine and pyrimidine metabolism can potentially impact brain function and contribute to the development or manifestation of ASD symptoms (Table 2).

Adenosine is a purine nucleoside that acts as a neuromodulator in the brain. It acts as an inhibitory neurotransmitter, inhibiting the release of neurotransmitters like dopamine, norepinephrine, serotonin, acetylcholine, and glutamate. It also regulates sleep-wake cycles by promoting sleepiness and initiating the sleep phase[109]. It helps to mitigate the harmful effects of excessive neuronal activity or excitotoxicity and acts as an anticonvulsant and a vasodilator, influencing blood flow in the brain. Adenosine has anti-inflammatory effects and is involved in the modulation of immune responses in the central nervous system. It can help regulate the inflammatory environment in the brain[110]. Adenosine interacts with neurotransmitters, including glutamate, dopamine, and serotonin, to regulate the development of oligodendroglia and myelination[111].

Altered adenosine signaling has been observed in individuals with ASD. Adenosine receptors have been implicated in regulating neurotransmitter release, synaptic plasticity, and neuronal excitability, all of which are relevant to ASD pathophysiology. Tanimura *et al*[112] showed that a combination of adenosine 2A receptor agonist and adenosine 1 receptor agonist reduces repetitive behaviors selectively and could aid with this core symptom in ASD. In contrast, either

Table 1 The changes observed in each amino acid metabolic pathway in individuals with autism spectrum disorder, along with relevant studies and their methods

Ref.	Study methods	Amino acid pathway	Changes in ASD
Boccuto *et al* [40]	Blood or CSF sample analysis	phenylalanine and glutamate	Elevated phenylalanine and glutamate levels
Ormstad *et al*[41]	Metabolomic profiling	phenylalanine and glutamate	Decreased glutathione and creatine levels
Almulla *et al*[42]	Peripheral blood analysis	Tryptophan pathway	Disrupted tryptophan metabolism
Higazi *et al* [56]	Genetic analysis of MAOA, *HAAO* and *AADAT* genes using real-time RT-qPCR.	tryptophan pathway	significant decrease in the expression of the selected genes within ASD children relative to children with learning disabilities and healthy controls
Naushad *et al*[66]	Genetic analysis	Tryptophan pathway	Lower serotonin synthesis
Naushad *et al*[66]	Enzyme activity measurement	Phenylalanine and tyrosine metabolism	Reduced tyrosine synthesis
Arum *et al* [71]	Dietary intake assessment	Phenylalanine and tyrosine metabolism	Abnormal phenylalanine-to-tyrosine ratio
Geier *et al* [84]	Peripheral blood analysis	Methionine and sulfur metabolism	reduced levels of serum glutathione, cysteine, methionine, cystathionine, and homocysteine in pre-pubertal children
Suh *et al*[85]	Genetic analysis	Methionine and sulfur metabolism	Reduced SAM levels
Guo *et al*[86]	Meta-analysis of metabolomic profiling	Methionine and sulfur metabolism	Impaired methylation capacity
Haghiri *et al* [89]	Genetic analysis	Methionine metabolism	mutations of methionine synthase in children with ASD

AADAT gene: Aminoadipate Aminotransferase gene; ASD: Autism spectrum disorder; *HAAO* gene: 3-Hydroxyanthranilate 3,4-dioxygenase is a monomeric cytosolic protein gene; *MAOA* gene: monoamine oxidase A gene; SAM: S-adenosylmethionine.

Table 2 The roles, impact, potential biomarkers, genetic associations, and therapeutic implications of both purine and pyrimidine metabolism in the context of autism spectrum disorder

Aspect	Purine metabolism	Pyrimidine metabolism
Functions	Building blocks for nucleic acid synthesis; Metabolic signals; Provide energy; Regulate cell growth; Participate in coenzymes; Contribute to sugar transport; Donate phosphate groups in phosphorylation reactions	Synthesizing DNA and RNA; Energy metabolism; Neurotransmitter signaling
Specific roles	Provide energy for membrane-active pumps like Na+/K+ ATPase; Vital for sustaining synaptic transmission; Facilitate collaboration between neurons and glial cells	Biosynthesis of polysaccharides and phospholipids; Participate in detoxification processes; Contribute to protein and lipid glycosylation
Impact on brain function	Altered purine metabolism may impact brain function and contribute to ASD symptoms; Adenosine acts as a neuromodulator, inhibiting neurotransmitter release and regulating sleep-wake cycles	Abnormalities in pyrimidine metabolism may be linked to ASD and neurodevelopmental issues; Disturbances in uracil metabolism could contribute to mitochondrial dysfunction in ASD
Potential biomarkers	Elevated adenosine levels and altered ADA activity observed in ASD; Abnormal levels of purine metabolites such as uric acid reported in ASD	Altered uracil levels and abnormal ratios of uracil to other pyrimidine bases reported in ASD; Abnormal levels of pyrimidine nucleotides observed in ASD
Genetic associations	Mutations in genes encoding enzymes involved in purine metabolism found in individuals with ASD	Genetic mutations in the gene encoding DPD identified in individuals with ASD
Therapeutic implications	Modulating adenosine signaling and targeting enzymes involved in purine metabolism could potentially improve neurochemical functioning in ASD	Supplementation with pyrimidine precursors such as uridine was explored as a possible intervention to improve mitochondrial function and neurodevelopmental outcomes in ASD

ADA: Adenosine deaminase; ASD: autism spectrum disorder; ATP: Adenosine Triphosphate; DPD: dihydro-pyrimidine dehydrogenase.

drug alone induces no or non-selective effect on behaviors, respectively. Some studies have reported elevated adenosine levels in the brains of individuals with ASD. Higher adenosine concentrations could influence neuronal activity and neurotransmitter release, potentially affecting cognitive and behavioral processes. Adenosine Deaminase (ADA) is an enzyme involved in purine metabolism that converts adenosine to inosine[113]. Altered ADA activity has been reported in some children with ASD. Dysregulation of ADA could impact adenosine levels and contribute to the neurochemical imbalances observed in ASD[114].

Abnormal levels of purine metabolites, such as uric acid, have been reported in individuals with ASD. Uric acid is the end product of purine degradation, and its dysregulation may reflect disturbances in the purine metabolic pathway[13]. Various studies analyzing metabolites have found an important reduction in uric acid levels in the urine of individuals diagnosed with ASD, especially those with food selectivity[115]. However, a study conducted by Page *et al*[116] found that some patients with certain types of autism showed high levels of uric acid, which was attributed to increased production of purines in these individuals. Certain genetic variations related to purine metabolism have been associated with an increased risk of ASD[117]. For example, mutations in genes encoding enzymes involved in purine metabolism, such as adenylosuccinate lyase, adenosine deaminase, and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC), have been identified in individuals with ASD[13]. Abnormalities in purine metabolism pathways may offer potential targets for therapeutic interventions in ASD. Modulating adenosine signaling or targeting enzymes involved in purine metabolism could potentially restore balance and improve neurochemical functioning.

Pyrimidines are molecules that play a vital role in synthesizing DNA and RNA, energy metabolism, and neurotransmitter signaling. While research on purine metabolism in children with ASD is more extensive, studies on pyrimidine metabolism are limited. Nevertheless, some evidence suggests that abnormalities in pyrimidine metabolism may be linked to ASD and neurodevelopmental and behavioral issues[118]. Uracil is a pyrimidine base, and disturbances in its metabolism have been observed in individuals with ASD. Uracil is involved in nucleotide synthesis, which is the building blocks of DNA and RNA. Disturbances in pyrimidine metabolism, including uracil, could potentially affect various cellular processes, including brain development and function[106]. Uracil is normally converted to thymine through the action of the enzyme dihydropyrimidine dehydrogenase (DPD). Altered DPD activity and imbalances in the uracil-to-thymine ratio have been reported in some children with ASD[119]. Genetic mutations in the gene encoding the enzyme DPD, which is involved in pyrimidine catabolism, have been found in some individuals with ASD[120].

Mitochondrial dysfunction has been implicated in a subset of individuals with autism. Since uracil and pyrimidine metabolism are closely linked to mitochondrial function, disturbances in uracil metabolism might contribute to mitochondrial dysfunction observed in some cases of ASD[10,121]. Additionally, abnormalities in folate metabolism, which is interconnected with pyrimidine synthesis, have been implicated in ASD[122]. Some studies have explored the possibility of using uracil disturbances as potential biomarkers for autism. Altered uracil levels or abnormal ratios of uracil to other pyrimidine bases have been reported in certain individuals with ASD[123]. However, further research is needed to validate these findings and determine their clinical significance. In addition, studies have reported alterations in the levels of pyrimidine nucleotides, such as uridine, cytidine, and deoxycytidine, in individuals with ASD[124]. These nucleotides are crucial for DNA and RNA synthesis and have important roles in neuronal development and function. Modulating pyrimidine metabolism pathways could potentially have therapeutic implications for ASD[125]. For instance, supplementation with certain pyrimidine precursors, such as uridine, has been explored as a possible intervention to improve mitochondrial function and neurodevelopmental outcomes in ASD[126].

Mitochondrial metabolic disorders in ASD

Mitochondria is a tiny structure located within nearly all body cells. It is the cellular powerhouse responsible for generating energy in the form of ATP. Due to their high energy needs, the muscle and the brain cells have a particularly high mitochondrial density to support their energy needs[127]. There is growing evidence of an increased prevalence of impaired functioning of the mitochondria in patients with ASD compared to the general population. This dysfunction can result from genetic mutations, environmental factors, or a combination of both[128]. A groundbreaking study by Giulivi *et al*[129] at the University of California in 2010 showed that 80% of the children with ASD enrolled in their study had blood tests indicating mitochondrial dysfunction, mtDNA overreplication, and mtDNA deletions. However, the estimates of the co-occurrence of mitochondrial disorders in individuals with ASD range from 5% to 80%[130]. Many factors increase the prevalence of mitochondrial dysfunction in children with autism, such as genetic mutations, dietary deficiencies of vitamins and minerals in the diet, certain chemicals and heavy metals exposure, some drugs, certain bacterial and viral infections, and stressful conditions[131]. Mitochondrial dysfunction can arise from genetic mutations in mitochondrial DNA (mtDNA) or nuclear DNA, affecting the function of mitochondrial proteins and enzymes[132,133]. Some studies have identified specific genetic variations associated with mitochondrial dysfunction in individuals with ASD, suggesting a potential genetic overlap between these conditions[134].

It's important to note that while mitochondrial dysfunction may be more common in individuals with ASD, it is not a defining feature of ASD. A significant number of people with ASD do not have mitochondrial dysfunction. However, mitochondrial dysfunction can lead to inadequate production of ATP, which is the energy currency of cells, including neurons in the brain. Insufficient energy can affect various organs and cellular processes, including synaptic plasticity, neuronal development, signaling, and maintenance, which may contribute to atypical brain functioning observed in individuals with ASD[135,136]. Mitochondrial dysfunction can also cause an imbalance between the production of ROS and the cellular antioxidant defense mechanisms, leading to oxidative stress[137]. Increased oxidative stress can damage cellular components, such as lipids, proteins, and DNA, impacting neuronal function and development[138]. Additionally, mitochondrial dysfunction can disrupt metabolic processes, such as abnormal amino acid metabolism, impaired fatty acid oxidation, and dysregulated carbohydrate metabolism. These metabolic alterations may further

contribute to the development of ASD and impact overall cellular energy balance[139].

Diagnosing mitochondrial dysfunction in individuals with ASD can be complex. Mitochondrial dysfunction is highly heterogeneous and can lead to a wide range of symptoms that may contribute to the development or severity of ASD symptoms. Although the manifestation of mitochondrial dysfunction in children with autism can vary widely, there is evidence to suggest that some individuals with ASD may experience abnormalities in mitochondrial function[140]. Children with mitochondrial dysfunction and autism may experience delays in reaching developmental milestones, such as walking, talking, or social interactions[141]. They may also have impaired language development and communication skills, including delayed speech, limited vocabulary, difficulties with expressive and receptive language, and challenges in social communication. Some children with mitochondrial dysfunction and autism may experience motor difficulties, including poor muscle tone (hypotonia), coordination problems, and gross or fine motor skill deficits. Mitochondrial dysfunction can also impact cognitive abilities, leading to intellectual disabilities, learning difficulties, or problems with attention and executive functioning[142]. Behavioral abnormalities, such as hyperactivity, repetitive behaviors, anxiety, aggression, or self-injurious behaviors, are also common in children with both mitochondrial dysfunction and autism [143]. Epileptic seizures may occur in some children with both conditions[144]. Gastrointestinal problems, such as chronic constipation, diarrhea, or gastrointestinal inflammation, have been reported in individuals with mitochondrial dysfunction and autism[145]. Children may also demonstrate heightened sensitivities or aversions to sensory stimuli, such as loud noises, bright lights, certain textures, or specific tastes or smells. It's important to note that these symptoms can also be present in individuals with autism without mitochondrial dysfunction[28].

Comprehensive evaluations involving clinical assessments, biochemical analyses, and genetic testing are often required. Specific diagnostic criteria, such as the "Mitochondrial Disease Criteria" or the "Mitochondrial Autism Criteria," have been proposed to aid in identifying individuals with both mitochondrial dysfunction and ASD[146]. As such, a comprehensive evaluation by healthcare professionals with expertise in both autism and mitochondrial disorders is necessary for accurate diagnosis and appropriate management[147]. Each child's experience with mitochondrial dysfunction and autism can be unique, and the severity and specific symptoms can vary. Early diagnosis and intervention, along with a multidisciplinary approach involving healthcare professionals from various specialties, can help address the specific needs of children with co-occurring mitochondrial dysfunction and autism[143].

Several laboratory tests can provide valuable insights into mitochondrial function and potential dysfunction. These may include blood tests to assess lactate, pyruvate, amino acids, creatine kinase, ammonia, total and free carnitine, and an acylcarnitine profile. Additionally, urine testing for organic acids can be performed[148]. Mitochondrial DNA analysis and nuclear DNA sequencing may be employed to identify specific genetic mutations associated with mitochondrial disorders[149]. Brain imaging techniques like magnetic resonance spectroscopy (MRS) can be used to assess brain chemistry and detect elevated lactate levels, which can indicate mitochondrial dysfunction[150]. In some cases, a muscle biopsy may be recommended to evaluate mitochondrial function directly. This involves obtaining a small sample of muscle tissue under local anesthesia for laboratory analysis[151]. Diagnosis of mitochondrial dysfunction in children with autism can be challenging due to the overlap of symptoms and the lack of specific diagnostic criteria. Therefore, a multidisciplinary approach involving pediatricians, neurologists, geneticists, and metabolic specialists is often necessary to arrive at an accurate diagnosis. Treatment strategies may include dietary interventions (such as specific nutritional supplements or ketogenic diets), vitamin and cofactor supplementation, antioxidants, and medications targeted at specific symptoms[152]. However, the effectiveness of these interventions in improving ASD symptoms associated with mitochondrial dysfunction is still an area of ongoing research[153,154]. Table 3 provides a concise summary of the prevalence, causes, impact, symptoms, diagnosis, management, challenges, and ongoing research related to mitochondrial metabolic disorders in individuals with ASD.

Oxidative stress and antioxidant metabolism

Antioxidant defense mechanisms counteract oxidative stress and minimize its harmful effects. These mechanisms include enzymatic and non-enzymatic antioxidants. Superoxide dismutase, catalase, glutathione peroxidase, and others are enzymes that neutralize ROS and protect against oxidative damage. Vitamins C and E, glutathione, coenzyme Q10, and various phytochemicals act as antioxidants, scavenging free radicals and preventing oxidative damage. Antioxidant enzymes work together in a coordinated manner to maintain redox balance and protect against oxidative stress[155,156]. Research has shown that oxidative stress and antioxidant metabolism are important factors in understanding ASD. Oxidative stress occurs when there is an imbalance between the production of ROS and the body's antioxidant defense systems. ROS are byproducts of cellular metabolism that include free radicals and other molecules. When ROS production exceeds the body's antioxidant capacity, oxidative stress occurs[157]. Several studies have found increased oxidative stress markers in children with ASD, indicating their role in the development of the disorder[158-160].

There are various reasons why children with autism may experience oxidative stress. The interaction between oxidative stress, mitochondrial dysfunction, inflammation, and immune dysregulation can contribute to the development of ASD[161]. One of the primary reasons is impaired mitochondrial function, which is frequently observed in individuals with autism. Mitochondria are crucial in generating cellular energy and are also a significant source of ROS. When mitochondrial function is compromised, it can lead to excessive ROS production, causing oxidative stress[162]. Additionally, chronic inflammation and immune system dysregulation, which are common in individuals with autism, can also contribute to oxidative stress[163]. Altered antioxidant metabolism, such as low levels of antioxidants like glutathione or reduced antioxidant enzyme activity, can weaken the body's ability to combat oxidative stress[164]. Bjørklund *et al*[165] showed that individuals with ASD have reduced glutathione levels, indicating a potential imbalance in their antioxidant metabolism. Additionally, some studies have found lower levels of vitamins C and E in children with ASD, which may affect their antioxidant capacity[166]. Furthermore, exposure to environmental toxins like heavy metals, pesticides, and pollutants can also increase oxidative stress levels in individuals with autism[167].

Table 3 The prevalence, causes, impact, symptoms, diagnosis, management, challenges, and ongoing research related to mitochondrial metabolic disorders in individuals with autism spectrum disorder

Aspect	Mitochondrial metabolic disorders in ASD
Overview	Mitochondria are cellular structures responsible for generating energy (ATP). High mitochondrial density in muscle and brain cells
Prevalence and causes	Increased prevalence of mitochondrial dysfunction in ASD compared to the general population. Can result from genetic mutations, environmental factors, or both
Evidence	About 80% of children with ASD show blood test indications of mitochondrial dysfunction and DNA abnormalities. Estimates of co-occurrence range from 5% to 80%
Contributing factors	Genetic mutations; Dietary deficiencies; Chemical and heavy metal exposure; Certain drugs; Bacterial and viral infections; Stressful conditions
Impact on ASD	Insufficient ATP production can affect synaptic plasticity, neuronal development, signaling, and maintenance. Oxidative stress and damage to cellular components may occur. Disruption of metabolic processes can further impact ASD development
Symptoms and diagnosis	Symptoms include delays in developmental milestones, impaired language and communication, motor difficulties, cognitive impairments, behavioral abnormalities, seizures, and gastrointestinal issues. Diagnosis involves comprehensive clinical assessments, biochemical analyses, genetic testing, and specific diagnostic criteria. Laboratory tests may include blood tests, urine tests, DNA analysis, brain imaging, and muscle biopsy
Management and treatment	Treatment strategies may include dietary interventions, nutritional supplements, antioxidants, and medications targeted at specific symptoms. A multidisciplinary approach involving healthcare professionals from various specialties is necessary for accurate diagnosis and management
Challenges and ongoing research	Diagnosis can be challenging due to overlapping symptoms and lack of specific criteria. The effectiveness of interventions in improving ASD symptoms associated with mitochondrial dysfunction is still under research

ASD: Autism spectrum disorder; ATP: Adenosine triphosphate; DNA: Deoxyribonucleic acid.

Oxidative stress is a harmful process affecting different cellular aspects and vital processes. ROS can cause damage to lipids, leading to harmful byproducts that disrupt cell membranes and hinder cellular function[168]. Oxidative stress can also alter proteins, affecting their structure and function and impacting enzymatic activity, signaling pathways, and cellular vital processes[169]. Furthermore, ROS can cause DNA damage, including DNA strand breaks and modifications, leading to genomic instability and impaired cellular function[170]. The brain is especially vulnerable to oxidative stress due to its high energy demand, high lipid content, and limited antioxidant defenses. This type of stress can disrupt neurodevelopmental processes, synaptic plasticity, neurotransmitter balance, and overall brain function, which may potentially contribute to the core symptoms of ASD[171].

Given the association between oxidative stress and ASD, there has been interest in exploring antioxidant interventions as a potential therapeutic approach[172]. However, clinical trials examining the efficacy of antioxidant supplementation in ASD have yielded mixed results. While some studies have shown improvements in certain symptoms, others have reported no significant effects[173]. The effectiveness of antioxidant therapies may depend on factors such as the specific antioxidants used, the individual's antioxidant status, underlying genetic factors, and the presence of other co-occurring conditions[173]. Further research is needed to understand better the role of oxidative stress and antioxidant metabolism in ASD. This includes investigating the underlying mechanisms and identifying reliable biomarkers of oxidative stress in individuals with autism[174]. Additionally, studies exploring personalized antioxidant therapies and interventions targeting mitochondrial dysfunction and inflammation in ASD are warranted.

Lipid metabolism

Lipid metabolism encompasses the body's synthesis, breakdown, and transportation of fats. Lipids are important components of cell membranes, energy storage, and signaling molecules. Abnormalities in lipid metabolism have been observed in children with ASD, suggesting a potential link between lipid dysregulation and the pathophysiology of the disorder[175]. Dyslipidemia, characterized by abnormal lipid levels in the blood, has been reported in several studies involving children with ASD[176]. These studies have revealed changes in various lipid parameters. For instance, some studies have found elevated levels of total cholesterol in children with ASD compared to typically developing individuals [177]. However, a Tunisian study found that decreased levels of total cholesterol and erythrocyte magnesium are risk factors for ASD[178]. Similarly, increased levels of low-density lipoprotein cholesterol (LDL-C), often referred to as "bad" cholesterol, have been observed in some individuals with ASD[176]. Conversely, reduced maternal postpartum plasma LDL levels were associated with an increased risk of ASD among children born to overweight or obese mothers, as found by Park *et al*[179]. Additionally, children with autism may exhibit decreased levels of high-density lipoprotein cholesterol (HDL-C), often called "good" cholesterol[180]. Elevated levels of triglycerides have also been observed in some individuals with ASD[181]. Furthermore, an increased ratio of LDL-C to HDL-C, considered a marker of increased cardiovascular risk, has been identified in children with autism[177]. Oxidative stress, a state of imbalance between the production of ROS and the body's antioxidant defenses, is frequently observed in individuals with ASD. This oxidative stress can lead to lipid peroxidation and the generation of oxidized lipids, which are increased in children with autism, suggesting heightened oxidative stress[182].

Several factors may contribute to lipid metabolism abnormalities in children with autism. Genetic variations in lipid metabolism-related genes (*e.g.*, EFR3A4) have been associated with an increased risk of non-syndromic idiopathic ASD. These genetic variants may influence lipid metabolism pathways and contribute to lipid dysregulation. Genetic syndromes of lipid metabolism, such as Smith–Melli–Opitz syndrome, are frequently associated with neurodevelopmental delay[183]. Oxidative stress, characterized by an imbalance between the production of ROS and the body's antioxidant defenses, is often observed in individuals with ASD. Oxidative stress can lead to lipid peroxidation and the generation of oxidized lipids[184]. Chronic inflammation is commonly reported in individuals with ASD and has been associated with altered lipid metabolism. Inflammatory processes can disrupt lipid homeostasis and contribute to dyslipidemia[185]. Emerging evidence suggests a link between gut microbiota composition and lipid metabolism. Alterations in the gut microbiota, which have been observed in some individuals with ASD, may influence lipid metabolism patterns [186]. Dietary factors, such as nutrient composition and quality, may influence lipid metabolism and contribute to lipid abnormalities in children with autism[176].

Since lipids play essential roles in brain development and function, abnormalities in lipid metabolism can have implications for neuronal membrane composition, myelination, synaptogenesis, and neurotransmitter signaling, potentially contributing to the neurodevelopmental abnormalities observed in ASD[187]. Lipid disorders, including dyslipidemia and increased oxidative stress, have been associated with chronic inflammation, a common feature in individuals with ASD[15]. Disruptions in lipid metabolism can also affect cellular energy production and utilization, potentially impacting brain function and development in individuals with ASD[126]. Oxidative stress resulting from lipid disorders can lead to lipid peroxidation and the generation of harmful byproducts, which may contribute to neuroinflammation and neuronal damage. In light of the potential role of lipid metabolism abnormalities in ASD, there is interest in exploring lipid-based interventions as potential therapeutic strategies[188]. However, further research is needed to determine the effectiveness of such interventions and their impact on lipid profiles and ASD symptoms. It is important to note that while there is evidence suggesting associations between lipid disorders and ASD, not all individuals with ASD will have lipid abnormalities, and not all individuals with lipid disorders will have ASD[189]. The relationship between these two conditions is complex and likely influenced by various genetic, environmental, and metabolic factors. Continued research is necessary to fully understand the mechanisms underlying lipid disorders in ASD and their specific effects on the disorder. Table 4 summarizes the prevalence, contributing factors, impact, therapeutic implications, and research needs related to lipid metabolism abnormalities in individuals with ASD.

Omega-3 fatty acids and autism

Extensive research has highlighted the potential implications of Omega-3 fatty acids for ASD. These essential fatty acids, which are mostly found in fish oil, have gained a lot of attention due to their supposed effects on neurodevelopment and cognitive function[190]. Omega-3s are a type of polyunsaturated fatty acids that play crucial roles in various biological processes. The two principal types of Omega-3s, eicosapentaenoic acid and docosahexaenoic acid (DHA), are essential components that help maintain neuronal membrane integrity, modulate neurotransmission, regulate inflammation, and promote overall brain health[191]. Omega-3 fatty acids play a crucial role in the early development of the brain as they help in the formation and functioning of neural cells. DHA, which is present in large amounts in the brain, is responsible for creating synapses and forming myelin sheaths around neurons[192]. These are essential processes that enable proper brain connectivity and communication. Additionally, omega-3s impact neurotransmitter signaling, which affects the production and release of key neurotransmitters like serotonin, dopamine, and GABA[193]. These neurotransmitters regulate mood, behavior, and cognitive function, which are often affected in individuals with ASD.

Inflammation and immune dysregulation have been implicated in ASD pathophysiology. Omega-3 fatty acids possess anti-inflammatory properties and can modulate immune responses, potentially mitigating neuroinflammatory processes associated with ASD[194]. While research on omega-3 supplementation's effects on ASD behavioral symptoms yields mixed findings, some studies report enhancements in social interaction, communication, and reduction in repetitive behaviors, emphasizing the variability in individual responses and the necessity for further investigation to delineate consistent effects[195]. Research suggests that inflammation and immune dysregulation may contribute to the development of ASD. Omega-3 fatty acids have anti-inflammatory properties and can regulate immune responses, potentially mitigating neuroinflammatory processes associated with ASD. However, studies on omega-3 supplementation's effects on ASD behavioral symptoms have yielded mixed results[196]. While some studies report improvements in social interaction, communication, and reduction in repetitive behaviors, individual responses vary greatly. Therefore, further investigation is necessary to delineate consistent effects. Cognitive deficits are prevalent among individuals with ASD. Omega-3 fatty acids have been scrutinized for their potential cognitive enhancement properties in ASD populations, with some studies indicating improvements in attention, executive function, and information processing[197]. However, conclusive evidence necessitates additional research to establish optimal dosages and supplementation durations.

ASD frequently co-occurs with conditions like attention deficit hyperactivity disorder (ADHD) and anxiety. Omega-3 fatty acids have been explored for their potential therapeutic benefits in managing these comorbidities, showing promise in reducing ADHD symptoms and enhancing attention, as well as ameliorating anxiety symptoms in individuals with ASD[198]. Nonetheless, further investigation is warranted to validate these findings. Omega-3 fatty acid supplementation is considered safe and well-tolerated[199]. However, caution should be exercised regarding high doses, which may lead to mild side effects such as gastrointestinal disturbances or increased bleeding risk, especially in individuals taking anticoagulant medications[200]. Consequently, consulting with healthcare professionals before initiating any supplementation regimen is advisable to ensure safety and efficacy.

Table 4 The prevalence, contributing factors, impact, therapeutic implications, and research needs related to lipid metabolism abnormalities in individuals with autism spectrum disorder

Aspect	Lipid metabolism in ASD
Overview	Lipid metabolism involves the synthesis, breakdown, and transportation of fats, which are crucial for cell membranes and energy
Dyslipidemia in ASD	Abnormal lipid levels observed in children with ASD. Variations include elevated total cholesterol and LDL-C, reduced HDL-C, and increased triglycerides. Increased LDL-C to HDL-C ratio, a marker of cardiovascular risk
Factors contributing to abnormalities	Genetic variations in lipid metabolism-related genes. Syndromes like Smith–Melli–Opitz syndrome linked to lipid metabolism and neurodevelopmental delay. Oxidative stress and chronic inflammation are common in ASD, affecting lipid metabolism. Gut microbiota alterations and dietary factors are also implicated
Impact on ASD	Abnormal lipid metabolism can affect brain development, myelination, synaptogenesis, and neurotransmitter signaling in ASD. Disruptions may lead to oxidative stress, neuroinflammation, and neuronal damage. Potential implications for cellular energy production and utilization in the brain
Therapeutic implications	Interest in lipid-based interventions for ASD, but effectiveness needs further research. Potential therapeutic targets to address lipid disorders and associated symptoms in ASD. Complex relationship between lipid disorders and ASD, influenced by genetic, environmental, and metabolic factors
Research needs	Further research is needed to understand the mechanisms underlying lipid disorders in ASD. Investigation required into the effectiveness of lipid-based interventions on ASD symptoms and lipid profiles. Recognition that not all individuals with ASD have lipid abnormalities, and vice versa

ASD: Autism spectrum disorder; HDL-C: High-density lipoprotein cholesterol; LDL-C: low-density lipoprotein-cholesterol.

Gut microbiome and ASD: A tale of altered metabolism

Teeming with diverse bacteria, the gut microbiome significantly influences a child's physical and mental development. This impact is particularly pronounced when the brain undergoes rapid growth during the perinatal period. These gut microbes influence the production of crucial neurotransmitters like GABA and serotonin through the gut-brain axis, playing a vital role in brain wiring and synaptogenesis. Serotonin, primarily produced by gut bacteria, heavily influences brain development and mood regulation. While it can't directly reach the brain, it still sends powerful signals through the peripheral nervous system[201]. Individuals with ASD exhibit a distinct gut microbiome composition in comparison to individuals without the disorder. Research has found that changes in the gut microbiome, including a reduction in microbial diversity, can impact gut health and potentially influence ASD development and symptoms[145]. Some bacterial groups, such as *Clostridium, non-spore-forming anaerobes, Faecalibacterium,* and *microaerophilic bacteria* such as *Desulfovibrio,* consistently increase in number, while beneficial species, such as *Bifidobacterium,* decrease. These changes can affect the production of metabolites like SCFAs and hydrogen sulfide, which can have both positive and negative effects on the host. Furthermore, it has been observed that the proportion of *Desulfovibrio* is correlated with the severity of autism symptoms[201,202].

The gut microbiome has a direct communication channel with the brain, known as the gut-brain axis. This two-way pathway involves neural, immune, and endocrine signals. The gut produces various metabolites like SCFAs, neurotransmitters, and neuroactive compounds, which can influence neuronal signaling, inflammation, and immune responses [203]. These effects can ultimately have an impact on behavior and cognition. Moreover, the gut-brain axis plays a significant role in regulating the immune system. Therefore, any disruptions in the gut microbiome can have serious consequences[204]. One such disruption is known as "leaky gut," which occurs when there is increased intestinal permeability. This condition can lead to reduced microbial diversity, pathogen overgrowth, and weakening of the mucus layer. As a result, tight junctions between intestinal cells get disrupted, allowing microbial products and inflammatory molecules to seep into the bloodstream, causing systemic inflammation. This inflammation can potentially affect brain function[205].

Several metabolic changes occur in the gut microbiome of children with ASD. These changes involve the gut bacteria's production and processing of various metabolites. SCFAs, which are produced by the fermentation of dietary fiber by gut bacteria, play important roles in energy metabolism and gut health. Studies have shown that individuals with ASD may have altered levels of SCFAs, including lower levels of butyrate, propionate, and acetate[206]. These changes in SCFA production may have implications for energy metabolism and gut-brain communication. Gut bacteria are involved in the metabolism of amino acids, which are crucial for various physiological processes[207].

Altered amino acid metabolism has been observed in individuals with ASD, where there may be disruptions in the breakdown of certain amino acids, such as tryptophan and phenylalanine, leading to imbalances in their levels. These imbalances can affect neurotransmitter synthesis and potentially contribute to ASD symptoms[208]. Gut bacteria can produce and modulate neurotransmitters like serotonin, dopamine, and GABA. Altered neurotransmitter metabolism in the gut microbiome has been associated with ASD. For instance, imbalances in the production of serotonin, a neurotransmitter involved in mood regulation, have been observed in individuals with ASD. These imbalances may influence brain function and behavior[208].

Gut bacteria can also metabolize bile acids, which play a role in fat digestion and absorption. Studies have found differences in bile acid metabolism in individuals with ASD, including changes in the composition and abundance of specific bile acid species[209]. These alterations may affect fat metabolism and potentially contribute to gastrointestinal symptoms in ASD. Individuals with ASD may have increased oxidative stress, which is an imbalance between the

production of reactive oxygen species and the body's ability to neutralize them with antioxidants[210]. The gut microbiome can influence oxidative stress through its metabolic activities. Altered antioxidant metabolism and imbalances in oxidative stress markers have been reported in individuals with ASD, suggesting a potential role of the gut microbiome in these processes[211].

Understanding these gut microbiome changes holds significant clinical weight. Gastrointestinal symptoms like pain, constipation, and diarrhea are common in ASD, and research suggests gut microbiome alterations may contribute[212]. Modulating the gut microbiome through dietary interventions or targeted therapies could potentially alleviate these symptoms and improve overall well-being. Furthermore, evidence suggests a link between gut microbiome alterations and behavioral symptoms in ASD, highlighting the potential of gut-based interventions to improve cognitive and behavioral outcomes[213].

Several dietary modifications targeting the gut microbiome have been studied in individuals with ASD. These interventions aim to alter the composition and function of the gut microbiome and include approaches such as probiotics, prebiotics, and dietary restrictions[211]. Probiotics are live bacteria or yeasts that are believed to have beneficial effects on the gut microbiome. Prebiotics are substances that promote the growth of beneficial bacteria in the gut. Some studies have shown positive effects of probiotic supplementation on gastrointestinal symptoms and behavioral outcomes in individuals with ASD[214]. However, the findings have been inconsistent, and further research is needed to determine the specific strains, dosages, and duration of treatment that may be most effective.

There have been studies conducted on individuals with ASD who have dietary restrictions, such as gluten-free and casein-free diets. Gluten is a protein commonly found in wheat, barley, and rye, while casein is found in milk and dairy products[215]. These diets aim to remove any possible dietary triggers that could worsen gastrointestinal symptoms or affect the behavior of individuals with ASD. Some studies have reported that a subset of individuals with ASD who follow these dietary restrictions have experienced improvements in their behavior and gastrointestinal symptoms[216]. However, the evidence is limited, and further research is necessary to understand the mechanisms better and identify which individuals may benefit from these dietary interventions.

Ongoing research is exploring the role of gut microbiome in ASD. Long-term studies can provide valuable insights into ASD development and progression by examining dynamic changes in the gut microbiome throughout life. Personalized interventions based on individual gut microbiome profiles show promise. However, well-designed clinical trials are crucial to assess the efficacy and safety of microbiome-based therapies and establish evidence-based guidelines for their use in ASD. By integrating gut microbiome research with genetics, epigenetics, and other omics approaches, we can better understand ASD and develop new therapeutic strategies.

Factors affecting the rate of metabolomic changes in children with autism

A complex interplay of various factors, including age, sex, genetic background, environmental interactions, dietary factors, gut microbiota, metabolic dysregulation, comorbid medical conditions, and pharmacological interventions, influences the rate of metabolomic changes in children with ASD (Figure 2).

The metabolic processes in children change dynamically during childhood, which can impact the rate of metabolomic changes in children with ASD. Studies have shown age-related differences in metabolomic profiles, indicating that metabolic pathways evolve as children grow and develop. For example, Sharma *et al*[217] showed that children with autism below five years had a greater brain metabolism than older children with autism with a linear decrease in brain metabolism with aging. These differences may be attributed to developmental changes in various metabolic processes, such as energy metabolism, neurotransmitter synthesis, and amino acid metabolism. Understanding the specific metabolic pathways that undergo age-related changes can offer insights into the metabolic dysregulations associated with ASD at different developmental stages[125,218].

Metabolomic profiles in children with ASD can be influenced by both sex and genetic background. Studies in metabolomics have revealed sex-specific differences in the metabolomes of individuals with ASD, suggesting the possibility of sex-specific metabolic dysregulation. For example, Xiong *et al*[219] found significantly increased adenine, creatinine, 2-methylguanosine, and 7-alpha-hydroxytestololactone levels and reduced creatine in females with ASD. These differences might be attributable to factors such as sex-specific hormonal influences, genetic variations in sex chromosomes, or sex-specific interactions between genes and the environment. Genetic factors also play a significant role in shaping metabolism and metabolomic profiles. Genome-wide association studies have identified genetic variants associated with ASD that are involved in metabolic pathways and neurotransmitter metabolism. Mutations in genes involved in metabolic pathways or regulatory genes can impact the rate and nature of metabolomic changes associated with ASD[71,220].

Environmental factors can interact with genetic factors and affect metabolic changes in children with ASD. Prenatal and postnatal environmental exposures have been found to alter metabolomic profiles. Prenatal factors, like maternal diet, infections, or exposure to toxins, can influence fetal development and program metabolic pathways in the child. Postnatal factors, including dietary patterns, medication exposure, toxins, pollutants, and gut microbiota, can further modify metabolomic profiles. These environmental factors can disrupt metabolic homeostasis, leading to alterations in metabolomic profiles associated with ASD[221,222].

The diet plays a significant role in shaping the metabolome, and the dietary patterns directly impact metabolic profiles in children with ASD. Studies on metabolomics have identified differences in nutrient intake and metabolism between individuals with ASD and typically developing individuals[223,224]. In individuals with ASD, amino acid metabolism, lipid metabolism, and energy metabolism alterations have been observed[225]. The imbalance in essential nutrients or amino acids can lead to metabolic dysregulations and contribute to differences in metabolomic profiles. Additionally, food sensitivities and intolerances that are commonly observed in individuals with ASD can impact metabolomic profiles by influencing nutrient absorption and gut function[226].

Figure 2 Factors that affect the metabolomic changes in individuals with autism.

Research has shown that the severity of autism symptoms can affect the metabolic profiles of individuals. Specifically, there are associations between the severity of core symptoms of ASD, such as deficits in social communication, restricted and repetitive behaviors, and certain metabolic alterations[30]. Studies have observed differences in metabolite levels and metabolic pathways between individuals with severe ASD and those with milder forms of the condition[126]. It is believed that the severity of ASD symptoms may reflect underlying metabolic dysregulation, which contributes to variations in metabolomic profiles[227]. Differences in cognitive and behavioral patterns found in individuals with ASD can lead to changes in their metabolomic profiles. Specific cognitive abilities, such as language skills or intellectual functioning, have been linked to distinct metabolomic alterations[226]. Moreover, particular behavioral features, such as self-injurious behaviors or hyperactivity, may be associated with specific metabolic dysregulations and metabolomic profiles[228]. In addition, certain behaviors can modify metabolic activity in children with autism. Ranieri *et al*[229] showed that exercise interventions and physical activity help to improve communication, social interaction and behaviors, and gut in children with autism.

The gut microbiota has emerged as a key metabolism modulator and can influence metabolomic changes in individuals with ASD. Metabolomics studies have identified differences in microbial-derived metabolites between individuals with ASD and neurotypical individuals[11]. Alterations in the gut microbiota composition, known as dysbiosis, are commonly observed in individuals with ASD. Dysbiosis can affect metabolic pathways by producing metabolites, such as short-chain fatty acids, neurotransmitters, and bile acids, which can have systemic effects on the host metabolome[230]. These microbial-derived metabolites can influence immune function, gut barrier integrity, and neurotransmitter synthesis, thereby impacting metabolomic profiles in children with ASD[12].

Metabolomics studies have consistently identified metabolic dysregulations in individuals with ASD. Abnormalities in energy metabolism, oxidative stress, mitochondrial dysfunction, and neurotransmitter metabolism have been reported [144]. These metabolic perturbations can lead to alterations in metabolomic profiles. For example, disruptions in energy metabolism can result in changes in metabolites involved in glucose metabolism, lipid metabolism, and the tricarboxylic acid cycle[231]. Mitochondrial dysfunction can lead to perturbations in energy production and the generation of reactive oxygen species, which can further impact metabolomic profiles through oxidative stress-related pathways[232]. These metabolic dysregulations are thought to contribute to the pathophysiology of ASD and may be linked to the core symptoms and associated comorbidities.

Children with ASD often present with comorbid medical conditions, including gastrointestinal disorders, immune dysregulation, and metabolic disorders. These comorbidities can influence metabolomic profiles and contribute to the heterogeneity of metabolomic changes observed in children with ASD[8]. For example, gastrointestinal disorders, such as inflammatory bowel disease or gastrointestinal inflammation, can alter nutrient absorption and metabolism, leading to changes in metabolomic profiles. Immune dysregulation and chronic inflammation can also impact metabolic pathways

and metabolomic profiles[233]. Metabolic disorders, such as abnormalities in lipid metabolism or energy metabolism, can directly affect metabolomic profiles in children with ASD[234].

Pharmacological interventions and treatments commonly used in children with ASD can influence metabolic pathways and contribute to changes in metabolomic profiles[235]. Medications, including psychotropic drugs, may directly impact metabolic processes by modulating neurotransmitter metabolism or affecting energy metabolism[236]. These medications can alter metabolomic profiles by influencing the levels of specific metabolites or by modulating metabolic pathways. Dietary supplements and specialized diets, such as the ketogenic diet or gluten-free/casein-free diet, can also affect metabolomic profiles by altering nutrient availability and metabolic pathways[237]. Behavioral interventions to improve dietary habits or lifestyle factors can indirectly influence metabolism and metabolomic profiles[238].

Understanding these factors and their specific effects on metabolomic profiles can provide valuable insights into the underlying metabolic dysregulations associated with ASD. It may help inform personalized treatment approaches for affected individuals. Further research is needed to elucidate the causal relationships and temporal dynamics between these factors and metabolomic changes in ASD and identify potential biomarkers for early detection and therapeutic monitoring.

Integration and clinical implications of metabolomic changes in children with ASD

Metabolomic profiling is an active area of research in children with ASD, with significant potential for improving diagnostic and therapeutic approaches. By comparing the metabolomic profiles of individuals with ASD to those of typically developing individuals, researchers have identified specific metabolites and metabolic pathways that show consistent differences[239]. These metabolic biomarkers may serve as objective measures to support clinical assessments and improve diagnostic accuracy, aiding in early detection, diagnosis, and subtyping of ASD[240].

Metabolomic analyses provide insights into the underlying metabolic dysregulations associated with ASD. This knowledge can help unravel the complex etiology of the disorder and inform the development of targeted interventions. Metabolomic profiling has the potential to enable personalized medicine approaches in the management of ASD[241]. By characterizing the unique metabolic profiles of individuals with ASD, clinicians may be able to tailor interventions and treatments to address specific metabolic dysregulations, potentially enhancing treatment efficacy and minimizing adverse effects[242].

Metabolomic profiling can also be used to monitor treatment response and assess the effectiveness of interventions in children with ASD[239]. By tracking changes in metabolomic profiles over time, clinicians can evaluate the impact of therapeutic interventions on metabolic dysregulations, guiding treatment adjustments and optimizing therapeutic strategies for individual patients[243]. The identified metabolic pathways may be potential therapeutic targets for developing novel interventions. Modulating the identified metabolic pathways through dietary modifications, supplementation, or pharmacological interventions may help restore metabolic balance and improve ASD symptoms[244].

Metabolomic profiling may also provide insights into the metabolic alterations associated with comorbid conditions commonly observed in individuals with ASD, such as gastrointestinal issues, epilepsy, or sleep disturbances[226]. Understanding these metabolic connections can guide the management of co-occurring conditions and inform strategies for comprehensive care[8]. Additionally, examining the metabolomic profiles of children with ASD at an early age may identify markers associated with different developmental trajectories, guiding early intervention strategies and providing prognostic indicators for personalized treatment planning[245]. While metabolomic profiling shows great potential, further research is needed to validate findings, establish standardized protocols, and overcome technical and analytical challenges. Nonetheless, the integration of metabolomic changes in children with ASD has the potential to enhance diagnostic accuracy, inform personalized treatment approaches, and improve outcomes for individuals with ASD[239].

Limitations of the study

Despite the comprehensive nature of this systematic review, several limitations should be acknowledged. Firstly, the inclusion criteria focused specifically on metabolomic changes in children with ASD, potentially excluding relevant studies that examined metabolomic alterations in other age groups or neurodevelopmental disorders. Additionally, although efforts were made to ensure a thorough search across various electronic databases up until January 2024, it is possible that some relevant studies may have been missed. The reliance on electronic databases may have also introduced publication bias, as studies not indexed in these databases were not included. Furthermore, the heterogeneity among the included studies, such as differences in methodology, sample size, and study design, may have influenced the interpretation and synthesis of results. Moreover, while the review aimed to provide a comprehensive overview of metabolomic changes in ASD, the complexity of metabolomic data and the diversity of factors influencing metabolism necessitate cautious interpretation. Finally, the absence of a quality assessment of included studies and the lack of meta-analytical techniques limit the robustness of the synthesized findings. Despite these limitations, this review provides valuable insights into the current understanding of metabolomic alterations in children with ASD and highlights avenues for future research.

Although we tried our best to conduct a comprehensive systematic review, it has several limitations that need to be acknowledged. Firstly, the inclusion criteria only focused on metabolomic changes in children with ASD, which means that relevant studies examining metabolomic alterations in other age groups or neurodevelopmental disorders may have been excluded. Additionally, even though efforts were made to ensure a thorough search across various electronic databases up until January 2024, it is likely that some relevant studies may have been missed. This reliance on electronic databases may have also introduced publication bias, as studies not indexed in these databases were not included. Furthermore, heterogeneity among the included studies, including differences in methodology, sample size, and study design, may have influenced the interpretation and synthesis of results. While the review aimed to provide a comprehensive systematic overview of metabolomic changes in ASD, the complexity of metabolomic data and the diversity of

factors influencing metabolism necessitate cautious interpretation. Finally, the absence of a quality assessment of included studies and the lack of meta-analytical techniques limit the robustness of the synthesized findings. Nevertheless, this systematic review provides valuable insights into the current understanding of metabolomic alterations in children with ASD and highlights areas for future research.

CONCLUSION

This systematic review provides an in-depth analysis of the changes in metabolism that occur in children with ASD. It combines the findings of various research studies, including reviews, meta-analyses, systematic reviews, case reports, editorials, and a book chapter, to offer a comprehensive overview of the topic. The review highlights the complex factors that contribute to metabolic changes in ASD and identifies potential biomarkers that could be used for diagnosis and personalized treatment. However, the review also acknowledges that there are limitations to the available literature, such as variations in study design and the possibility of publication bias. Despite these limitations, the review emphasizes the importance of further research to validate the findings, establish standardized protocols, and overcome technical challenges. By incorporating metabolomic changes into clinical practice, healthcare professionals can improve diagnosing, treating, and monitoring children with ASD. The review concludes that continued research into metabolomic alterations in ASD holds promise for advancing our understanding of the disorder and improving the lives of affected individuals and their families.

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FOOTNOTES

Author contributions: Al-Beltagi M, Saeed NK, Bediwy AS, and Elbeltagi R contributed to this systematic review exploring metabolomic changes in children with autism spectrum disorder (ASD). Al-Beltagi M, Saeed NK, and Elbeltagi R conceptualized and designed the review, with Al-Beltagi M specifically proposing, designing, and conducting the systematic search in electronic databases, as well as synthesizing the included studies' findings. Bediwy AS, along with Al-Beltagi M and Saeed NK, screened studies, extracted data, and contributed to the analysis and interpretation of the results. Both Al-Beltagi M and Saeed NK made crucial and indispensable contributions to the project, qualifying them as co-first authors of the review. Elbeltagi R, along with Al-Beltagi M, played important and indispensable roles as co-corresponding authors, with Elbeltagi R providing oversight and guidance throughout the review process, as well as contributing to the interpretation of the results and drafting the manuscript. Furthermore, Al-Beltagi M and Elbeltagi R collaborated closely in synthesizing the findings, identifying specific metabolites and metabolic pathways associated with ASD, and discussing the clinical implications of the results. This collaboration between Al-Beltagi M and Elbeltagi R was crucial for the completion and publication of this systematic review, which aims to enhance our understanding of metabolic dysregulations in ASD and their clinical implications.

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SYSTEMATIC REVIEWS

Fecal calprotectin in pediatric gastrointestinal diseases: Pros and cons

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Abstract

BACKGROUND

Fecal calprotectin is a valuable biomarker for assessing intestinal inflammation in pediatric gastrointestinal diseases. However, its role, pros, and cons in various conditions must be comprehensively elucidated.

AIM
To explore the role of fecal calprotectin in pediatric gastrointestinal diseases, including its advantages and limitations.

METHODS
A comprehensive search was conducted on PubMed, PubMed Central, Google Scholar, and other scientific research engines until February 24, 2024. The review included 88 research articles, 56 review articles, six meta-analyses, two systematic reviews, two consensus papers, and two letters to the editors.

RESULTS
Fecal calprotectin is a non-invasive marker for detecting intestinal inflammation and monitoring disease activity in pediatric conditions such as functional gastrointestinal disorders, inflammatory bowel disease, coeliac disease, coronavirus disease 2019-induced gastrointestinal disorders, gastroenteritis, and cystic fibrosis-associated intestinal pathology. However, its lack of specificity and susceptibility to various confounding factors pose challenges in interpretation. Despite these limitations, fecal calprotectin offers significant advantages in diagnosing, monitoring, and managing pediatric gastrointestinal diseases.

CONCLUSION
Fecal calprotectin holds promise as a valuable tool in pediatric gastroenterology, offering insights into disease activity, treatment response, and prognosis. Standardized protocols and guidelines are needed to optimize its clinical utility and mitigate interpretation challenges. Further research is warranted to address the identified limitations and enhance our understanding of fecal calprotectin in pediatric gastrointestinal diseases.

Key Words: Fecal calprotectin; Pediatric gastrointestinal diseases; Functional gastrointestinal disorders; Inflammatory bowel disease; Coeliac disease; COVID-19-induced gastrointestinal disorders; Infectious gastroenteritis; Cystic fibrosis

Core Tip: The core findings of this systematic review underscore the pivotal role of fecal calprotectin as a non-invasive marker for assessing intestinal inflammation in pediatric gastrointestinal diseases. Through an extensive systematic literature review, it was evident that fecal calprotectin provides valuable insights into disease activity, treatment response, and prognosis across various conditions, including functional gastrointestinal disorders, inflammatory bowel disease, coeliac disease, coronavirus disease 2019 induced gastrointestinal disorders, gastroenteritis, and cystic fibrosis-associated intestinal pathology. Despite its limitations in specificity and susceptibility to confounding factors, fecal calprotectin remains a valuable tool in pediatric gastroenterology, offering clinicians a means to diagnose, monitor, and manage gastrointestinal diseases effectively.

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INTRODUCTION

Pediatric gastrointestinal (GI) diseases encompass a wide range of conditions affecting the digestive system in children from infancy through adolescence. These diseases can manifest with diverse symptoms, including abdominal pain, diarrhea, vomiting, and poor weight gain, and they can significantly impact a child's overall health and well-being[1]. Understanding the significance of pediatric GI diseases is essential for healthcare professionals, caregivers, and society as a whole, as it underscores the importance of early detection, diagnosis, and management to optimize outcomes for affected children. Pediatric GI diseases are a global health concern[2]. According to the World Health Organization, GI infections are one of the leading causes of illness and death in children under five years of age, especially in low- and middle-income. Chronic GI conditions such as inflammatory bowel disease (IBD), celiac disease, and gastroesophageal reflux disease (GERD) can also impair a child's quality of life and require ongoing medical management[3]. Pediatric GI diseases also have significant socioeconomic implications for families and healthcare systems. Additionally, missed school days and parental work absences due to caring for a sick child can disrupt family routines and impact household income[4].

Diagnosing and treating pediatric GI diseases can be challenging due to the complexities of the developing digestive system and the varied symptoms that children may experience. Unlike adults, children may not always be able to express their symptoms clearly, making it difficult for healthcare providers to obtain an accurate medical history and perform a thorough physical examination[5]. Consequently, pediatric GI diseases may be underdiagnosed or misdiagnosed, leading

to delays in appropriate treatment and potential complications. Early intervention and multidisciplinary care are essential, as chronic GI conditions can lead to growth and developmental delays in children[6]. Non-invasive diagnostic tools are necessary in pediatric gastrointestinal diseases due to their safety, accessibility, and accuracy. They minimize discomfort, enhance safety, and offer comprehensive evaluation of the gastrointestinal tract without invasive exploration. They also facilitate longitudinal monitoring of disease progression and treatment response, ensuring timely diagnosis and treatment[7]. An ideal diagnostic marker for pediatric gastrointestinal diseases should be non-invasive, sensitive, specific, quantifiable, predictive, cost-effective, feasible for pediatric populations, stable, reproducible, and with limited interference from external factors. Ethical considerations regarding sample collection comfort for pediatric patients are also important[8].

The primary objective of the review is to assess the utility of fecal calprotectin as a diagnostic marker, specifically in pediatric patients with gastrointestinal diseases, and to evaluate its advantages and limitations in this population, focusing on its role as a non-invasive and efficient indicator of gastrointestinal inflammation. Additionally, the article likely seeks to provide insights into the practical implications of using fecal calprotectin in pediatric clinical practice, including its ability to provide early indications of gastrointestinal pathology, aid in disease monitoring, differentiate between organic and functional disorders, and predict disease relapse. Furthermore, the review likely intends to address the challenges and considerations associated with interpreting fecal calprotectin results in pediatric patients.

MATERIALS AND METHODS

We conducted a systematic literature search to identify studies examining the role of fecal calprotectin in pediatric gastrointestinal diseases and evaluating its pros and cons. The search was performed across various electronic databases, including PubMed, PubMed Central, Google Scholar, and other scientific research engines. The search strategy utilized combinations of keywords and Medical Subject Headings terms related to fecal calprotectin, pediatric gastrointestinal diseases, functional gastrointestinal disorders, inflammatory bowel disease, coeliac disease, coronavirus disease 2019 (COVID-19)-induced gastrointestinal disorders, infectious gastroenteritis, and cystic fibrosis. The search was conducted up to February 24, 2024, with no restrictions on publication date.

Articles were included if they provided relevant information on using fecal calprotectin as a biomarker in pediatric gastrointestinal diseases, including studies examining its diagnostic, prognostic, or therapeutic implications. Both original research articles and review articles were considered for inclusion. We also included articles discussing fecal calprotectin's advantages and disadvantages (pros and cons) in pediatric gastrointestinal diseases. We excluded articles that did not focus on pediatric populations or specifically address fecal calprotectin's role in gastrointestinal diseases. Studies not available in English were also excluded from the review.

Two reviewers performed Data extraction independently using a standardized data extraction form. The extracted data were synthesized to provide a comprehensive overview of the role of fecal calprotectin in pediatric gastrointestinal diseases, focusing on its advantages and disadvantages. Findings from the included studies were analyzed, summarized, and presented in the subsequent sections of this review. The quality of included studies was assessed based on study design, methodology, sample size, and the reliability of reported findings. Both quantitative and qualitative studies were included, and the strength of evidence provided by each study was considered during data synthesis and interpretation.

RESULTS

The literature search identified 90 research articles, 60 review articles, 6 meta-analyses, 2 systematic reviews, 2 consensus papers, and 2 Letters to the editors. Figure 1 shows the study's flow chart. These studies were selected based on their relevance to the role of fecal calprotectin in pediatric gastrointestinal diseases and its pros and cons. The included studies covered various pediatric gastrointestinal conditions such as functional gastrointestinal disorders, IBDs, coeliac disease, COVID-19-induced gastrointestinal disorders, infectious gastroenteritis, and cystic fibrosis. The included studies explored the role of fecal calprotectin as a biomarker in pediatric gastrointestinal diseases. The studies consistently found that fecal calprotectin was a sensitive marker of intestinal inflammation, aiding in diagnosing, monitoring, and managing various gastrointestinal conditions in children. Elevated fecal calprotectin levels were associated with the presence and severity of gastrointestinal inflammation, helping differentiate between functional, inflammatory, and non-inflammatory conditions.

The studies identified several advantages of fecal calprotectin use in pediatric gastrointestinal diseases. These included its non-invasive nature, making it well-tolerated by pediatric patients, and its sensitivity in detecting intestinal inflammation accurately. Fecal calprotectin levels were found to correlate with disease activity, treatment response, and clinical outcomes in conditions such as IBD, coeliac disease, and cystic fibrosis. Additionally, fecal calprotectin provided valuable insights into disease prognosis and helped guide therapeutic interventions. Despite its utility, fecal calprotectin testing presented specific challenges and limitations. One notable limitation was the lack of specificity of fecal calprotectin elevation, as it could occur in various inflammatory and non-inflammatory conditions, leading to potential false positives and diagnostic ambiguity. Interpretation of fecal calprotectin levels was also complicated by factors such as variations in average values among different age groups, confounding factors like diet and medication use, and the lack of standardized cutoff values for specific conditions.

The included studies highlighted variability in clinical practice regarding the routine use of fecal calprotectin in pediatric gastrointestinal diseases. While fecal calprotectin was widely utilized for diagnosing and monitoring certain conditions, there was a lack of consensus among expert groups regarding its appropriate use in specific patient popula-

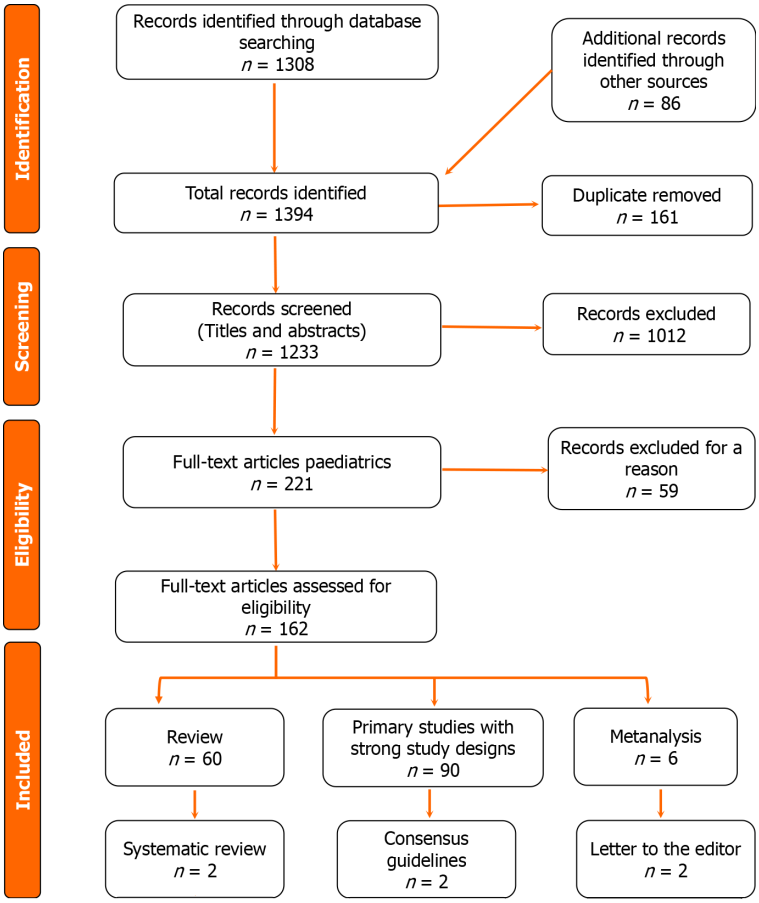

Figure 1 The flow chart of the included studies.

tions, such as those with acute gastroenteritis. This variability underscored the need for standardized guidelines and protocols for fecal calprotectin testing in pediatric practice. Overall, the results of the included studies emphasized the significant role of fecal calprotectin as a valuable biomarker in pediatric gastrointestinal diseases. While it offered several advantages, including its non-invasive nature and sensitivity in detecting intestinal inflammation, fecal calprotectin testing posed challenges related to its specificity and interpretation. Further research and establishing standardized guidelines are needed to optimize the clinical utility of fecal calprotectin in pediatric gastrointestinal practice.

DISCUSSION

Calprotectin is a complex of calcium-binding leucocyte proteins that belongs to the S100 protein family. It can bind to calcium, zinc, and manganese ions and plays a significant role in the innate immune response and inflammation. Calprotectin is also a heat-stable protein and can resist bacterial and enzymatic degradation. It is primarily found in neutrophils, forming about 60% of cytosol-soluble proteins in human neutrophils. It is also present in monocytes, macrophages, and epithelial cells[9,10].

Calprotectin comprises two primary proteins: S100A8 and S100A9 (Figure 2). S100A8, also known as Calgranulin A, is a 10.8 kDa protein mainly found in myeloid cells such as neutrophils, monocytes, and macrophages[11]. It is critical in the innate immune response, particularly in host defense against microbial pathogens and inflammation. S100A8 interacts with receptors such as toll-like receptor 4 (TLR4) and receptors for advanced glycation end products (RAGE)[12]. Similarly, S100A9, also known as Calgranulin B, is a 13.2 kDa protein found primarily in myeloid cells and upregulated during inflammation. It creates homodimers and heterodimers with S100A8 to form the calprotectin complex[13]. This complex is released into the extracellular environment during inflammation, with antimicrobial activity (against bacteria and fungi) and modulating inflammatory responses[14]. Calprotectin also has pro- and anti-tumor properties related to DNA damage response, angiogenesis, cell survival, growth, and the remodeling of the extracellular matrix[15].

While calprotectin is typically formed by the heterodimeric combination of S100A8 and S100A9, homodimers of either S100A8 or S100A9 can also exist. These homodimers may have distinct functions or roles compared to the heterodimeric form of calprotectin [11]. In certain disease states or physiological conditions, calprotectin complexes may contain other proteins or molecules besides S100A8 and S100A9. These mixed complexes could arise due to interactions with other proteins in the cellular environment or alterations in the composition of immune cells[16].

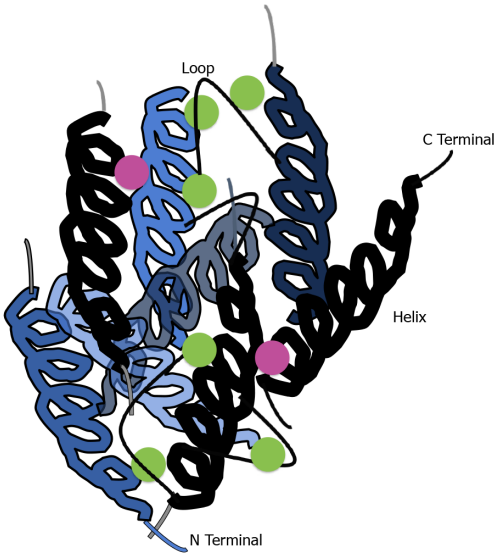

Figure 2 Diagrammatic representation of calprotectin dimer. The crystal two S100A8-S100A9 Dimer structure of calprotectin loaded with Mn^{2+} and Ca^{2+}. The black and blue chains represent S100A8 and S100A9, respectively. Purple spheres represent Mn^{2+} and green spheres represent Ca^{2+}. Only one manganese ion can bind per calprotectin dimer.

Calprotectin is a critical component of the innate immune response, exhibiting antimicrobial activity by chelating essential metals needed for bacterial growth, such as calcium, zinc, and manganese ions. Calprotectin inhibits many zinc-dependent enzymes, including matrix metalloprotease, inducing anti-proliferative & apoptosis effects in both normal and transformed cells[17]. Additionally, calprotectin modulates inflammatory responses by interacting with receptors like TLR4 and RAGE, influencing cytokine production and immune cell recruitment[18]. Moreover, it contributes to tissue homeostasis and repair by regulating cell differentiation and proliferation processes, thereby promoting wound healing [14].

Activated neutrophils and other myeloid cells release calprotectin as part of the innate immune response to inflammation[19]. Inflammatory stimuli, such as infection, tissue damage, or autoimmune processes, trigger the migration of neutrophils to the site of inflammation, where they release calprotectin into the surrounding tissues or body fluids[20]. Clinically, calprotectin, particularly fecal calprotectin, is a sensitive marker for inflammation, aiding in diagnosing and monitoring various gastrointestinal conditions. Its multifaceted roles in immunity, inflammation modulation, tissue repair, and diagnostic applications underscore its significance in health and disease[21].

Calprotectin in systemic diseases

Calprotectin is a protein linked to various systemic inflammatory and infectious diseases affecting different organ systems, highlighting its potential as a biomarker of inflammation and disease activity[22]. In patients with rheumatoid arthritis, calprotectin levels are elevated in both the synovial fluid and serum and correlate with disease activity and severity. Calprotectin may thus serve as a marker of joint inflammation and could be used to monitor disease progression and response to treatment in rheumatoid arthritis[23]. In addition, Cheng *et al*[24] found that serum calprotectin correlates with the duration of psoriatic arthritis disease and is independently associated with the presence of carotid plaque in these patients. Emerging evidence suggests that calprotectin may also play a role in cardiovascular diseases, specifically atherosclerosis and coronary artery disease. Elevated levels of calprotectin have been associated with an increased risk of cardiovascular events and may serve as a prognostic marker in these conditions[25].

In patients with psoriasis, a chronic inflammatory skin disorder, elevated calprotectin levels have been found in skin lesions. Calprotectin may contribute to the inflammatory process in psoriatic skin lesions, and serum calprotectin could serve as a biomarker for disease activity[26]. Calprotectin levels have also been investigated as a potential marker of inflammation in cystic fibrosis, a genetic disorder characterized by lung and digestive system problems. Elevated fecal calprotectin levels have been observed in patients with cystic fibrosis and may reflect gastrointestinal inflammation in this population[27]. Calprotectin levels have been reported to be elevated in patients with chronic kidney disease (CKD), particularly those with progressive renal impairment and inflammation. Calprotectin may serve as a marker of systemic inflammation and could be associated with the progression of CKD and cardiovascular complications[28,29].

Studies have shown elevated levels of calprotectin in patients with liver diseases such as hepatitis, cirrhosis, and non-alcoholic fatty liver disease. Calprotectin may reflect hepatic inflammation and injury and could potentially be used as a marker of disease severity and prognosis in liver diseases[30]. Emerging evidence suggests a potential role for calprotectin in neurological disorders such as multiple sclerosis (MS) and Alzheimer's disease. Elevated levels of calprotectin have been observed in the cerebrospinal fluid and brain tissues of patients with MS and Alzheimer's disease, respectively, indicating neuroinflammation and neurodegenerative processes[31,32]. Calprotectin has also been investigated as a biomarker for sepsis, a life-threatening condition characterized by systemic inflammation in response to infection. Elevated calprotectin levels have been observed in patients with sepsis and may reflect the severity of the inflammatory response and organ dysfunction[33].

Calprotectin in gastrointestinal disorders

Both serum and fecal calprotectin can be used to assess GI disorders, particularly those involving inflammation of the gastrointestinal tract. Elevated serum calprotectin levels indicate systemic inflammation that could be associated with GI disorders. It can be related to various conditions, including IBD, such as Crohn's disease and ulcerative colitis, as well as other inflammatory conditions like rheumatoid arthritis. Serum calprotectin levels can be a marker of disease activity and severity in certain gastrointestinal disorders. Fecal calprotectin is a marker of intestinal inflammation derived from neutrophils infiltrating the intestinal mucosa. It is measured in stool samples and can provide valuable information about the presence and severity of inflammation in the gastrointestinal tract. Fecal calprotectin can be preserved and easily measured in stools for relatively long periods, sufficient enough to allow for easy collection and analysis[34]. Elevated fecal calprotectin levels are associated with various gastrointestinal conditions, including IBD, infectious gastroenteritis, and colorectal cancer. Fecal calprotectin testing is particularly useful in distinguishing between the gastrointestinal tract's inflammatory and non-inflammatory conditions and monitoring disease activity and response to treatment in patients with IBD. Fecal calprotectin can be used to screen, diagnose, monitor, and predict relapse of these disorders. Table 1 compares serum and fecal calprotectin.

Fecal sampling

A good-quality stool sample of about 5 gm is essential to ensure accurate fecal calprotectin measurement. It is better to use the morning sample as Calafat *et al*[35] found that samples from the first stool in the morning obtained the highest calprotectin levels within-day value in about 33.3% of cases and the lowest values in about 38.9% of cases. However, the exact reason for this day variability is unclear, and it is better not to depend on a single sample determination. The stool sample should not be contaminated with urine, toilet water, or other foreign substances, as this can interfere with the analysis and lead to inaccurate results. Obtaining a clean stool sample in children could be sometimes challenging. Liquid stool samples can still be used for fecal calprotectin measurement, as in many gastrointestinal disorders, diarrhea is a frequent symptom[36]. The collection containers or kits should be clean and dry. The containers inside should not be touched by gloves, tissues, or other materials to avoid contamination. The amount of stool in fecal samples should be adequate as per the laboratory's requirements. Generally, a pea-sized amount or a small spoonful of stool is sufficient for testing. The samples should be clearly labeled with the patients' information according to the laboratory standard.

Properly collecting stool samples as soon as possible after defecation is essential to prevent calprotectin degradation. If the sample needs to be transported, it should be delivered promptly and following the laboratory's instructions. This includes using appropriate transport media or packaging to maintain sample integrity during transit. Fresh stool samples provide more accurate results compared to samples that have been stored for an extended period. However, some literature suggests that fecal calprotectin can remain stable at room temperature for up to 3 to 7 d[37,38]. Nonetheless, a study by Haisma *et al*[34] revealed that fecal calprotectin levels decreased by 35-45% when stored at room temperature for six days compared to the baseline level detected immediately after sampling but remained stable when stored at 4°C. This can lead to false reassurance for children with IBD and/or their caretakers due to falsely low calprotectin values. To avoid this problem, refrigerating stool samples until they can be delivered to the hospital laboratory for testing is recommended as a standardized pre-analytical calprotectin handling procedure. According to the European Society for Paediatric Gastroenterology and Nutrition Gastroenterology (ESPGHAN) Committee, fecal samples can be used up to 2 to 3 d when kept at room temperature, 5 to 7 d when kept in a fridge (4°C) or longer when the samples are kept frozen (−20°C or −4°C). Proper storage helps maintain the stability of calprotectin levels until analysis[39]. There are different methods of measuring fecal calprotectin, including enzyme-linked immunosorbent assay (ELISA), turbidimetric immunoassay, fluorescence immunoassay, lateral flow immunoassay, chemiluminescent immunoassay, and quantitative polymerase chain reaction, as shown in Table 2. Although most tests have very high sensitivity for mucosal inflammation detections, these tests significantly differ in their specificity and absolute values[40].

The cutoff level for fecal calprotectin in children can vary depending on several factors, including age, laboratory methodology, and the specific clinical context. Generally, cutoff levels are established to distinguish between normal and elevated fecal calprotectin levels, indicating intestinal inflammation. Cutoff values for fecal calprotectin may not be comparable among different kits, even those produced by the same manufacturer[41]. Therefore, it is highly recommended that the same testing kit and extraction methodology be used for diagnosis, disease activity monitoring, and follow-up procedures in the same patient[42].

In pediatric patients, cutoff levels for fecal calprotectin are often higher than those used for adults. This is because normal calprotectin levels can vary depending on age, with higher baseline levels observed in infants and young children than adults, with a tendency towards lower values with increasing age[43]. However, there are no well-established cut-off levels for specific age ranges. Additionally, disease presentation and severity may differ in pediatric patients compared to adults, necessitating age-appropriate cutoff levels to accurately interpret fecal calprotectin results in children[44]. Therefore, laboratories often establish pediatric reference ranges or cutoff levels to account for these age-related differences and ensure appropriate clinical interpretation. Oord *et al*[45] found cutoff values of 538 μg/g for infants between 1 and 6 months, 214 μg/g for children between 6 months and three years, and 75 μg/g for children between 3 and 4 years. On the other hand, Fagerberg *et al*[46] found that the cutoff level for adults (< 50 μg/g) can be used for children aged from 4 to 17 years, regardless of sex. A fecal calprotectin concentration > 50 μg/g warrants follow-up. However, the ESPGHAN expert group has strongly recommended that the laboratory in each center should establish its own normal fecal calprotectin cutoff values and account for variations in calprotectin levels throughout childhood and adolescence with range stratification into different age groups, such as infants, toddlers, children, and adolescents, with corresponding cutoff levels for each group[39].

Table 1 Comparison between serum and fecal calprotectin

Aspect	Serum calprotectin	Fecal calprotectin
Purpose	Marker of systemic inflammation	Marker of intestinal inflammation
Source	Neutrophils in the bloodstream	Neutrophils in the intestinal mucosa
Production	Neutrophils release calprotectin into the bloodstream during systemic inflammation	Neutrophils migrate to gut mucosa during inflammation, releasing calprotectin into the intestinal lumen
Method of measurement	Blood tests (serum) using techniques like ELISA or immunoassays	Stool samples using techniques like ELISA
Sample stability	Stable at room temperature for shorter periods	Requires refrigeration and prompt analysis
Clinical utility	Less commonly used. Monitor overall systemic inflammatory status; response to treatment	Widely used. Distinguish between GI disorders; assess disease activity
Role in pediatric GI disorders	Less specific to GI disorders, it may not reflect the severity of GI inflammation	Highly specific to GI inflammation; aids in diagnosis and monitoring of GI disorders
Advantages	Provides systemic inflammation status	Non-invasive; reflects intestinal inflammation accurately
Limitations	Less specific to GI disorders; not as accurate for GI evaluation, influenced by systemic inflammation	Invasive; requires stool sample collection; influenced by extraintestinal factors
Reference range	Lower levels, < 50 μg/mL	Higher levels, < 50 μg/g
Interpretation	Limited evidence for clinical interpretation	Established cutoffs for clinical interpretation
Cost	Typically, higher cost	Generally lower cost
Availability	May require specialized testing facilities	Widely available

ELISA: Enzyme-linked immunosorbent assay; GI: Gastrointestinal.

It's essential to interpret fecal calprotectin levels in the context of clinical symptoms, patient history, and other diagnostic findings. Elevated fecal calprotectin levels above the established cutoff may indicate intestinal inflammation, prompting further evaluation and management[22]. Laboratories may use different assays and methodologies for fecal calprotectin testing, leading to variations in cutoff levels (Table 2). Limited evidence suggests potential gender differences in fecal calprotectin cutoff values in children. While some studies have observed slight variations in calprotectin levels between males and females, the differences are generally not considered significant enough to warrant separate cutoff values based solely on gender. Fecal calprotectin levels are primarily influenced by factors such as age, intestinal inflammation, and gastrointestinal conditions rather than gender[47,48].

Cutoff values for fecal calprotectin in children are typically determined according to age-specific reference ranges instead of gender-specific ones. It is essential to follow the reference ranges and cutoff values provided by the analysis laboratory. Interpretation of fecal calprotectin levels in children should be based on the clinical context, established guidelines, and laboratory standards[44]. Due to significant interindividual variability, especially in young children, clinical decisions should consider fecal calprotectin levels and the overall clinical context. In preterm infants and children younger than 1 year, fecal calprotectin levels may be elevated without apparent inflammation, so careful interpretation is necessary until a normal range for this age group is established. For children older than 4 years, cutoff values of 50 μg/g, as in adults, can be used, although healthy children may have fecal calprotectin levels up to 100 μg/g or higher[39].

Fecal calprotectin in some pediatric gastrointestinal disorders

Functional gastrointestinal disorders: Fecal calprotectin, a marker of intestinal inflammation, has garnered attention in the context of functional gastrointestinal disorders (FGIDs) in pediatric patients. FGIDs are characterized by chronic or recurrent gastrointestinal symptoms without identifiable structural or biochemical abnormalities[49]. While traditionally considered non-inflammatory conditions, emerging evidence suggests a potential role for low-grade inflammation in some FGIDs, challenging conventional views[50]. Studies investigating fecal calprotectin levels in pediatric patients with FGIDs have yielded conflicting results. Some studies report elevated fecal calprotectin levels in subsets of patients with FGIDs, suggesting the presence of subclinical inflammation[51]. However, other studies, such as Flagstad *et al*[52], who studied children between 4 and 15 years with FGIDs, found no significant differences in fecal calprotectin levels between children with FGIDs and those without.

The interpretation of fecal calprotectin levels in pediatric FGIDs remains challenging due to several factors. The most crucial factor is the heterogeneity of FGIDs. FGIDs encompass a diverse group of disorders, including irritable bowel syndrome (IBS), functional abdominal pain disorders, and functional constipation[53]. Subtypes within FGIDs may exhibit distinct pathophysiological mechanisms[54], potentially influencing fecal calprotectin levels differently. In addition, the symptoms of FGIDs, such as abdominal pain, bloating, and altered bowel habits, overlap with those of IBD. Differential diagnosis between FGIDs and IBD is crucial but challenging, particularly in cases of atypical presentation [55]. Moreover, psychosocial factors, such as stress and anxiety, play a significant role in FGIDs and may contribute to symptom severity. However, the impact of psychological factors on fecal calprotectin levels in FGIDs remains unclear

Table 2 The different methods for measuring fecal calprotectin, including their descriptions and key characteristics

Method	Description
ELISA	The most common method for fecal calprotectin measurement
	Detection achieved through enzyme-linked secondary antibodies to quantify calprotectin in stool samples
	Provides quantitative results using colorimetric or fluorescent detection techniques
	Widely available and standardized
	High sensitivity and specificity
	Relatively expensive
	Requires laboratory equipment and trained personnel
Turbidimetric immunoassay	Measures turbidity produced when antibodies react with calprotectin in the stool sample
	Simple and automated, suitable for high-throughput analysis
Fluorescence enzyme immunoassay	Similar to ELISA but uses fluorescent markers to detect calprotectin-antibody complexes in the stool sample
	High sensitivity and specificity comparable to ELISA
	Faster than traditional ELISA
	Suitable for point-of-care testing
	Less widely available than ELISA
	Requires specialized equipment and trained personnel
Lateral flow immunoassay	Rapid and user-friendly method where stool migrates along a membrane containing immobilized antibodies specific to calprotectin
	Provides quick visual results
	Suitable for point-of-care settings or resource-limited environments
Chemiluminescent immunoassay	Utilizes chemiluminescent labels to detect calprotectin-antibody complexes in the stool sample.
	Often used in automated laboratory platforms for high sensitivity and a wide dynamic range of detection
Immunochromatographic tests	Similar to pregnancy tests, uses colored lines to indicate calprotectin levels
	Easy to use, requires minimal training
	Portable and potentially suitable for home use
	Less sensitive and specific than ELISA
	Requires visual interpretation, susceptible to user error
Mass spectrometry	A highly accurate and sensitive method for fecal calprotectin measurement
	Considered the gold standard for research but not widely used in clinical practice
	Very expensive, complex technique, not readily available
Quantum dot-based assay	Utilizes quantum dots, nanocrystals that emit fluorescent signals, for measuring fecal calprotectin
	Offers enhanced sensitivity and multiplexing capabilities
Quantitative polymerase chain reaction	Measures calprotectin mRNA in the stool sample, correlating with fecal calprotectin levels
	Provides high sensitivity and specificity, suitable for research purposes
	Provides quantitative results
Point-of-care tests	Rapid tests performed at the clinic or even at home
	Faster results (minutes to hours)
	Convenient for patients and healthcare providers
	Lower sensitivity and specificity compared to ELISA
	Limited availability and higher cost per test

ELISA: Enzyme-linked immunosorbent assay.

[56]. Furthermore, there is a lack of specificity in fecal calprotectin for inflammatory conditions. It could be elevated in various gastrointestinal disorders, infections, and even non-gastrointestinal conditions[22]. Despite these challenges, assessing fecal calprotectin levels in pediatric patients with FGIDs may offer valuable clinical insights. Elevated fecal calprotectin levels in FGIDs could indicate underlying inflammation or mucosal immune activation, prompting further evaluation or consideration of alternative diagnoses[57]. However, normal fecal calprotectin levels do not rule out the presence of FGIDs, highlighting the multifactorial nature of these disorders[52].

Infant colic: Infant colic, characterized by excessive crying and fussiness in otherwise healthy infants, remains a perplexing and challenging condition for both parents and healthcare providers. It is believed to be a self-limiting condition that typically resolves by the age of 3-4 months. Despite its prevalence, the exact cause of colic remains unclear, with various factors such as immature gastrointestinal function, infant temperament, gastrointestinal inflammation, gut microbiota composition, and feeding patterns being implicated[58]. While fecal calprotectin may be elevated in some cases of infant colic, it is not routinely used as a diagnostic test for this condition. The diagnosis of colic is usually based on the presence of specific criteria, such as crying for at least three hours a day, at least three days a week, in the preceding week[59]. Evaluating an infant with colic may involve a thorough medical history, physical examination, and sometimes additional tests to rule out other potential causes of the symptoms. Here, we summarize several studies investigating fecal calprotectin levels and gut microbiota in infants with colic, shedding light on potential associations with this enigmatic condition. Sommermeyer *et al*[60] observed significantly elevated fecal calprotectin levels equal to or greater than 100 μg/g in infants with colic. Interestingly, factors such as gender, type of feeding, gestational age, and birth weight did not appear to influence calprotectin levels. However, infants delivered *via* cesarean section showed significantly higher fecal calprotectin levels. Rhoads *et al*[61] corroborated these findings, noting that fecal calprotectin levels were approximately twice as high in infants with colic compared to control infants, measuring 413 +/- 71 μg/g *vs* 197 +/- 46 μg/g, respectively. Furthermore, they observed an increased presence of Klebsiella species and a decreased presence of Enterobacter/Pantoea species in the stool samples of infants with colic compared to control infants. Notably, these differences in gut microbiota were not influenced by factors such as breast *vs* formula feeding, consumption of elemental formula, or exposure to antibiotics. Additionally, Karabayır *et al*[62] reported significantly higher fecal calprotectin levels in infants with typical infant colic than control infants, with median values of 651 μg/g and 354 μg/g, respectively. Follow-up revealed that four infants developed food allergies. Fayed *et al*[63] found that infants with colic exhibited significantly higher fecal calprotectin levels and rates of Escherichia coli infection than infants without colic. Moreover, those with Escherichia coli infection demonstrated significantly higher fecal calprotectin levels than those without, highlighting the role of gastrointestinal inflammation and infection in infantile colic. However, Olafsdottir *et al* [64] did not find a significant difference in fecal calprotectin levels detected by enzyme-linked immunosorbent assay kit between infants with classic infant colic and healthy infants (278 +/- 105 μg/g *vs* 277 +/- 109 μg/g).

Functional constipation: Functional constipation refers to a condition in which children experience difficulty with regular bowel movements and infrequent evacuation of hard and painful stools without any underlying structural or organic cause. It is frequently accompanied by fecal incontinence and/or abdominal pain. It is a common condition in children and is often related to factors such as dietary habits, inadequate fluid intake, lack of physical activity, and psychological factors. However, gut inflammation is not one of the possible underlying mechanisms[65]. In some cases, functional constipation may be associated with low-grade inflammation or other underlying conditions that can be detected through tests like fecal calprotectin. Rashed studied fecal calprotectin in 40 children with functional constipation out of 180 children with various gastrointestinal disorders. Rashed found that the mean fecal calprotectin was 23.6 ± 21.8 μg/g with no significant differences compared with healthy control with sensitivity and specificity of 89% and 81%, respectively[66]. In addition, Mahjoub *et al*[67] compared fecal calprotectin in children with functional constipation with those with Hirschsprung's disease. They found that children with functional constipation had values below the predetermined cutoff value of 50 μg/g with a median value of 4 μg/g. While fecal calprotectin levels may be normal in individuals with functional constipation, elevated levels could indicate the presence of inflammation in the gastrointestinal tract[39].

Gastroesophageal reflux in infants and children: Functional gastroesophageal reflux (GER) in infants and children refers to regurgitating stomach contents into the esophagus without associated complications or underlying structural abnormalities[68]. While traditionally considered a non-inflammatory condition, emerging evidence suggests a potential link between GER symptoms and low-grade intestinal inflammation, as reflected by fecal calprotectin levels[69]. Studies investigating fecal calprotectin in functional GER have yielded variable results, with some reporting elevated calprotectin levels in affected individuals compared to healthy controls, suggesting a possible association with subclinical inflammation. Shelly *et al*[70] found significantly higher fecal calprotectin levels in preterm babies with GERD than in their peer controls. As GER disease is usually associated with more degrees of gastroesophageal pathology and, consequently, inflammation, it is expected to have these higher levels. Therefore, fecal calprotectin can help to differentiate GER from GERD. However, there is a lack of research about the value of fecal calprotectin in functional GER. Further research is needed to elucidate the role of fecal calprotectin in functional GER, its clinical implications, and its potential utility as a diagnostic or prognostic marker in this context. Understanding the relationship between fecal calprotectin and functional GER may provide insights into the pathophysiology of GER symptoms and inform personalized management approaches for affected individuals.

Functional abdominal pain disorders: Functional abdominal pain (FAPDs) in children encompasses chronic or recurrent abdominal pain without evidence of organic pathology that falls into four categories: functional dyspepsia, abdominal migraine, irritable bowel syndrome, and functional abdominal pain—not otherwise specified. While traditionally

considered a non-inflammatory condition, emerging research suggests a potential role for low-grade intestinal inflammation in some cases of FAPDs[50]. Studies investigating fecal calprotectin in FAPDs have produced mixed results, with some demonstrating elevated calprotectin levels in affected individuals compared to healthy controls, indicating possible subclinical inflammation. Moorman *et al*[71] found that children with FAPDs have significant gastrointestinal inflammation as indicated by the high fecal calprotectin, especially those with a clinically complex FAPDs profile (such as with increased rate and degree of anxiety, disability, and pain) than those with two or fewer elevations. However, other studies have not found significant differences in calprotectin levels between FAP patients and controls. Olafsdottir *et al*[64] found no significant differences in fecal calprotectin levels in healthy children and children with recurrent abdominal pain without an identifiable organic disease. At the same time, it was significantly lower than children suffering from IBD. Flagstad *et al*[52] also found no significant differences in fecal calprotectin levels in children with FGID, including those with FAPD and healthy children. In a relatively recent study, Pieczarkowski *et al*[72] found no significant differences in fecal calprotectin between children with FGIDs, including dysfunctional abdominal pain, and those with healthy control. In contrast, fecal calprotectin was significantly lower when compared with children with IBD. The interpretation of fecal calprotectin in FAP remains challenging due to the heterogeneous nature of the condition and the lack of standardized diagnostic criteria. ESPGHAN stated that fecal calprotectin levels in children with different forms of FAPDs are similar to or slightly higher than healthy but lower compared with children with IBD. ESPGHAN experts strongly recommended using fecal calprotectin to distinguish functional abdominal pain disorders from organic diseases [39].

Further research is needed to elucidate the relationship between fecal calprotectin and FAP, as well as its potential clinical implications in diagnosis and management. Understanding the role of intestinal inflammation in FAP may provide insights into its pathophysiology and guide the development of targeted therapeutic approaches.

Cow milk protein allergy: Cow milk protein allergy (CMPA) is a common food allergy among infants and young children, affecting around 2%-3% of infants in developed countries. This allergy is an immune-mediated reaction to specific proteins found in cow's milk, mainly casein and whey[73]. The gastrointestinal symptoms associated with CMPA are often nonspecific and typically not mediated by IgE antibodies. Therefore, the definitive diagnosis relies on eliminating milk from the diet to observe symptom resolution and assess for relapse upon milk reintroduction[74]. In infants and children with CMPA, increased levels of fecal calprotectin indicate underlying intestinal inflammation, likely caused by the immune system's response to cow milk proteins[75]. Studies have shown that infants with CMPA, particularly those with non-IgE-mediated disease, tend to have higher fecal calprotectin concentrations than healthy controls [76]. However, a study by Díaz *et al*[77] presented conflicting findings, with no significant difference in fecal calprotectin levels between infants with non-IgE-mediated CMPA and healthy controls. On the other hand, Zain-Alabedeen *et al*[78] found that infants with positive cow's milk-related-symptom- scores (CoMiSS) had higher fecal calprotectin levels than those with negative CoMiSS scores with a positive correlation between fecal calprotectin and CoMiSS scores. Because of the heterogeneous results about the role of fecal calprotectin in diagnosing CMPA, ESPGHAN stated that fecal calprotectin can be elevated in patients with CMPA with significant individual variability[39].

Monitoring fecal calprotectin levels in infants and children suspected of or diagnosed with CMPA can provide valuable insights into the presence and severity of intestinal inflammation and the response to elimination therapy. Qiu *et al*[79] observed a significant decrease in fecal calprotectin levels after dietary intervention in infants suffering from milk protein allergy, indicating that fecal calprotectin can be a helpful monitoring tool in this context. Elevated fecal calprotectin levels after the elimination diet may indicate the need for further diagnostic evaluation, including endoscopic assessment, to determine the extent and nature of inflammation. Additionally, fecal calprotectin levels can be used as a practical gauge to track the effectiveness of dietary management and therapeutic interventions for CMPA. However, the ESPGHAN expert group did not recommend using fecal calprotectin as a diagnostic or monitoring marker for CMPA in children[39].

Inflammatory bowel diseases: IBDs are characterized by chronic inflammation of the gastrointestinal tract, with two main categories affecting children: Crohn's disease and ulcerative colitis[80]. Diagnosing and managing IBD in children often requires a multidisciplinary approach involving pediatric gastroenterologists, dietitians, and other specialists. Fecal calprotectin has been extensively studied as a vital inflammatory biomarker that helps diagnose, monitor, and manage IBD[81]. As previously mentioned, calprotectin is released by neutrophils during gastrointestinal inflammation and offers a good indicator of the severity and extent of inflammation within the intestines. Clinically, fecal calprotectin can help distinguish inflammatory from non-inflammatory bowel conditions and guide the need for further evaluation, such as endoscopy[82,83]. It is also superior as a screening tool for identifying IBD in undiagnosed patients to blood inflammatory markers like C-reactive protein (CRP) or ESR[84]. However, according to the ESPGHAN, diagnostic endoscopy should not be delayed in cases where IBD is strongly suspected, and fecal calprotectin results are not promptly available [39].

There is no consensus on the acceptable fecal calprotectin levels for disease management. A meta-analysis of 9 studies by Degraeuwe *et al*[85] found that the best cut-off value to screen for IBD was 212 μg/g, with a sensitivity and specificity of 0.90 and 0.87, respectively. Relying on a cut-off value of 50 μg/g yields a false positive in 17% and a false negative in 2% of cases. However, many studies used the cut-off value of 100 μg/g as a trigger to investigate the possibility of IBD[86, 87]. However, we should consider that young children may have typically high fecal calprotectin levels. While fecal calprotectin is a useful biomarker for diagnosing IBD, it has its limitations in distinguishing between different types of IBD, such as Crohn's disease and ulcerative colitis. Although fecal calprotectin levels usually indicate the presence and severity of intestinal inflammation in IBD, they do not consistently differentiate between Crohn's disease and ulcerative colitis. Both Crohn's disease and ulcerative colitis can result in elevated fecal calprotectin levels due to the underlying

inflammatory processes in the gastrointestinal tract. Thus, fecal calprotectin alone cannot definitively distinguish between Crohn's disease and ulcerative colitis[88]. However, some studies have suggested that fecal calprotectin levels may differ depending on the specific characteristics of the disease. For instance, fecal calprotectin levels may be higher in Crohn's disease patients with colonic involvement than those with isolated small bowel disease[89]. Similarly, fecal calprotectin levels may be lower in ulcerative colitis patients with isolated proctitis than those with more extensive colonic involvement[90].

Moreover, serial Fecal calprotectin measurements help assess the disease activity, treatment response, and the risk of relapse, with decreasing levels often indicative of successful treatment and remission[83]. Elevated fecal calprotectin levels at diagnosis or during treatment are associated with increased risks of disease progression and adverse outcomes [91]. The sensitivity and specificity of fecal calprotectin testing and its negative and positive predictive values vary depending on patient cohorts and potential confounding factors leading to elevated fecal calprotectin levels, such as bacterial or viral gastroenteritis or juvenile polyps[92].

Fecal calprotectin elevation generally correlates with histological inflammation, but absolute levels don't categorize disease activity without endoscopy[93]. While capsule endoscopy scores may not directly correlate with fecal calprotectin values, higher fecal calprotectin levels are associated with a greater likelihood of detecting lesions[94]. This suggests that elevated fecal calprotectin levels can indicate increased disease severity and the need for further investigation. Despite fecal calprotectin's utility, patients strongly suspected of IBD, especially those with alarm symptoms, should undergo endoscopic examination regardless of fecal calprotectin values[95]. Other neutrophil-derived markers of IBD, such as lactoferrin, myeloperoxidase, matrix metalloproteinase 9, or S100A12, perform similarly to fecal calprotectin but offer no added value when used alongside it[96].

Notably, fecal calprotectin testing offers a non-invasive means of monitoring intestinal inflammation, reducing the reliance on invasive procedures like endoscopy. Its integration into clinical practice guidelines has optimized IBD management, facilitating early detection of disease recurrence, treatment optimization, and cost-effective healthcare utilization[97]. Though rare, normalization of FC is seen in patients with ulcerative colitis in clinical remission, while absolute fecal calprotectin levels in acute severe colitis don't predict prognosis[98]. Therapeutic interventions such as Infliximab or exclusive enteral nutrition (EEN) have rapidly reduced fecal calprotectin levels. Notably, Foster *et al*[99] found that fecal calprotectin levels above 250 mg/g in children with Crohn's disease on Infliximab therapy may signify a risk of clinical relapse within three months. On the other hand, Logan *et al*[100] found that fecal calprotectin reduction during exclusive enteral nutrition may be less clear and more gradual. Exclusion diets are also associated with mucosal healing and decreased fecal calprotectin levels. Overall, fecal calprotectin plays a pivotal role in the holistic care of individuals with IBD, enhancing diagnostic accuracy, treatment monitoring, and prognostication[101]. Further research is needed to establish standardized cut-off values and optimize the clinical utility of fecal calprotectin in pediatric IBD management.

Coeliac disease: Coeliac disease is a chronic autoimmune condition that causes inflammation in the small intestine when individuals with a genetic susceptibility consume gluten. This inflammation primarily damages the intestinal mucosa, leading to villous atrophy, crypt hyperplasia, and infiltration of inflammatory cells[102]. The immune system is involved in this process, and both innate and adaptive mechanisms contribute to it. This leads to the release of cytokines and immune cell activation, causing tissue damage[103]. Fecal calprotectin is often used as a biomarker of inflammation, but there is limited, scarce, and inconsistent data on its usefulness in diagnosing and managing coeliac disease in children. Due to the presence of significant inflammation in patients with celiac disease, fecal calprotectin levels tend to be significantly elevated at the time of diagnosis, particularly in those presenting with higher levels of serological markers or classic symptoms. Balamtekın *et al*[104] found significantly higher fecal calprotectin levels in newly diagnosed children with celiac disease than in those on gluten-free diets and healthy controls, respectively. They also observed significantly higher levels in children presenting with gastrointestinal symptoms than in those without.

Although the coeliac disease is primarily characterized by small intestinal inflammation rather than the large intestinal inflammation seen in IBD, fecal calprotectin levels are elevated in patients with active coeliac disease compared to those in remission or healthy controls[105]. This suggests that fecal calprotectin may reflect the degree of mucosal inflammation in coeliac disease, offering potential insights into disease activity and reflecting disease histopathological findings and response to treatment[104]. However, conflicting findings have emerged regarding the correlation between fecal calprotectin levels and anti-tissue transglutaminase antibody levels. Shahramian *et al*[106] found a significant correlation between fecal calprotectin level and IgA anti-tissue transglutaminase titer in 70 newly diagnosed children with coeliac disease. On the other side, Montalto *et al*[107] found no significant differences in fecal calprotectin levels between untreated adults with coeliac disease and the control and no significant relation between fecal calprotectin and lesion severity, clinical score, or degree of neutrophil infiltration. However, this study was performed in adult patients with late-onset coeliac disease than usually observed in children. Meanwhile, according to the Marsh classification, Szaflarska-Popławska *et al*[108] also found no significant relationship between fecal calprotectin and both the clinical picture and small intestinal lesions. In fifty-five children recently diagnosed with coeliac disease.

While fecal calprotectin values in coeliac disease patients at diagnosis typically average around 100 mg/g across many studies, they significantly increase compared to controls[39]. However, it's important to note that fecal calprotectin elevation in coeliac disease may not be specific to mucosal inflammation related to gluten exposure[108]. Other factors, such as concomitant gastrointestinal conditions or dietary habits, could influence fecal calprotectin levels, potentially confounding its interpretation in coeliac disease patients[104].

Fecal calprotectin can also be used as a useful non-invasive tool to assess mucosal healing and monitor disease activity in coeliac disease. Studies have shown that fecal calprotectin levels decrease following a gluten-free diet, the primary treatment for coeliac disease, indicating a reduction in intestinal inflammation[109]. Furthermore, persistent elevation of

fecal calprotectin levels despite adherence to a gluten-free diet may suggest ongoing mucosal inflammation or non-responsive coeliac disease, prompting further evaluation and management[110]. Notably, this difference diminishes within four to 12 months after initiating a gluten-free diet. Despite these observations, fecal calprotectin values exhibit wide individual variability, leading to overlap between active coeliac disease and control groups[39]. Although there has been interest in utilizing fecal calprotectin to assess dietary compliance and histological recovery, no significant association has been established between fecal calprotectin levels and histological lesions. Published results suggest that fecal calprotectin measurement does not confer additional benefits beyond the currently employed serological markers in the diagnostic or follow-up phases. In addition, the ESPGHAN expert group recommended against using fecal calprotectin as a marker for diagnosing or monitoring coeliac disease[39]. Despite these challenges, fecal calprotectin holds promise as a complementary tool in managing coeliac disease, providing valuable information about intestinal inflammation and treatment response. Further research is needed to elucidate fecal calprotectin's clinical utility in coeliac disease and establish standardized protocols for its use in clinical practice. Additionally, future studies should explore the potential utility of fecal calprotectin as a prognostic marker and its role in predicting long-term outcomes in coeliac disease patients.

COVID-19-induced pediatric gastrointestinal disorders: COVID-19 is a respiratory illness caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, it is increasingly recognized that gastrointestinal symptoms such as diarrhea, vomiting, and abdominal pain are also present, particularly in children[111]. Fecal calprotectin is a marker of intestinal inflammation and can provide insights into the pathophysiology and clinical course of gastrointestinal involvement, especially in severe cases of children with COVID-19[112]. Studies suggest that elevated fecal calprotectin levels are associated with varying degrees of intestinal inflammation with gastrointestinal symptoms in children with COVID-19. This ongoing inflammation can contribute to the overall disease severity and prognosis[113]. However, even patients without gastrointestinal symptoms may have elevated fecal calprotectin levels, indicating subclinical intestinal inflammation, especially in severe COVID-19 cases, as shown by Ojetti *et al*[114]. In addition, Shokri-Afra *et al*[115] found serum and fecal calprotectin levels are not correlated with diarrhea or other gastrointestinal symptoms. Fecal calprotectin levels could be a non-invasive tool for monitoring disease activity and treatment response in children with COVID-19 patients with gastrointestinal involvement. Serial fecal calprotectin measurements may help clinicians assess the resolution of intestinal inflammation over time and guide therapeutic interventions[116].

It's important to note that elevated fecal calprotectin levels are not specific to COVID-19 and can be influenced by various factors such as other infections, inflammatory conditions, or dietary factors[117,118]. In addition, some COVID-19-associated gastrointestinal manifestations are due to autonomic changes and not inflammation, in which fecal calprotectin could be normal despite gastrointestinal symptoms[119]. Therefore, fecal calprotectin should be interpreted in conjunction with clinical findings, other laboratory tests, and imaging studies to comprehensively assess gastrointestinal health in pediatric COVID-19 patients[120]. Further research is needed to establish standardized protocols for fecal calprotectin's clinical use in COVID-19-induced pediatric gastrointestinal disorders. Nevertheless, the preliminary evidence suggests that fecal calprotectin could be a useful biomarker for monitoring this population's intestinal inflammation and disease progression.

Gastrointestinal infections

Infectious gastroenteritis, caused by various pathogens, including viruses, bacteria, and parasites, is a common condition characterized by inflammation of the gastrointestinal tract[121]. Fecal calprotectin has emerged as a valuable biomarker in diagnosing and managing infectious gastroenteritis, aiding in differentiating between bacterial and viral causes, helping to assess the severity and predict prognosis of infection, and differentiating between infectious and non-infectious causes of gastrointestinal symptoms[122]. Viral gastroenteritis, often caused by viruses such as norovirus, rotavirus, and adenovirus, is a leading cause of gastroenteritis worldwide, particularly in children[123]. Studies have demonstrated elevated fecal calprotectin levels in patients with viral gastroenteritis, indicating the presence of intestinal inflammation. However, fecal calprotectin elevation in viral gastroenteritis may be transient and resolve as the infection clears. Despite this, fecal calprotectin can still be useful in monitoring disease activity and assessing the need for further evaluation or intervention[124].

Bacterial gastroenteritis, commonly caused by pathogens like *Salmonella, Campylobacter, Escherichia coli,* and *Shigella*, is characterized by inflammation of the intestinal mucosa. Fecal calprotectin levels are elevated in patients with bacterial gastroenteritis, reflecting the severity of mucosal inflammation. Combining fecal calprotectin with occult blood in the stool is a useful marker for diagnosing bacterial etiology in children with acute gastroenteritis[125]. In cases of bacterial gastroenteritis, fecal calprotectin elevation may be higher and persist longer than viral infections, correlating with the serenity and persistence of symptoms and the extent of mucosal damage[126]. Sýkora *et al*[127] found that fecal calprotectin can help tell if the AGE is caused by bacteria or viruses in children under 3 years old. They found that combining fecal calprotectin with other inflammatory markers, such as CRP, can accurately differentiate bacteria (median fecal calprotectin was 219 μg/g) from viral (median fecal calprotectin was 49.3 μg/g) AGE in children. In addition, Duman *et al* [128] showed that fecal calprotectin levels are higher in patients with certain bacterial infections like *Salmonella* and *Shigella* compared to viral infections like Rotavirus and Norovirus. They found that fecal calprotectin can be a good marker for diagnosing bacterial AGE, with high sensitivity and specificity, when a cutoff value of fecal calprotectin of 710 μg/g is used. However, in a severe form of acute gastroenteritis, fecal calprotectin cannot differentiate between viral and bacterial gastroenteritis, especially in those who need hospitalization[129]. However, the ability of fecal calprotectin to assess the severity of acute gastroenteritis remains uncertain. While it seems to be linked to the severity of symptoms in children, it may not be as useful in adults with *Clostridium difficile* infection. In *Clostridium difficile*, both fecal calprotectin

and fecal lactoferrin increase, but they don't seem to reflect how serious the infection is and cannot differentiate between *Clostridium difficile* infection and antibiotic-associated diarrhea[130,131].

Parasitic gastroenteritis, caused by parasites such as *Giardia lamblia, Cryptosporidium* spp., and *Entamoeba histolytica*, can also lead to intestinal inflammation[132]. Studies have shown that fecal calprotectin levels are elevated in patients with parasitic gastroenteritis, indicating ongoing mucosal inflammation. Fecal calprotectin measurement may help differentiate parasitic infections from other causes of gastroenteritis and assess the response to anti-parasitic therapy[133,134]. Overall, fecal calprotectin is a valuable tool in evaluating infectious gastroenteritis, providing clinicians with important information about the presence and severity of intestinal inflammation[126]. While fecal calprotectin elevation is not specific to infectious gastroenteritis and can occur in other gastrointestinal conditions, its measurement can aid in diagnosing, managing, and monitoring patients with infectious gastroenteritis of viral, bacterial, or parasitic origin. However, The ESPGHAN expert group recommended against the routine use of fecal calprotectin in acute gastroenteritis, aiming to distinguish viral from bacterial viral gastroenteritis in children[39]. Further research is needed to elucidate the role of fecal calprotectin in specific pathogens and its utility in guiding treatment strategies for infectious gastroenteritis.

Cystic fibrosis: Cystic fibrosis is a prevalent genetic disorder characterized by multi-organ involvement due to dysfunctional ion transport across epithelial cells, leading to thick and sticky mucus production, primarily affecting the pancreas (85%), respiratory system, and gastrointestinal tract[135]. While respiratory symptoms dominate the clinical picture, gastrointestinal manifestations are also common in cystic fibrosis, including malabsorption, pancreatic insufficiency, and liver disease[136]. Mounting evidence suggests that cystic fibrosis-associated intestinal pathology is characterized by mucous accumulation, dysmotility, and dysbiosis, contributing to chronic inflammation and microbial colonization[137]. Notably, studies employing whole gut lavage and capsule endoscopy have highlighted increased levels of inflammatory biomarkers, including interleukin-8, in cystic fibrosis patients, indicative of ongoing intestinal inflammation[138].

Fecal calprotectin is a promising biomarker for assessing gastrointestinal inflammation and mucosal integrity in children with cystic fibrosis. Elevated fecal calprotectin levels in children with cystic fibrosis reflect bacterial overgrowth, which induces intestinal inflammation and correlates with the severity of gastrointestinal symptoms and disease progression, as evidenced by Rumman *et al*[139]. Therefore, oral probiotic administration can reduce bacterial overgrowth-induced intestinal inflammation and significantly reduce fecal calprotectin levels[140,141]. Additionally, fecal calprotectin levels have been found to correlate with markers of nutritional status and pancreatic function, suggesting a link between intestinal inflammation and malnutrition in cystic fibrosis. A meta-analysis by Talebi *et al*[142] found that median fecal calprotectin levels were inversely associated with body mass index (BMI) and BMI Z score, reflecting the nutritional status of the patients. However, Adriaanse *et al*[143] found this negative correlation in adult patients and not in children. Studies have shown that fecal calprotectin levels are higher in patients with cystic fibrosis and pancreatic insufficiency, indicating a potential association between pancreatic status and intestinal inflammation[27,142]. However, studies also observed increased fecal calprotectin in children with or without pancreatic insufficiency. This increase in fecal calprotectin may be due to the pulmonary production of calprotectin, which is swallowed into the gut, potentially confounding its interpretation, especially in the presence of pulmonary infections[144]. This observation can also explain why fecal calprotectin levels may inversely correlate with lung function in adult patients with cystic fibrosis[145]. However, the relationship is less clear in children. However, there is a lack of conclusive evidence regarding the extent to which sputum calprotectin contributes to fecal calprotectin levels due to the possible denaturation of sputum calprotectin by gastric acidity[146]. Another important confounding to fecal calprotectin in patients with cystic fibrosis is that the underlying genetic abnormalities in cystic fibrosis could affect the S100A8 and S100A9 gene expression, causing altered epithelial barrier function and impaired innate immunity, particularly at respiratory and gastrointestinal epithelium[147].

Furthermore, fecal calprotectin has shown utility in monitoring response to therapy and predicting clinical outcomes in cystic fibrosis patients[148]. High fecal calprotectin can also affect the quality of life of children, with poor quality of life with higher levels[149]. Changes in fecal calprotectin levels over time can indicate treatment efficacy and disease progression, guiding therapeutic interventions and optimizing patient care. Fecal calprotectin may also serve as a non-invasive tool for assessing intestinal inflammation in cystic fibrosis patients, reducing the need for invasive procedures such as endoscopy[150]. However, it's important to note that fecal calprotectin levels can be influenced by factors other than cystic fibrosis-related intestinal inflammation, such as infection, dietary factors, and certain medications, such as antibiotics[39]. Therefore, fecal calprotectin measurements should be interpreted considering the clinical symptoms and other laboratory findings to avoid misinterpretation. Despite these challenges, fecal calprotectin has shown promise as a marker of intestinal inflammation in cystic fibrosis and has been used to monitor treatment response and predict clinical outcomes[139]. However, more research is needed to establish its reliability in assessing intestinal inflammation and guiding treatment decisions in patients with cystic fibrosis. Further studies should also explore its correlation with endoscopic and histological findings for a better understanding of its clinical utility in cystic fibrosis management.

Pros and cons of fecal calprotectin use in pediatrics gastrointestinal diseases

Fecal calprotectin is a laboratory test commonly used to diagnose gastrointestinal disorders in children and adults. It offers significant advantages in diagnosing, monitoring, and managing pediatric gastrointestinal diseases[9]. However, when interpreting results and making clinical decisions, we must also consider its limitations and challenges. One of the significant advantages of fecal calprotectin is that it provides a non-invasive means of assessing intestinal inflammation. This makes it particularly valuable in pediatric patients who may be averse to or unable to undergo invasive procedures like endoscopy[83]. The non-invasive nature of the test enhances patient comfort and compliance with monitoring protocols. Elevated fecal calprotectin levels can indicate the presence of intestinal inflammation even before clinical symptoms manifest[151]. This allows for early detection and diagnosis of gastrointestinal diseases in pediatric patients. Early identification facilitates prompt initiation of treatment and potentially prevents disease progression[152]. Fecal

calprotectin also helps differentiate between inflammatory and non-inflammatory gastrointestinal conditions. This aids in the diagnosis of conditions like IBD and cystic fibrosis-associated intestinal pathology[153]. Overall, fecal calprotectin is a valuable tool in diagnosing and monitoring pediatric gastrointestinal diseases, but its limitations and challenges must be considered when interpreting results and making clinical decisions. Table 3 outlines the changes in fecal calprotectin levels across different gastrointestinal diseases and populations.

While fecal calprotectin levels indeed vary across different gastrointestinal diseases, the question of whether calprotectin is directly involved in the occurrence and development of these diseases is a complex one. Calprotectin, a calcium-binding protein predominantly found in neutrophils, plays a crucial role in the innate immune response, particularly in inflammation and host defense mechanisms in the gastrointestinal tract [22]. For example, in IBD, calprotectin is often used as a marker of intestinal inflammation. Elevated fecal calprotectin levels are associated with active disease and correlate with disease severity in both Crohn's disease and ulcerative colitis[154]. However, whether calprotectin directly contributes to the pathogenesis of IBD or is merely a consequence of ongoing inflammation remains a subject of investigation. Similarly, in coeliac disease, where intestinal inflammation is triggered by gluten consumption in genetically susceptible individuals, fecal calprotectin levels are elevated, reflecting mucosal inflammation. While calprotectin may contribute to the inflammatory response, its exact role in the pathogenesis of coeliac disease requires further elucidation [21].

In infectious gastroenteritis, including viral, bacterial, and parasitic infections, fecal calprotectin levels rise in response to intestinal inflammation caused by the invading pathogens. Calprotectin likely serves as a marker of the host's immune response to infection rather than being directly involved in disease occurrence[10]. In cystic fibrosis-associated intestinal pathology, characterized by mucous accumulation, dysmotility, and dysbiosis, calprotectin levels are elevated due to bacterial overgrowth-induced inflammation[155]. Calprotectin may contribute to perpetuating inflammation in the intestinal mucosa, exacerbating disease severity[142]. Overall, while fecal calprotectin is a valuable biomarker for assessing gastrointestinal inflammation, its precise role in the occurrence and development of specific gastrointestinal diseases remains incompletely understood. It likely represents a component of the host's immune response to mucosal injury and inflammation rather than being a primary driver of disease pathogenesis[156]. Further research is needed to clarify the mechanistic role of calprotectin in gastrointestinal diseases and its potential as a therapeutic target.

On the other hand, fecal calprotectin has many cons that should be considered while being interpreted. Elevated fecal calprotectin levels are not specific to any particular gastrointestinal condition. They can occur in various inflammatory and non-inflammatory conditions, including infectious gastroenteritis, irritable bowel syndrome, and even non-gastrointestinal conditions like asthma and non-inflammatory conditions such as celiac disease. This lack of specificity may lead to false-positive results and diagnostic ambiguity[115]. Interpreting fecal calprotectin levels in pediatric patients with gastrointestinal diseases can be challenging due to factors such as variations in normal values among different age groups, the presence of confounding factors, and the lack of established cutoff values for certain conditions[43]. In certain pediatric gastrointestinal diseases, the specificity of fecal calprotectin as a marker of intestinal inflammation may be limited due to factors such as pulmonary production of calprotectin, genetic abnormalities affecting gene expression, and overlap with respiratory symptoms[157].

Inconsistent levels of fecal calprotectin in various pediatric gastrointestinal disorders stem from a multitude of factors. Firstly, demographic factors such as age can influence calprotectin levels, alongside diet, medication usage (*e.g.*, nonsteroidal anti-inflammatory drugs or antibiotics), gastrointestinal bleeding, and concurrent infections. Infants and young children may have naturally higher levels of calprotectin compared to older children and adults due to factors such as the ongoing development of the gastrointestinal tract and the maturation of the immune system[118]. Disease-specific factors also contribute to variation. IBD, characterized by dysregulated immune responses and chronic inflammation, typically exhibit consistently elevated calprotectin levels[158]. Conversely, functional gastrointestinal disorders like IBS often lack significant inflammation, resulting in normal-range calprotectin levels[57]. Disease severity further complicates matters; acute infectious gastroenteritis or IBD flare-ups generally correlate with markedly elevated fecal calprotectin levels[159]. Host immune responses, including genetic predispositions and individual inflammation susceptibilities, also influence calprotectin production[160]. Methodological considerations, such as sampling timing and assay techniques, introduce additional variability[161]. Moreover, lifestyle factors, medication usage, and dietary habits can impact calprotectin expression[118]. Grasping these intricacies is essential for accurately interpreting calprotectin measurements and discerning their clinical significance in pediatric gastrointestinal disease diagnosis and management. Table 4 provides a concise overview of the diverse factors influencing fecal calprotectin levels in pediatric gastrointestinal disorders, helping to understand the complexities involved in result interpretation and diagnosis.

In addition, collecting stool samples for fecal calprotectin testing may be challenging in younger pediatric patients or those with certain conditions affecting bowel habits[36]. While fecal calprotectin testing is generally considered cost-effective for diagnosing and monitoring certain conditions, it may still represent an added cost, particularly if repeated testing is necessary over time. Clinicians should be aware of these limitations when using fecal calprotectin in these populations. While fecal calprotectin is widely used in clinical practice, there is a lack of consensus among expert groups regarding its routine use in certain pediatric gastrointestinal conditions, such as acute gastroenteritis[162]. The absence of standardized guidelines may lead to variability in clinical practice and uncertainty regarding its appropriate use in specific patient populations. Table 5 summarizes the pros and cons of using fecal calprotectin in children with gastrointestinal disorders.

Limitations of the study

The primary limitation of this review is inherent to the selection of articles from databases and search engines. Despite efforts to include a comprehensive range of studies, there may be a risk of selection bias, wherein certain articles or perspectives were inadvertently excluded. Additionally, the inclusion of only English-language articles may have

Table 3 The changes in fecal calprotectin levels across different gastrointestinal diseases and populations

Cited literature	Gastrointestinal disease	Population	Calprotectin level	Changes in fecal calprotectin levels
Flagstad *et al*[52], 2010	FGID	Children between 4 and 15 yr	16 μg/g	No significant differences in FC levels between children with FGIDs and those without
Rhoads *et al*[61], 2009	Infant colic	Infants	413 +/- 71 μg/g *vs* 197 +/- 46 μg/g	FC levels were approximately twice as high in infants with colic compared to control infants
Karabayır *et al* [62], 2021	Infant colic	Infants	651 μg/g and 354 μg/g, respectively	Significantly higher FC typical infant colic than in control infants
Olafsdottir *et al* [64], 2002	Infant colic	Infants	278 +/- 105 μg/g *vs* 277 +/- 109 μg/g	No significant difference in FC levels was detected between infants with classic infant colic and healthy infants
Pieczarkowski *et al* [72], 2018	FGID and IBD	Children	1191.5 μg/g. in IBD and 100 μg/g.in controls and patients with FIGDs	Patients with IBD and other inflammatory GI disorders had a significantly higher FC level than those in control
Rashed *et al*[66], 2022	Functional constipation	Children	23.6 ± 21.8 μg/g	No significant differences compared with healthy control
Mahjoub *et al*[67], 2013	Functional constipation	Children	< 50 μg/g	FC was below the predetermined cutoff value of 50 μg/g
Shelly *et al*[70], 2021	GERD	Preterm babies	-	High levels of FC in preterm babies with GERD than in their peer controls
Moorman *et al*[71], 2021	FAPD	Children	≥ 50 μg/g	Children with FAPDs have significantly high FC, especially those with a clinically complex FAPD profile
Díaz *et al*[77], 2018	Non-IgE-mediated CMPA	Infants	-	No significant differences with healthy control
Zain-Alabedeen *et al*[78], 2023	CMPA	Infants	2934.57 μg/g *vs* 955.13 μg/g	Infants with positive CoMiSS had higher FC levels than those with negative CoMiSS scores with positive correlation between CoMiSS & FC
Qiu *et al*[79], 2021	CMPA	Infants	-	Significant FC reduction after dietary intervention
Degraeuwe *et al* [85], 2015	IBD	Children	was 212 μg/g	The best cut-off value to screen for IBD was 212 μg/g, with a sensitivity and specificity of 0.90 and 0.87, respectively
Foster *et al*[99], 2019	Crohn's disease	Children	250 μg/g	FC levels above 250 μg/g in children with Crohn's disease on Infliximab therapy may signify a risk of clinical relapse within three months
Balamtekın *et al* [104], 2012	Coeliac disease	Newly diagnosed children	117.2 μg/g in patients *vs* 3.7 μg/g in controls	Elevated levels compared to healthy controls and those on gluten-free diets. It is also higher in children with GI symptoms than those without
Shahramian *et al* [106], 2019	Coeliac disease	Newly diagnosed children	239.1 ± 177.3 μg/g *vs* 38.5 ± 34.6 μg/g in controls	a significant correlation between FC level and IgA ATGA titers
Montalto *et al* [107], 2007	Coeliac disease	Adults	-	No significant differences in FC levels between untreated adults with coeliac disease and the control and no significant relation between FC and lesion severity, clinical score, or degree of neutrophil infiltration
Szaflarska-Popławska *et al* [108], 2020	Coeliac disease	Newly diagnosed children	91.7 ± 144.8 μg/g	No significant relationship between FC and both the clinical picture and small intestinal lesions
Ojetti *et al*[114], 2020	COVID-19-induced gastrointestinal disorders	Adults with COVID-19	> 50 μg/g	Elevated levels associated with varying degrees of intestinal inflammation, including subclinical cases
Shokri-Afra *et al* [115], 2021	COVID-19-induced gastrointestinal disorders	Adults with COVID-19	124.3 *vs* 25.0 μg/g	Serum and FC levels are not correlated with diarrhea or other gastrointestinal symptoms
Sýkora *et al*[127], 2010	Acute gastroenteritis	Children under 3 yr	219 μg/g in bacterial *vs* 49.3 μg/g in viral	FC can help tell if the AGE is caused by bacteria or viruses
Duman *et al*[128], 2015	Infectious gastroenteritis	Children with bacterial gastroenteritis	710 μg/g	Higher and persistent elevation compared to viral gastroenteritis, correlates with the severity and persistence of symptoms

Czub *et al*[129], 2014	Infectious gastroenteritis	Hospitalized children with severe gastroenteritis	20 (viral) *vs* 55 (Bacterial) *vs* 4 (healthy control) ug/mL	FC cannot differentiate between severe viral from bacterial gastroenteritis
Rumman *et al* [139], 2014	Cystic fibrosis	Children with cystic fibrosis	94.29 μg/g	Elevated levels reflect bacterial overgrowth and correlate with the severity of gastrointestinal symptoms

CMPA: Cow milk protein allergy; CoMiSS: Cow'S milk-related-symptom- scores; FAPD: Functional Abdominal pain disorders; FC: Fecal calprotectin; GERD: Gastroesophageal reflux disease; IBD: Inflammatory bowel diseases; ATGA: Anti-tissue transglutaminase; GI: Gastrointestinal; COVID-19: Coronavirus disease 2019.

Table 4 Factors influencing inconsistent levels of fecal calprotectin in various pediatric gastrointestinal disorders

Factors	Influence on fecal calprotectin levels
Demographic factors	Age; higher in infants and younger children
Dietary factors	The diet that increases FC includes inflammatory Foods (such as saturated fats, refined sugars, and processed ingredients), food Sensitivities and Allergies, Alcohol and Caffeine, and dehydration. The diet that decreases FC includes hydration, high fiber intake (such as fruits, vegetables, whole grains, and legumes), Omega-3-containing foods (such as fatty fish (*e.g.*, salmon, mackerel, sardines), flaxseeds, and walnuts), and prebiotics and probiotics
Medication usage	Drugs that could increase FC levels: Prolonged use of NSAIDs, antibiotics, PPIs, and antidiarrheal medications such as loperamide. Drugs that could reduce FC levels: Corticosteroids, immunosuppressants, such as azathioprine, and methotrexate, biological agents like infliximab and adalimumab, and Probiotics
Gastrointestinal conditions	Gastrointestinal bleeding, concurrent infections
Disease-specific factors	Disease etiology (*e.g.*, IBD *vs* functional GI disorders like IBS)
Disease severity	Severity of inflammation (*e.g.*, active inflammation in acute infectious gastroenteritis or IBD flare-ups)
Host immune responses	Genetic factors, individual susceptibility to inflammation
Methodological considerations	Sampling timing, assay methodologies
Lifestyle factors	Medication usage, dietary habits

FC: Fecal Calprotectin; GI: Gastrointestinal; IBD: Inflammatory bowel disease; IBS: Irritable bowel syndrome; NSAIDs: Nonsteroidal anti-inflammatory drugs; PPI: Proton pump inhibitors.

Table 5 The advantages (pros) and disadvantages (cons) of using fecal calprotectin in various pediatric gastrointestinal disorders

Aspect	Pros	Cons
Non-invasive	Well-tolerated by pediatric patients	Collection may be challenging in certain patients
Sensitive marker	Detects intestinal inflammation accurately	Elevated in various conditions, leading to potential false positives. Elevated levels can occur in non-gastrointestinal conditions. Limited utility in certain conditions like acute infectious gastroenteritis or functional disorders
Disease monitoring	Helps monitor disease activity and treatment response	Does not provide information on specific cause or location of inflammation
Early detection	Allows for early detection and intervention	Interpretation may vary depending on age. Variability in cutoff values across laboratories
Differentiation	Aids in differentiating between inflammatory and non-inflammatory conditions	Limited specificity for diagnosis. Interpretation challenges requiring clinical expertise
Cost-effective	Generally considered cost-effective for diagnosis and monitoring	Represents added cost for repeated testing

introduced language bias. Another limitation relates to the quality and reliability of the included studies. While efforts were made to select high-quality research articles, review articles, meta-analyses, and consensus papers, variations in study design, sample sizes, methodologies, and reporting standards across studies may have influenced the robustness of the findings. There is a possibility of publication bias, whereby studies with positive or significant results are more likely to be published than those with null or negative findings. This bias could impact the comprehensiveness of the evidence base and skew the interpretation of fecal calprotectin's role in pediatric gastrointestinal diseases. The interpretation of fecal calprotectin levels in pediatric gastrointestinal diseases is complex and multifaceted. While elevated levels are generally indicative of intestinal inflammation, other factors such as age, diet, medication use, and concurrent infections can influence results. Therefore, the findings of this review should be interpreted with caution, considering the contextual factors surrounding fecal calprotectin testing in clinical practice. The generalizability of the findings may be limited by the heterogeneity of the included studies and the specific populations under investigation. The role of fecal calprotectin in pediatric gastrointestinal diseases may vary across different patient demographics, disease severities, and geographical regions, which should be considered when applying the findings to specific clinical settings. Despite these limitations, this review provides valuable insights into the pros and cons of fecal calprotectin use in pediatric gastrointestinal diseases. Future research should aim to address the limitations identified herein by conducting well-designed prospective studies with standardized methodologies, larger sample sizes, and diverse patient populations.

Recommendations

After conducting a comprehensive analysis, we have identified several recommendations to make fecal calprotectin testing more effective in diagnosing and managing pediatric gastrointestinal diseases. To begin with, standardizing protocols for fecal calprotectin testing can improve the consistency and comparability of results across different studies and clinical settings. Developing age-specific reference ranges for fecal calprotectin levels can account for variations in pediatric populations, which can help accurately interpret test results. Integrating fecal calprotectin testing with other clinical parameters such as symptoms and imaging studies can enhance diagnostic accuracy and facilitate personalized management strategies for pediatric patients. Additionally, incorporating serial fecal calprotectin measurements into routine monitoring protocols can enable clinicians to track disease progression, assess treatment response, and detect early signs of relapse in pediatric gastrointestinal diseases. We believe that continued research efforts are necessary to explore the clinical utility of fecal calprotectin in specific pediatric populations, optimize cutoff values for different conditions, and elucidate its role in predicting long-term outcomes and guiding therapeutic interventions. Finally, efforts to establish consensus guidelines and protocols for fecal calprotectin testing in pediatric practice would contribute to the optimization of its clinical utility. By implementing these recommendations, healthcare professionals can harness the full potential of fecal calprotectin as a valuable biomarker in the management of pediatric gastrointestinal diseases, ultimately improving patient care and outcomes.

CONCLUSION

In conclusion, fecal calprotectin is a valuable biomarker for assessing intestinal inflammation and mucosal integrity in pediatric gastrointestinal diseases. Despite its limitations, including lack of specificity and variability in interpretation, fecal calprotectin offers significant advantages in diagnosing, monitoring, and managing various conditions such as functional gastrointestinal disorders, IBDs, coeliac disease, COVID-19-induced gastrointestinal disorders, gastroenteritis, and cystic fibrosis-associated intestinal pathology. Its non-invasive nature, sensitivity in detecting inflammation, and ability to aid in disease monitoring and early detection make it a valuable tool in pediatric gastroenterology. However, the interpretation of fecal calprotectin levels should consider clinical context, patient demographics, and potential confounding factors. Additionally, further research is needed to establish standardized protocols, cutoff values, and guidelines for fecal calprotectin testing in pediatric practice. Future studies should focus on addressing the limitations identified in this review, such as selection bias, quality of included studies, publication bias, and interpretation challenges. By doing so, clinicians can optimize the clinical utility of fecal calprotectin in pediatric gastrointestinal diseases, leading to improved patient outcomes and quality of care.

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CASE REPORT

Unique presentation of neonatal liver failure: A case report

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Abstract

BACKGROUND

Acute fulminant liver failure rarely occurs in the neonatal period. The etiologies include viral infection (15%), metabolic/genetic disease (10%), hematologic disorders (15%), and ischemic injury (5%). Gestational alloimmune liver disease usually manifests as severe neonatal liver failure, with extensive hepatic and extrahepatic iron overload, sparing the reticuloendothelial system. Empty liver failure is a rare cause of liver failure where a patient presents with liver failure in the neonatal period with no hepatocytes in liver biopsy.

CASE SUMMARY

A 5-week-old male presented with jaundice. Physical examination revealed an alert but deeply icteric infant. Laboratory data demonstrated direct hyperbilirubinemia, a severely deranged coagulation profile, normal transaminase, and normal ammonia. Magnetic resonance imaging of the abdomen was suggestive of perinatal hemochromatosis. Liver biopsy showed histiocytic infiltration with an absence of hepatocytes. No hemosiderin deposition was identified in a buccal mucosa biopsy.

CONCLUSION

Neonatal liver failure in the absence of hepatocellular regeneration potentially reflects an acquired or inborn defect in the regulation of hepatic regeneration.

Key Words: Liver; Hyperbilirubinemia; Le foie vide; Neonatal hemochromatosis; Case report

Core Tip: We report a rare case of liver failure in which a term infant, with no history of perinatal complication, presented at age of 4-wk with an insidious onset of liver failure. We speculated that a severe liver insult may have occurred sub-clinically in the first week of life which was not detected. Regardless of the etiology of the marked hepatocyte destruction, there appears to be a. complete absence of hepatocellular regeneration, indicating a possible acquired or inborn defect in the regulation of hepatic regeneration.

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INTRODUCTION

Neonatal liver failure presents a unique clinical challenge as it is usually part of broader systemic disorder. Identifying of liver disease in newborns is difficult since biochemical findings such as hyperbilirubinemia and coagulopathy may be due to various physiological and pathophysiological processes. The leading cause of acute liver failure in the neonatal period is gestational alloimmune liver disease (GALD), previously known as perinatal hemochromatosis (PH). Other causes are viral infections, metabolic diseases, hemophagocytic lymphohistiocytosis, and other rare disorders[1]. Here, we describe a term infant who developed hepatic failure at the age of 28 d. Liver pathology showed a hepatic parenchyma lacking hepatocytes, as well as iron accumulation distributed in a hemochromatotic pattern.

CASE PRESENTATION

Chief complaints

Worsening jaundice at 28 d old.

History of present illness

The infant was doing well at birth but was noticed to be jaundiced at the age of 14 d. However, he was gaining weight, and the parents did not have any concerns until 28 d when the jaundice worsened.

History of past illness

Pregnancy was spontaneous and was the first pregnancy for the 28-year-old mother (no history of previous pregnancy losses or stillbirth). The mother has taken a prenatal multivitamin.

A full-term male newborn had been delivered by elective cesarean section owing to malpresentation after an uneventful pregnancy and had a birth weight of 2400 g and no perinatal complications.

Personal and family history

There was no family history of liver failure, metabolic disease, or any chronic illness.

Physical examination

The patient was well nourished and not dysmorphic, and there was no hepatosplenomegaly or features of encephalopathy.

Laboratory examinations

The patient's total bilirubin was 553 μmol/L (normal value < 17 μmol/L), out of which, the direct bilirubin was 250 μmol/L (normal range < 17 μmol/L). The infant was anemic with hemoglobin of 84 g/L, platelets were low at 74 × 10^3 μL (normal range 140–400 × 10^3/μL) while white blood cell counts were normal at 9 × 10^9/L. Work up for hemolysis including Coombs test, reticulocyte count, and haptoglobin were normal. However, the patient's coagulation profile was significantly deranged with a prothrombin time of > 120 s (normal range 12–15 s), partial thromboplastin time at > 102 s (normal range 27–42 s), and international normalized ratio > 10 (normal range 0.7–1.1). Initial serum glucose was normal at 4 mmol/L (normal range 2.5–7.00 mmol/L). Liver enzymes remained normal with aspartate aminotransferase (AST) at 35 IU/L (normal range < 63 IU/L) and alanine aminotransferase (ALT) at 19 IU/L (normal range < 46 IU/L). Serum ammonia was normal at 45 mmol/L and serum albumin was low at 27 g/L (normal range 34–54 g/L). Work up for infectious etiology was negative for rubella polymerase chain reaction (PCR), cytomegalovirus PCR, herpes simplex PCR, enteroviruses PCR, Epstein-Barr virus (EBV) PCR, hepatitis B surface antigen, hepatitis B virus PCR, human immunodeficiency virus PCR, and toxoplasmosis serum serology. Blood and stool cultures were negative, but urine culture grew *Klebsiella pneumonia*. Metabolic evaluation demonstrated that urine was negative for reducing substance and succinylacetone, red blood cells had normal galactose-1-phosphate uridylyl-transferase activity, and serum amino profile

and lactate were normal. In addition, alpha fetoprotein, thyroid function test, serum cortisol level, serum ferritin, and serum triglycerides were all normal. Urine testing for organic acid and amino acid were normal.

The patient underwent trans-jugular liver biopsy because of severe coagulopathy, and histopathology of the liver biopsy demonstrated histiocytic infiltration between the portal areas (Figure 1A), diffusely positive staining for CD68 (a histiocytic marker) (Figure 1B), and negative staining for HbPar (a hepatocyte marker; Figure 1C). These HbPar results were indicative of an absence of hepatocytes. Iron staining showed mild iron deposition, predominantly in the proliferating ducts and histiocytes (Figure 1D).

Similar to light microscopy, electron microscopy showed histiocytic infiltration (Figure 2). Adenovirus and EBV stains were negative. A buccal biopsy was obtained to look for extrahepatic iron deposition, and no hemosiderin-laden macrophages were observed. Bone marrow aspirate was also normal. Whole-exome sequencing and mitochondrial genome analysis were normal.

Imaging examinations

Abdomen ultrasound showed an unremarkable liver with normal size, echogenicity, and vascularity. Magnetic resonance imaging (MRI) of the abdomen showed a markedly shrunken liver, moderate splenomegaly, mild prominence of the common bile duct, and marked reduction in pancreatic signal intensity in comparison to the spleen, suggestive of extensive pancreatic iron deposition with splenic sparing. A skeletal survey showed no lytic lesions.

FINAL DIAGNOSIS

Acute neonatal liver failure of unknown etiology.

TREATMENT

The patient's unstable clinical condition precluded liver transplant. Although PH was unlikely and because of the critical condition of the patient, intravenous immunoglobulin (IVIG), pulse steroid, and empirical anti-viral medication and maximal medical support in intensive care were initiated.

OUTCOME AND FOLLOW-UP

Over the following 4 wk, the patient's hospital course was complicated by encephalopathy, high blood pressure, bradycardia, and seizure. The patient required invasive ventilation and died from pulmonary hemorrhage at the age of 66 d.

DISCUSSION

We report an unusual case of liver failure in which a term infant-with no history of perinatal complication-presented at the age of 4 wk with an insidious onset of liver failure. There was no history of acute illness or ischemic insult that could precipitate liver failure. Histology of the liver showed an absence of hepatocytes. Laboratory data revealed profound liver synthetic dysfunction, direct hyperbilirubinemia, and profound coagulopathy, while serum aminotransferases were constantly normal or near normal despite significant liver failure which is attributed to reduced liver parenchymal mass, rather than normal hepatocytes or liver cirrhosis.

The initial presumptive clinical diagnosis was GALD, where the mother produces IgG antibodies against fetal hepatocytes, resulting in complement-mediated hepatocytes injury leading to markedly decreased or absent hepatocytes [2]. GALD usually manifests as acute severe neonatal liver failure with extensive hepatic and extrahepatic iron overload, sparing the reticuloendothelial system. GALD is rare and typically presents early in the newborn period[2], often occurring in slightly preterm or low-birth-weight infants, whereas the reported patient was full term of normal birth weight.

GALD is characterized by severe liver disease in a newborn accompanied by extrahepatic siderosis[3]. A diagnosis of GALD is established by the presence of iron overload in the serum, liver, and other organs, with sparing of the reticuloendothelial system, along with the classic clinical picture. This situation was not found in the reported patient; his blood ferritin level was normal, and no iron deposits were found in buccal mucosa. The liver biopsy did not detect any identifiable hepatocytes but showed mild iron deposition, predominantly in the proliferating ductules. Typically, in GALD, surviving hepatocytes show coarsely granular siderosis, and severe pan-lobular parenchymal fibrosis is a dominant feature; however, all these features were absent in the reported case.

GALD has been reported to cause acute liver injury to the fetal liver, resulting in stillbirth or neonatal demise[4]. In affected infants, liver pathology revealed hepatocyte necrosis without evidence of collapse, fibrosis, or inflammation, indicating a hyperacute process, consistent with the reported case. There may not be any siderosis in the liver or other tissues. The reason why certain infants experience this hyperacute liver failure while others present with congenital

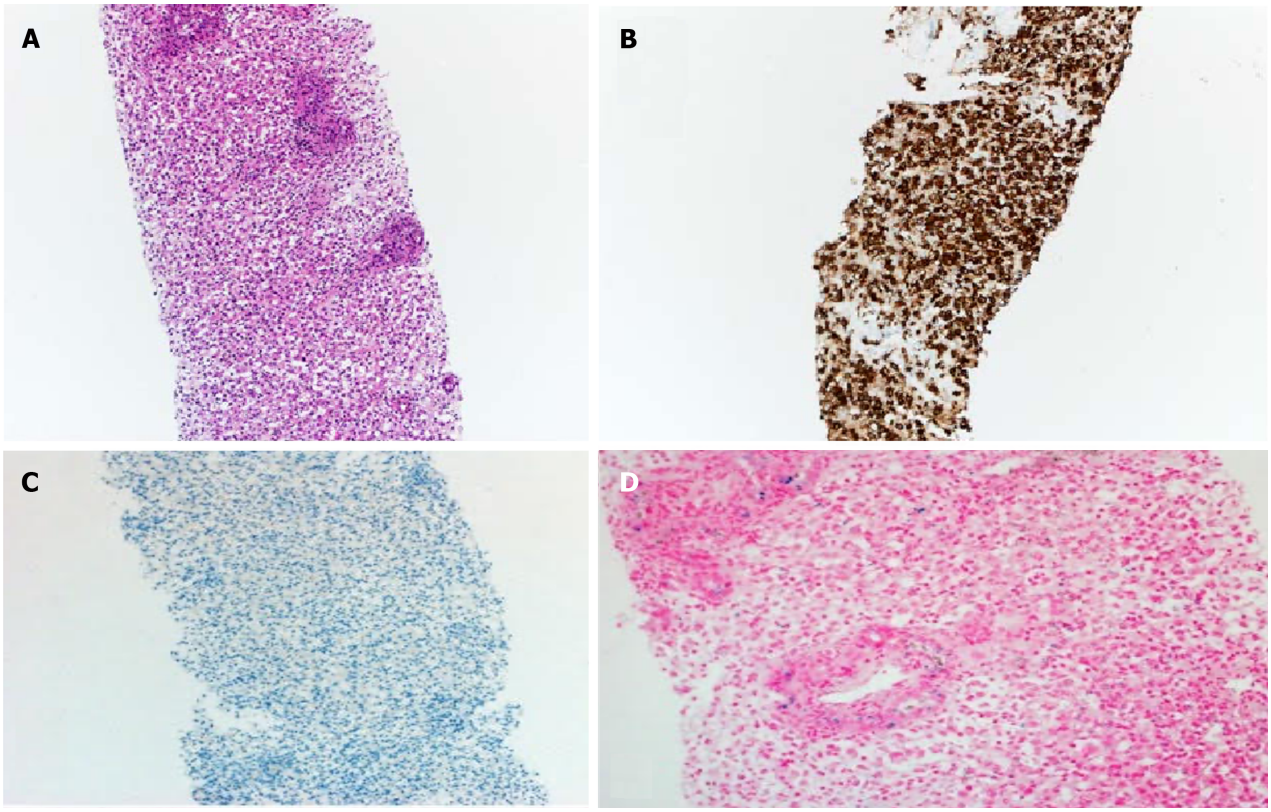

Figure 1 The liver biopsy. A: The liver biopsy shows prominent histiocytic proliferation between the portal areas (hematoxylin and eosin, × 100); B: The proliferating cells are diffusely positive for CD68 (a histiocytic marker) (CD68 stain, × 100); C: The cells are negative for hepPar confirming the absence of hepatocytes (× 100); D: Iron stain shows mild iron deposition in the proliferating ductules and histiocytes (iron stain, × 200).

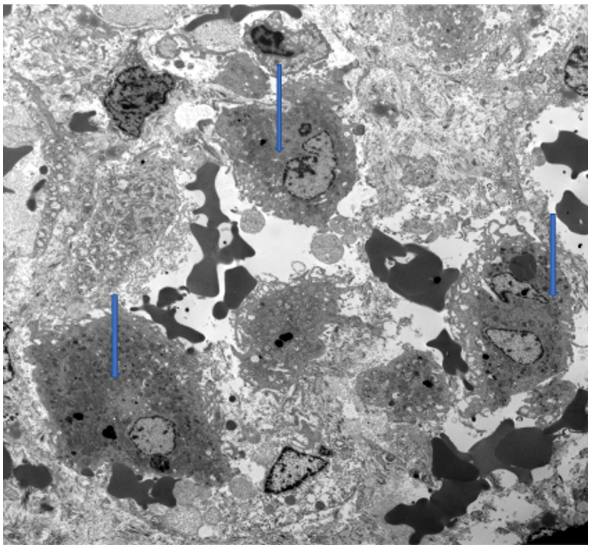

Figure 2 Electron microscopy highlights the presence of abundant histiocytes (highlighted by arrows) with no hepatocytes.

cirrhosis remains unclear.

In addition, for patients with GALD, the T2-weighted MRI scan is often used as a noninvasive approach to show iron accumulation in the liver and other organs[4]. The MRI of the patient in this case report did not show any iron deposition in the liver; however, severe loss of hepatocytes can give a false impression of low iron levels on MRI scans. Pancreatic siderosis was detected by the patient's MRI; however, this is a nonspecific finding and can be observed in other conditions including hemolytic disease, galactosemia, and sepsis, all of which were ruled out in the reported patient.

Following the delivery of an infant of GALD, the likelihood of recurrence in subsequent pregnancies exceeds 90%[5], which can be prevented by treatment with IVIG during gestation[5]. In this case, the mother of the reported patient subsequently gave birth to a healthy baby who outgrew the infancy period with no issues and the mother did not receive any preventive treatment[6].

Hemophagocytic lymphohistiocytosis was unlikely because serum ferritin and triglycerides were persistently normal and bone marrow aspirate did not detect hemophagocytosis.

Although neonatal hemochromatosis was unlikely, the critical condition of the patient meant that intravenous immunoglobin, pulse steroid, and empirical anti-viral medication and maximal medical support in intensive care were initiated. Over the following 4 wk, the patient's hospital course was complicated by encephalopathy, high blood pressure, bradycardia, and seizure. The patient required invasive ventilation and died of pulmonary hemorrhage at the age of 66 d.

A rare liver disease, termed empty liver syndrome (Le Foi Vide), was reported by Gilmour *et al*[4] in 1996, and had similar findings to our case, with a 5-wk-old female patient who presented at the age of 2 d with liver failure and profound coagulopathy. The hospital course of Gilmour *et al*'s patient[4] was complicated by hypoglycemia and seizure, and the infant died at the age of 39 d after pulmonary hemorrhage. Histological examination of the liver showed a total absence of hepatocytes with minimal stromal proliferation. Gilmour *et al*[4] discussed theories that could lead to complete absence of hepatocytes, including infection, severe hypoxic-ischemic insult, hepatic tumors, or inborn error of metabolism (IEOM). In our reported patient, extensive studies for bacterial and viral infections were negative. IEOM leading to perinatal liver failure will usually show fatty changes in hepatocytes rather than absent hepatocytes. Gilmour *et al*[4] speculated that a severe hepatic insult occurred sub-clinically in the first week of life which was not detected. Regardless of the etiology of the marked hepatocyte destruction, there appears to be total lack of hepatocellular regeneration, which may reflect an acquired or inborn defect in the regulation of hepatic regeneration[4].

CONCLUSION

In conclusion, GALD presents as a severe liver disease in a newborn accompanied by extrahepatic siderosis. A diagnosis of GALD is established by detecting iron overload in the serum, liver, and other organs, while sparing of the reticuloendothelial system, along with the classic clinical picture[7]. Early diagnosis and prompt medical intervention can lead to an improved prognosis.

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FOOTNOTES

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