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Contents

Quarterly Volume 14 Number 4 December 20, 2024

EDITORIAL

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Retrospective Study

World Journal of Experimental Medicine

Quarterly Volume 14 Number 4 December 20, 2024

Contents

World Journal of Experimental Medicine

Quarterly Volume 14 Number 4 December 20, 2024

Contents

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EDITORIAL

Harnessing aryl hydrocarbon receptor dynamics: Unveiling therapeutic pathways in esophageal squamous cell carcinoma

Chun-Han Cheng, Wen-Rui Hao, Tzu-Hurng Cheng

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Abstract

This editorial discusses the insightful minireview by Rahmati *et al*. The mini-review delves into the role of the aryl hydrocarbon receptor in the development and progression of esophageal squamous cell carcinoma, highlighting its potential as a promising therapeutic target. The authors concisely summarize the current understanding of how aryl hydrocarbon receptor modula-tion influences immune responses and the tumor microenvironment, offering fresh perspectives on therapeutic strategies. This editorial aimed to emphasize the significance of these findings and their potential impact on future research and clinical practices for the management of esophageal squamous cell carcinoma.

Key Words: Aryl hydrocarbon receptor; Esophageal squamous cell carcinoma; Immune modulation; Therapeutic opportunities; Tumor microenvironment

Core Tip: This editorial highlighted the critical insights from the minireview by Rahmati *et al* on the role of aryl hydrocarbon receptor (AHR) in esophageal squamous cell carcinoma, especially with regards to the modulation of immune responses and the potential of AHR as a therapeutic target. By discussing these findings, this editorial highlighted the importance of AHR dynamics in the development of innovative treatment strategies for esophageal squamous cell carcinoma, paving the way for future research and clinical applications.

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INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive cancers, often diagnosed late with a poor prognosis. Understanding the molecular mechanisms driving its progression is essential for developing effective therapies. The aryl hydrocarbon receptor (AHR) has recently been recognized as a key factor in the pathogenesis of several cancers, including ESCC. Rahmati *et al*'s mini-review offers a comprehensive analysis of AHR's role in ESCC, emphasizing its potential as a therapeutic target[1]. This editorial highlighted the key insights from their work, exploring the implications of AHR dynamics in ESCC and its potential to influence future treatment approaches.

AHR ACTIVATION AND ITS EFFECTS ON GENE EZPRESSION IN ESCC

The activation of the AHR plays a pivotal role in regulating gene expression in ESCC. Upon ligand binding, AHR translocates to the nucleus and binds to xenobiotic response elements in the promoters of target genes. This transcriptional regulation affects several pathways involved in cancer progression, including cell proliferation, migration, and immune evasion. AHR activation notably modulates genes linked to cell proliferation and metastasis. For instance, AHR upregulates cytochrome P450 1A1, a gene involved in xenobiotic detoxification, which is also associated with oxidative stress, DNA damage, and carcinogenesis[1]. This highlights AHR's dual role in both protecting against environmental toxins and promoting tumor development in contexts such as ESCC. Another key gene influenced by AHR is ATP-binding cassette subfamily G member 2, which contributes to multidrug resistance. Constitutive AHR activation has been shown to increase ATP-binding cassette subfamily G member 2 expression in cisplatin-resistant ESCC cells, enhancing drug efflux and reducing chemotherapy efficacy[2]. This demonstrates AHR's role not only in tumor progression but also in treatment resistance, underscoring its importance as a therapeutic target. AHR activation also influences genes involved in epithelial-mesenchymal transition, a process critical for cancer invasion and metastasis. Studies have shown that AHR activation can inhibit RhoA/Rho-associated protein kinase 1 signaling, reversing the mesenchymal phenotype and reducing ESCC cell invasiveness[3]. This indicates that AHR plays a nuanced role, balancing tumor suppression and promotion depending on the signaling context. Moreover, AHR activation regulates genes that facilitate immune escape. For example, it promotes the expression of indoleamine 2,3-dioxygenase 1, an enzyme that metabolizes tryptophan into kynurenine, which in turn activates AHR and fosters an immunosuppressive environment. This promotes the recruitment of regulatory T cells (Tregs) and suppresses cytotoxic T cell activity[1]. The kynurenine/sialic acid binding immunoglobulin like lectin 15 (Siglec-15) axis, as identified by Zhang *et al*[4], further contributes to immune evasion in squamous cell carcinomas, suggesting a similar mechanism in ESCC. By creating an immunosuppressive microenvironment, AHR helps cancer cells evade immune detection. In addition, AHR modulates macrophage polarization within the tumor microenvironment (TME). Activation of AHR drives macrophages toward an M2-like phenotype, which is associated with pro-tumorigenic activities such as angiogenesis and tissue remodeling[5]. These macrophages secrete cytokines that support tumor growth and metastasis, further emphasizing how AHR shapes the TME to favor cancer progression. AHR activation also interacts with molecular chaperones, with ligand specificity influencing these interactions. Narita *et al*[6] demonstrated that different toxic and non-toxic ligands recruit distinct sets of chaperone proteins to the AHR complex, impacting transcriptional outcomes. This ligand-specific regulation underscores the complexity of AHR's role in gene expression and presents opportunities for therapeutic intervention by manipulating ligand-AHR interactions. Overall, AHR activation in ESCC leads to the upregulation of genes associated with oxidative stress, drug resistance, immune evasion, and tumor progression. Targeting AHR and its downstream pathways offers a promising strategy for disrupting these mechanisms, potentially improving patient outcomes by inhibiting tumor growth and enhancing immunotherapy efficacy. Continued research into the specific gene networks regulated by AHR in ESCC will be critical for developing more effective AHR-targeted therapies[1,7].

IMMUNE MODULATION BY AHR

The AHR plays a crucial role in shaping immune responses within the TME, creating an immunosuppressive environment that promotes tumor progression. Beyond its direct effects on tumor cells, AHR profoundly influences both the innate and adaptive immune systems, particularly affecting Tregs, dendritic cells, and macrophages. As Rahmati *et al*[1] note, AHR activation fosters an immune-suppressive microenvironment conducive to tumor growth in ESCC. AHR's impact on dendritic cells includes the induction of immunosuppressive cytokines such as interleukin 10 and transforming growth factor-β, which impair T cell activation and proliferation, thereby weakening the immune response against tumor cells[1]. Moreover, AHR signaling promotes Tregs, which inhibit effector T cells and reinforce immune suppression in tumors[8]. This dampening of the immune response further compromises the body's ability to combat cancer effectively. In addition to Tregs, AHR also modulates macrophages within the TME. Activation of AHR polarizes macrophages toward an M2-like phenotype, characterized by tumor-promoting activities such as the secretion of growth factors, angiogenic mediators, and tissue-remodeling enzymes[5]. These M2-like macrophages support metastasis and immune evasion, contributing to tumor progression, as shown in studies across multiple cancers, including ESCC. While AHR's role in immune suppression and tumor promotion presents challenges, it also offers therapeutic opportunities. AHR-driven immune modulation can hinder immunotherapies by promoting immune tolerance, but targeting AHR could enhance cancer treatments. Blocking AHR may reduce Treg-mediated suppression and reprogram macrophages toward an anti-tumor M1 phenotype, thereby restoring immune surveillance and strengthening the anti-tumor response[7]. Interestingly, AHR's effects on immune cells appear to be ligand-dependent. Narita *et al*[6] demonstrated that different AHR ligands, whether toxic or non-toxic, elicit distinct immune responses. This ligand-specific activation suggests that not all forms of AHR engagement lead to immunosuppression, which holds significant implications for developing selective therapies targeting AHR. Furthermore, dietary components such as tryptophan metabolites can activate AHR and influence immune function in non-cancerous contexts, indicating that modulating AHR could have preventative benefits as well[9]. In sum, AHR's ability to shape the immune landscape in cancer, particularly through its effects on Tregs and macrophages, makes it a compelling target for novel therapeutic strategies. By inhibiting its immunosuppressive actions, we could disrupt the tumor-supportive environment, enhancing immunotherapy efficacy and improving patient outcomes. The growing understanding of AHR's role in immune regulation underscores the need for therapies that account for its complex, context-dependent effects on various immune cells[10].

THERAPEUTIC POTENTIAL OF TARGETING AHR

The exploration of the AHR as a therapeutic target in ESCC has garnered significant attention, with various studies highlighting its potential to enhance cancer treatment strategies. AHR plays a pivotal role in modulating immune responses and influencing tumor progression, making it an attractive candidate for targeted therapies. The editorial accompanying the mini-review by Rahmati *et al*[1] emphasizes novel strategies for inhibiting AHR activity in ESCC, including the development of small molecule inhibitors and the exploration of natural compounds to disrupt AHR signaling pathways. Targeting these pathways could inhibit tumor growth and improve patient outcomes. AHR's immune-modulatory effects are a crucial aspect of its therapeutic potential. AHR activation often leads to an immunosuppressive TME, enabling cancer cells to evade immune detection and proliferate unchecked[1]. Research has shown that inhibiting AHR could reverse these effects, potentially restoring immune surveillance and enhancing the body's natural ability to combat tumors. This has broad implications for improving the efficacy of existing therapies, especially when AHR inhibitors are combined with conventional treatments like chemotherapy and immunotherapy, which may yield synergistic effects. The study by Malany *et al*[5] underscores this potential, highlighting how AHR-driven macrophage functions can be modulated to favor anti-tumor responses. Moreover, AHR's role extends beyond immune modulation to influence tumor progression and metastasis. Helmbrecht *et al*[7] suggest that the modern human AHR, when ancestralized through genome editing, exhibits higher activity levels. This discovery points to new avenues for therapeutic interventions, as manipulating AHR activity could enhance its effectiveness as a target in cancer treatment. Similarly, Narita *et al*[6] provide valuable insights into how different ligands, whether toxic or non-toxic, differentially affect the AHR-molecular chaperone complex. This understanding could guide the development of more precise AHR inhibitors that selectively target cancer-promoting activities while minimizing adverse effects. Integrating AHR inhibitors into combination therapies represents another promising strategy for improving treatment outcomes in ESCC. Although AHR has been studied as a drug target in other cancers, including advanced prostate cancer, the challenges and insights from those investigations are equally relevant to ESCC[10]. Overcoming obstacles like drug resistance and improving inhibitor efficacy remain critical. The potential for AHR-targeted therapies to complement other modalities, such as immune checkpoint inhibitors, opens up a multi-faceted approach to cancer treatment that could address both the tumor and its immune microenvironment. Recent studies further support the innovative use of AHR inhibitors in modulating the metabolic landscape of tumors. The kynurenine/Siglec-15 axis has been explored in head and neck squamous cell carcinoma, revealing crucial interactions that may also occur in ESCC[4]. Targeting this axis could potentially prevent immune evasion and enhance the therapeutic efficacy of AHR inhibitors, highlighting a broader scope for AHR-targeted therapies that extend beyond traditional immune modulation to integrate metabolic pathways. In summary, targeting AHR in ESCC presents a multifaceted therapeutic strategy with the potential to improve patient outcomes by directly inhibiting tumor growth and modulating the immune environment. The research reviewed here demonstrates that AHR's role in both immune suppression and tumor progression makes it a valuable target for developing novel treatments. Continued investigation into AHR inhibitors, their interactions with other therapeutic modalities, and the exploration of

specific metabolic pathways will be essential for advancing cancer therapies and improving patient outcomes in ESCC. The integration of AHR-targeted strategies into the current cancer treatment landscape represents a promising direction for future clinical applications.

FUTURE DIRECTIONS IN AHR RESEARCH

Research into the AHR in ESCC has gained significant momentum, presenting opportunities to advance cancer therapy. As discussed by Rahmati *et al*[1], targeting AHR may modulate immune responses and inhibit tumor progression. However, the mechanisms through which AHR influences tumor biology and immune modulation in ESCC remain incompletely understood, necessitating further investigation. One critical area for future research involves dissecting the molecular pathways by which AHR impacts both oncogenic signaling and immune surveillance in ESCC. Understanding AHR's role in immune evasion is particularly important, as it may help identify biomarkers for treatment resistance or response. The kynurenine/Siglec-15 axis, highlighted in studies of head and neck squamous cell carcinoma[4], suggests potential parallels in ESCC. Investigating whether similar metabolic interactions occur in ESCC could provide valuable insights for developing more effective therapeutic strategies. The development of AHR inhibitors represents another promising therapeutic approach, though challenges remain. As Helmbrecht *et al*[7] indicate, modifying AHR to mimic ancestral versions could enhance receptor activity and therapeutic efficacy. This concept raises the possibility of designing inhibitors finely tuned to modulate AHR signaling without compromising physiological functions, thereby reducing off-target effects. Furthermore, Mosa *et al*[11] emphasize the potential of repurposing clinically approved drugs as AHR modulators, which could expedite the development of new treatment options for ESCC. However, safety concerns related to AHR inhibition must also be addressed. Narita *et al*[6] explain that the composition of the AHR-chaperone complex varies depending on whether the ligand is toxic or non-toxic, highlighting the need for thorough preclinical testing to mitigate potential adverse effects. Understanding the toxicological profiles of AHR inhibitors is crucial to ensuring their safe application in cancer therapy. As well as Malany *et al*[5] pointed out, AHR-driven macrophage polarization could lead to unintended immune consequences, necessitating a careful balance between immune modulation and anti-tumor effects. Delivery mechanisms also warrant critical attention in future research. Effective delivery of AHR inhibitors to tumor cells while minimizing systemic exposure is essential for maximizing therapeutic benefit. Targeted delivery systems, potentially employing nanoparticle-based approaches, could enhance drug accumulation in the TME and reduce off-target toxicity - a significant challenge that must be addressed for these therapies to succeed in clinical settings. Moreover, the interplay between AHR and other oncogenic pathways in ESCC deserves further investigation. Recent studies, such as those by Zhang *et al*[12], suggest that AHR may intersect with pathways like Wnt/β-catenin and mitogen-activated protein kinase, known to drive cancer progression. Targeting these intersections could yield novel combination strategies for treating ESCC, as indicated by similar approaches in other cancers[13]. Finally, clinical trials evaluating AHR inhibitors in combination with standard-of-care therapies, such as chemotherapy or immune checkpoint inhibitors, are essential to determine whether these agents can improve patient outcomes. Optimizing such combinatory regimens could enhance therapeutic efficacy by simultaneously targeting multiple facets of ESCC biology, a strategy currently being explored with other molecular targets[14]. Overall, the work of Rahmati *et al*[1] lays a foundation for advancing AHR research in ESCC. By addressing these future directions, researchers can develop more effective and safer therapeutic strategies that leverage AHR's unique role in cancer biology.

CONCLUSION

This editorial emphasizes the significant insights provided by Rahmati *et al*[1] regarding the AHR and its multifaceted role in ESCC. Their work positions AHR as a promising therapeutic target with the potential to enhance treatment strategies for ESCC. Building on this foundation, Helmbrecht *et al*[7] suggest that ancestralizing of AHR through genome editing could fine-tune its modulation and lead to more effective therapeutic agents. Also, Zhang *et al*'s research on the kynurenine/Siglec-15 axis in head and neck squamous cell carcinoma highlights critical metabolic interactions that may offer valuable insights for ESCC, paving the way for targeting immune evasion mechanisms[4]. The development of potent, selective AHR inhibitors remains a key priority as the potential for clinical application increases. However, Narita *et al*[6] caution that variations in the components of the AHR-molecular chaperone complex - depending on ligand toxicity - necessitate careful evaluation of the safety and efficacy of these inhibitors. Furthermore, research by Malany *et al*[5] underscores the importance of optimizing AHR modulation to balance macrophage-driven immune responses, further highlighting the therapeutic potential of AHR-targeted strategies. In conclusion, Rahmati *et al*[1] have established a strong foundation for future research into harnessing AHR as a therapeutic target in ESCC. Continued investigation into its metabolic and immunological mechanisms, alongside the development of safe and effective inhibitors, will be critical for advancing AHR-based therapies and improving patient outcomes in ESCC.

FOOTNOTES

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REVIEW

Exploring the impact of hepatitis B immunoglobulin and antiviral interventions to reduce vertical transmission of hepatitis B virus

Dhita Prabasari Wibowo, Agustiningsih Agustiningsih, Sri Jayanti, Caecilia H C Sukowati, Korri Elvanita El Khobar

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Abstract

Hepatitis B virus (HBV) infection is a major public health burden. In HBV endemic regions, high prevalence is also correlated with the infections acquired in infancy through perinatal transmission or early childhood exposure to HBV, the so-called mother-to-child transmission (MTCT). Children who are infected with HBV at a young age are at higher risk of developing chronic HBV infection than those infected as adults, which may lead to worse clinical outcome. To reduce the incidence of HBV MTCT, several interventions for the infants or the mothers, or both, are already carried out. This review explores the newest information and approaches available in literature regarding HBV MTCT prevalence and its challenges, especially in high HBV endemic countries. This covers HBV screening in pregnant women, prenatal intervention, infant immunoprophylaxis, and post-vaccination serological testing for children.

Key Words: Hepatitis B virus; Hepatitis B immunoglobulin; Mother-to-child transmission; Vertical transmission; Antiviral prophylaxis

Core Tip: Mother-to-child transmission (MTCT) of the hepatitis B virus (HBV) is still a problem in HBV endemic countries. This review explores the newest information and approaches available in literature to overcome MTCT and its challenges, including screening of pregnant women, prenatal intervention, infant immunoprophylaxis, and post-vaccination serological testing.

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INTRODUCTION

Hepatitis B virus (HBV) infection continues to be a leading cause of morbidity and mortality worldwide. HBV infection causes a wide range of disease manifestations and clinical outcomes from acute asymptomatic to chronic hepatitis B (CHB). Despite the availability of safe and effective vaccines to control viral transmission, more than two billion people in the world are estimated to be affected by HBV infection[1]. CHB leads to long-term inflammation and liver damage that can progress to serious liver diseases, including liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)[2]. Currently, over 292 million people globally are chronically infected with hepatitis B. Additionally, an estimated 58 million people have occult hepatitis B infection (OBI). Thus, CHB causes significant health problems, leading to over 880000 deaths annually from cirrhosis and HCC[3].

CHB is estimated to affect 4.1% of people worldwide across all age groups. CHB prevalence varies notably in different regions according to the Global Burden Disease Study 2019[4]. In Africa, the HBV infection rate is particularly high, with an estimated 6.5% of the population affected. The Eastern Mediterranean and Southeast Asia regions also face a considerable challenge, with 3.1% of its population living with CHB. In the European and Americas regions, the prevalence is relatively lower at 1.1% and 1.2%, respectively[4].

As the Asia-Pacific region is home to more than half of the global population, it has the highest number of deaths due to HBV. CHB was a major cause of death from cirrhosis in the Asia-Pacific region in 2015. Also, in that year, more than two-thirds of all cases of acute viral hepatitis in the world occurred in this region[5]. Most of the burden of HBV-related diseases in the Asia-Pacific region is mainly due to the infections acquired in infancy *via* perinatal transmission or early childhood exposure. Children who are infected with HBV at a young age are much more likely to develop CHB than adults. The risk of CHB infection is highest in infants who are infected in the first year of life (80%–90%) compared to children infected between 1 and 5 years of age (30%–50%) and people who are infected as adults (< 5%)[6,7].

There are limited accurate and large-scale data on the prevalence of hepatitis B surface antigen (HBsAg) in pregnant females in the Asia-Pacific region[7]. Varying rates of HBsAg positivity in pregnant women have been reported, from 0.1%–1.0% in Japan, 3% in South Korea, 4% in Mongolia, and up to 6% in China. In the Western Pacific region, perinatal transmission is estimated to cause 180000 new HBV infections in infants each year[8]. A study in Indonesia found that 2.76% of almost 70000 pregnant women across 12 provinces had HBV infection in 2015. Prevalence was found to be lowest in West Sumatra (1.6%) and highest in West Papua (8.0%)[9]. In addition, a study of pregnant women conducted in 37 midwifery clinics and one private obstetric clinic from July 2018 to April 2019 in Bandung, West Java, Indonesia found that 6.1% were HBsAg seropositive[10]. A review of studies conducted across Southeast Asia and the Western Pacific regions between 1983 and 2016 found that the prevalence of HBsAg in children born to mothers who were also hepatitis B e antigen (HBeAg) seropositive ranged from 2.7% to 53.0%[11].

Managing hepatitis B in pregnant women requires careful consideration for both the mother's health and the risk of viral transmission to the infant. The World Health Organization (WHO) has established global targets to eliminate viral hepatitis as a significant public health threat by 2030. These goals include: (1) Reduction of new chronic HBV infections by 90% (which means less than 0.1% of 5-year-olds with HBsAg seropositive); (2) Increase the coverage of the hepatitis B vaccine birth-dose (HepB-BD) within 24 hours of birth (to protect the newborns) by 90%; and (3) Increase coverage of the third dose HepB in infants by 90%[12].

HBV VERTICAL TRANSMISSION ROUTE

Mother-to-child transmission (MTCT) of HBV involves the transfer and reproduction of HBV from the mother to the child, resulting in the generation of new viral particles[7]. HBV vertical transmission is defined as the transfer of infection from mother to child either during pregnancy, childbirth, or after delivery[1], as depicted in Figure 1.

While the specific prevalence of transmission routes remains uncertain, delivery-related transmission appears to be the primary cause of vertical transmission. Notably, the presence of HBeAg and high HBV DNA viral load (VL) in mothers are significant risk factors for mother-to-infant transmission[13]. Following the availability of hepatitis B immunoglobulin (HBIG) and effective HepB, the rates of MTCT in children born to mothers with CHB, whether they are HBeAg seropositive or seronegative, have decreased dramatically to approximately 4%–10% and less than 0.1%, respectively[14,15].

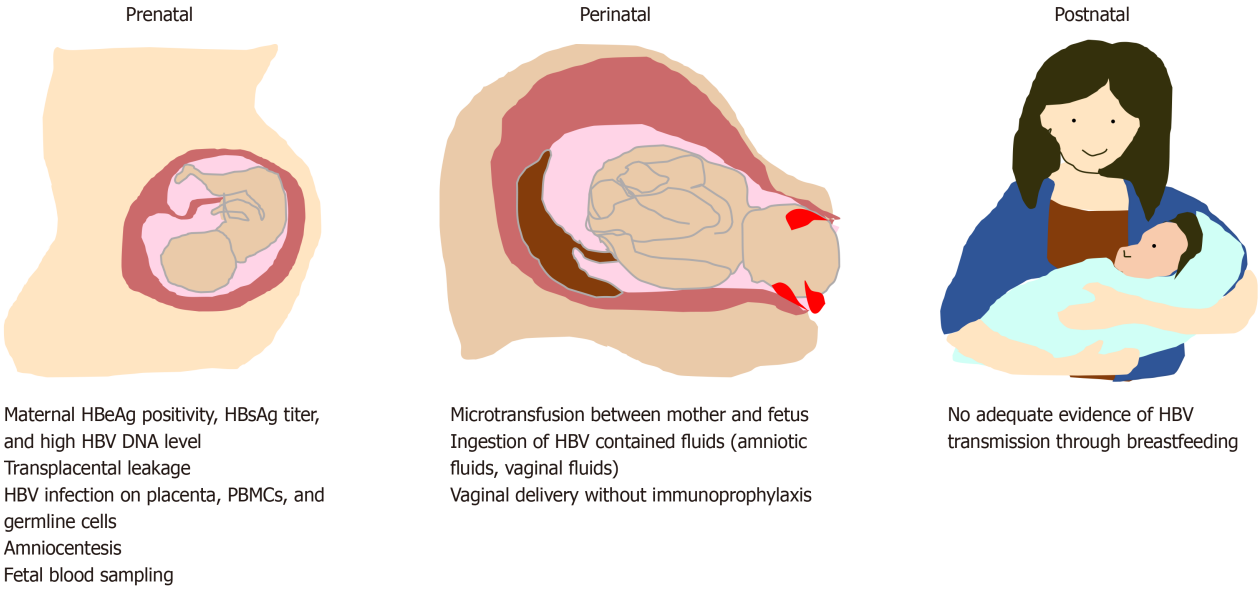

Figure 1 Modes of mother-to-child transmission of hepatitis B virus. HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen.

Prenatal transmission

HBV transmission during pregnancy refers to intrauterine transmission. The risk factors for HBV intrauterine transmission are still poorly understood. Various reports have identified potential factors associated with intrauterine transmission of HBV including method of delivery, prior abortion history, antepartum hemorrhage, and maternal HBV DNA load, although with conflicting results[16-18]. The intrauterine HBV infection rate was found to be linear with maternal serum HBsAg titers and HBV DNA concentrations[17]. There are several mechanisms that would allow for intrauterine HBV transmission, these include viral infection of the placenta, maternal HBeAg positivity, extrahepatic reservoirs, transplacental leakage and the impact of medical procedures[19,20].

Viral infection of the placenta

The placenta typically protects against the transmission of blood-borne pathogens to the fetus. However, several studies have reported the presence of HBV particles, HBsAg, and HBV DNA, in the four distinct layers of the placenta, with varying rates of HBV infection among the different layers[21]. The rate of HBV infection diminishes gradually within the maternal-to-fetal placental layer, with HBV detection rate in decidua cells, trophoblastic cells, villous mesenchymal cells, and villous capillary endothelial cells 55.4%, 51.0%, 46.5%, and 29.9%, respectively[21]. HBV was also present in both the endothelial cells of the villous capillaries and the trophoblasts within the placenta, supporting the theory that a breach of the placental barrier as one of the pathways for intrauterine infection[17,22]. Thus, HBV has the potential to breach the placental barrier, leading to viral infection and replication across various types of placental cells prior to reaching the fetus[23]. The rates of HBV infection in the placenta increase with the stage of pregnancy. The HBV infection rates during the first trimester, second trimester, and full-term were found to be 4.2%, 16.6%, and 44.6%, respectively[24].

Maternal HBeAg positivity

More recent research has identified maternal HBeAg positivity and high HBV DNA VL as the most critical risk factors for HBV vertical transmission. In a study by Xu *et al*[17], maternal HBeAg positivity, HBsAg titer, and HBV DNA level were observed to be risk factors for transplacental HBV transmission[17]. A more recent study has also shown that the placental infection rate was higher in HBeAg-positive mothers compared to the HBeAg-negative group [odds ratio (OR) = 15.56, 95%CI: 2.5-95.7], in addition, placental infection was significantly related to intrauterine transmission of HBV (OR = 4.6, 95%CI: 2.29-9.4)[25]. Thus, HBeAg positivity in mothers might indicate increased intrauterine transmission risks for the infant. However, Chalid *et al*[26] noted a 3.1% occurrence of high HBV DNA level (> 5.3 Log_{10} copies/mL) in the cord blood of HBeAg seronegative mothers, indicating the limited effectiveness of HBeAg detection in predicting intrauterine transmission[26]. Thus, relying solely on HBeAg status to predict intrauterine transmission may not be sufficient. Other factors, such as HBV DNA levels and placental infection rates, also play a crucial role in transmission risk, indicating the need for a more comprehensive approach to assess and manage HBV transmission risk during pregnancy.

Extrahepatic reservoirs

Extrahepatic reservoirs and germline infections also increase the risk of HBV prenatal transmission. Peripheral blood mononuclear cells (PBMCs) might serve as extrahepatic reservoirs as HBV DNA has been detected in these cells. A 2015 study showed that infants born to mothers with HBV DNA-seronegative but HBV DNA-positive PBMCs have a 5-fold higher risk of HBV infection compared to those born to mothers with HBV DNA-negative PBMCs[27]. HBV presence in germline cells has also been demonstrated[20]. In male CHB patients, HBV may be present as either free viral particles in the seminal plasma or as integrated DNA in the genome of spermatozoa[28]. This HBV presence has been associated with

adverse effects on the motility and fertilizing abilities of sperm and may even induce chromosomal aberrations during embryo development due to the high incidence of integrated viral DNA[29]. In female CHB patients, HBV has been shown to infect the ovum at different stages of development and may even replicate within the ovum[30]. HBV expression in the oocyte has been associated with HBV DNA level and infection status of the mother[31]. Despite all these findings, there is still limited understanding on whether HBV-infected germlines from the parent can be passed directly to the infant.

Transplacental leakage and the impact of medical procedures

Transplacental leakage may occur when the placental barriers are ruptured, which allows for direct exchange between the fetal and maternal blood. This might occur in early pregnancy due to immaturity of the placenta. The transfer of HBeAg-positive maternal blood across the placenta, which can be triggered by uterine contractions during pregnancy or disruptions in placental barriers (such as threatened preterm labor or spontaneous abortion), is a probable pathway for HBV intrauterine infection.

Invasive medical procedures, such as amniocentesis and chorionic sampling during pregnancy have also been reported to increase the risk of HBV prenatal transmission[20]. Amniocentesis has been shown to significantly increase the rate of MTCT (OR = 21.3, 95%CI: 2.90-153.775) on studies in HBsAg- and HBeAg-positive mothers with a very high HBV DNA level (≥ 7 Log_{10} copies/mL)[32]. Needle penetration during amniocentesis can damage chorionic villi, leading to the mixing of maternal and fetal blood, and may cause bleeding from fetal-maternal capillaries within the fetal membranes [23]. These studies indicated that in pregnant mothers with high HBV DNA VL, amniocentesis procedures should not be encouraged or only be considered after proper risk and benefit assessment to reduce viral transmission rate to the infant [19,33].

A recent case report assessing the impact of fetal blood sampling during pregnancy on MTCT rate showed that, like amniocentesis, fetal blood sampling in HBsAg-positive women also increased the risk of MTCT. Performing fetal blood sampling for prenatal diagnosis prior to postnatal immunoprophylaxis can heighten the likelihood of intrauterine HBV infection due to potential placental disruption and maternal blood contamination[34,35]. This was evident by persistent HBsAg positivity up to 12 months in the infants born to untreated, HBeAg-positive, and high HBV DNA VL mothers. In addition, the infants born to antiviral-treated, HBeAg-positive, and high HBV DNA VL mothers were HBsAg seronegative with antibody to HBsAg (anti-HBs) seropositive status until the end of the follow-up period[36]. More studies are warranted to verify these findings as the study included a very low sample size.

Perinatal transmission

Infection during childbirth may arise due to micro transfusion between the mother and fetus or ingestion of infectious fluid[37]. A previous study showed the presence of HBsAg in a significant proportion in both vaginal epithelial cells (55%–98%) and cervicovaginal cells (12%), along with detectable HBV DNA[38]. Moreover, HBsAg was detected in amniotic fluid samples (26%) and vaginal fluid samples (96%)[39]. These findings suggest that direct exposure to infective fluids in the maternal genital tract could be a possible route of HBV transmission to infants[38,39].

Several studies have explored the impact of delivery mode on HBV transmission risk. Findings from a 1988 study in China showed that among 447 infants born to HBsAg-positive women, around 25% of those delivered vaginally were infected with HBV at birth, compared to less than 10% of those delivered *via* cesarean section[40]. However, a later study found that the incidence of CHB among infants who were born by spontaneous vaginal delivery, either with the use of forceps or vacuum extraction, and by cesarean section and who had received HBIG and HepB vaccination did not differ significantly, at 7.3%, 7.7%, and 6.8%, respectively. These findings indicate that the cesarean section mode of delivery was not sufficient to decrease the MTCT rate, particularly during the occurrence of possible immune prophylaxis failure[16]. However, performing elective cesarean sections for HBeAg seropositive mothers with pre-delivery HBV DNA levels of ≥ 6 Log_{10} copies/mL might decrease the risk of viral transmission from mother to child[41].

Post-natal transmission

In late 1980s, Wong *et al*[39] first reported that breastfeeding poses a significant concern for postpartum HBV transmission, with HBsAg detected in 72% of breast milk samples[39]. However, a more recent study by Chen *et al*[42] suggests that while HBsAg, HBeAg, and HBV DNA are present in both colostrum and breast milk, there is no concrete evidence that breastfeeding increases the risk of HBV MCTC. Their study also found that the prevalence of HBsAg in breastfed infants was 1.5%, compared to 4.7% in formula-fed infants, with no significant statistical difference[42]. These findings suggest that the risk of viral transmission *via* breast milk is minimal, compared to the risk posed by direct exposure to maternal blood or fluids during delivery. Several hepatitis experts have also evaluated the potential danger posed by a significant daily intake of breast milk for the infant, given the delicate condition of the gastrointestinal mucosa and the incomplete development of the digestive tract[43]. However, to date, there is no evidence to show an elevated risk of viral transmission through direct contact with bleeding nipples or open sores on the breast, especially when appropriate immunoprophylaxis has been administered at birth[33,44,45].

CURRENT STRATEGIES TO REDUCE MTCT RATE

The strategy for MTCT elimination differs between different geographical regions, thus, it is important to understand the specific circumstances in each region to find solutions in the local context to eliminate HBV infection[46,47]. Different strategies may be adopted to reduce and prevent the risk of HBV MTCT. These approaches may include targeted in-

tervention only for the infants or the mothers. However, concerted actions for both the infants and the mothers are believed to be more effective in reducing the vertical viral transmission rate. There are four general approaches that need to be included in any HBV MTCT prevention program, especially in high HBV endemic countries: (1) Screening of pregnant women; (2) Prenatal intervention in pregnant women; (3) Timely infant immunoprophylaxis, and (4) Post-vaccination serological testing (PVST) for children[46,47].

Screening of pregnant women

Screening for HBV infection during pregnancy is necessary to identify mothers whose infants are at risk of perinatal transmission. Detection of HBsAg has been demonstrated to be an economical approach and easily applicable even in areas with limited resources. Indeed, HBsAg detection by qualitative rapid detection tests is commonly used for HBV screening programs in pregnant women in Asia and Africa[48-50]. Detection of HBV DNA VL, by quantitative PCR assay, however, can provide a more accurate picture of the maternal HBV infection status. In addition, HBV DNA VL can also be used to identify which mothers need antiviral prophylaxis as high maternal HBV DNA level has been identified as a significant risk factor for HBV MTCT[51,52].

As HBV DNA VL quantification is more costly and requires a proper instrument set-up, use of this method is inefficient particularly in areas with few resources, thus not many HBV screening programs recommend its use[53]. Alternatively, the detection of other HBV serological markers, such as HBeAg and quantitative HBsAg levels, has been identified as possible surrogate markers for HBV DNA level detection in pregnant women. HBeAg is a marker of viral replication, thus HBeAg detection can help to identify high-risk pregnant women in a resource-limited setting. In addition, a meta-analysis has shown that HBeAg detection was accurate in classifying women with high HBV DNA titers (> 5.3 Log_{10} IU/mL), with a sensitivity of 99.5% (95%CI: 91.7-100) and specificity of 62.2% (95%CI: 55.2-68.7), respectively [54].

Additionally, the use of quantitative HBsAg detection in areas of limited resources is also encouraged. A 2016 study showed a positive correlation between maternal serum quantitative HBsAg level and HBV DNA VL, which can accurately predict maternal HBV DNA level, particularly in those with high VL levels (6–8 Log_{10} IU/mL)[55]. A more recent study showed that maternal serum quantitative HBsAg level was also strongly correlated with HBeAg status, with higher quantitative HBsAg levels in the HBeAg seropositive group compared to the HBeAg seronegative group (3.88 *vs* 2.86 Log_{10} IU/mL). Serum quantitative HBsAg level can also be used to predict HBV infection in the placenta (with 90% sensitivity and 83% specificity) and umbilical cord blood (with 82% sensitivity and 96% specificity). Therefore, maternal serum HBsAg level can be used as a surrogate test for HBeAg and HBV DNA VL tests in pregnant women with HBV infection[25].

Prenatal intervention

HBIG is a purified solution of human immunoglobulin containing high titers of anti-HBs. HBIG is widely administered to rapidly neutralize HBV by activating the complement system and strengthening humoral immunity. Prenatal injection of HBIG in pregnant women could theoretically prevent intrauterine infection by transferring maternal antibodies to the fetus. HBIG injection in CHB mothers (either HBsAg seropositive or HBeAg seropositive) will offer protection to infants by passive diffusion of the antibody through the placenta[56-62]. The effect of this passive diffusion of the antibody was found to be greatest during the last trimester of pregnancy[63].

A meta-analysis in 2017 showed that HBIG administration was effective in preventing hepatitis B occurrence in newborn infants born to mothers who were HBsAg- and HBV DNA-positive[63]. However, another study found that HBIG administration alone did not prevent MTCT, especially in mothers with high HBV DNA VL[64]. As there is an additional potential risk of immune complex disease due to the specific binding of HBIG to HBsAg, the European Association of the Study of The Liver (EASL) still does not indicate the use of HBIG in HBsAg-positive mothers[65].

Instead of administering HBIG in the third trimester of pregnancy, antiretroviral therapy (ART) may be a better choice in preventing MTCT. The use of ART has been widely accepted and applied in clinical practice and has a good result in blocking HBV vertical transmission from mother to infant. It has been reported that antiviral therapy in mothers with high HBV DNA VL (≥ 7 Log_{10} IU/mL) significantly reduced the rate of MTCT from 14.3% to 0%[66]. Several types of nucleos(t)ide analogue (NA) drugs including lamivudine (3TC), telbivudine (LdT), and tenofovir disoproxil fumarate (TDF) have been used in CHB pregnant women to study the safety and efficacy of these antiviral prophylaxes in mothers and infants to reduce HBV MTCT rate[60-62].

Antiviral prophylaxis for CHB pregnant women has now been recommended by the three major liver study associations, the Asia Pacific Association for the Study of the Liver (APASL), EASL, and the American Association for the Study of the Liver (AASLD). The APASL has recommended that pregnant females with HBV DNA levels ≥ 5.3 Log_{10} IU/mL should receive antiviral prophylaxis to prevent MTCT. TDF is the recommended antiviral prophylaxis, which should be initiated at 24-28 weeks of the gestation period, in addition to appropriate immunoprophylaxis for the infants[7]. Similarly, the EASL also advised TDF treatment as antiviral prophylaxis for pregnant women with HBV DNA levels of ≥ 5.3 Log_{10} IU/mL or HBeAg-positive at week 24-28 of the gestation period, which should be continued for up to 12 weeks after delivery[65]. The AASLD specifically recommend the use of TDF to prevent the risk of MTCT in HBsAg-positive pregnant women with high HBV DNA levels (≥ 5.3 Log_{10} IU/mL) and to minimize the risk of viral resistance emergence during treatment. However, the AASLD discourages the use of ART to reduce the risk of perinatal HBV transmission in HBsAg-positive pregnant women with low HBV DNA levels (< 5.3 Log_{10} IU/mL)[33].

In line with this, the WHO has just released their new guidelines for CHB infection prevention, diagnosis, care, and treatment, and updated their recommendation for antiviral prophylaxis in pregnant women. To prevent MTCT of HBV, TDF treatment is recommended for high HBV VL (≥ 5.3 Log_{10} IU/mL) or HBeAg-positive, HBsAg-positive pregnant women, starting from the second trimester of pregnancy until delivery or until completion of vaccination in the infants.

Where testing of HBV DNA and/or HBeAg is lacking, TDF treatment for all HBV-infected (HBsAg-positive) pregnant women is recommended[59].

A 2019 meta-analysis compared the effectiveness of 3TC, LdT, and TDF in reducing MTCT rate. It was shown that only LdT treatment was associated with a reduced rate of HBsAg positivity in infants; and higher rates of HBeAg loss, reduced HBV DNA levels, and normalization of alanine aminotransferase (ALT) levels in mothers. None of the NA drug treatments were associated with any preterm births, congenital malformations or low birthweight. Furthermore, LdT treatment also significantly lowered both the MTCT rate and infants' HBV DNA positivity at birth, and increased HBV DNA level suppression in mothers[67]. A 2020 real-world study also compared the efficacy and safety of 3TC and LdT treatment in more than 2000 pregnant mothers with high HBV DNA levels (> 6 Log_{10} IU/mL) in China to prevent MTCT. They found that antiviral treatment, either with 3TC or LdT, which was initiated in the third trimester, greatly reduced the MTCT rate compared to untreated mothers. There were also no differences in perinatal complications in the mothers or growth parameters in the infants[68]. These results indicate the recommended use of LdT treatment for CHB pregnant mothers to prevent MTCT. However, there are concerns regarding the low genetic barrier of LdT, which may allow for the emergence of viral resistance-associated mutations[7]. As such, NA drug treatment history in pregnant women is crucial to determine which type of drugs can be administered. In treatment-naïve pregnant mothers, 3TC or LdT treatment is preferred, as antiviral resistance to 3TC or LdT is rare due to the brief length of drug exposure. However, in previously treated subjects, TDF treatment is advisable due to its favorable resistance profile. Furthermore, TDF has demonstrated a good long-term fetal safety profile[65].

Recently, more studies have compared the efficacy and safety of TDF treatment in pregnant women. A 2020 observational study in 644 pregnant women who were HBeAg-positive and had a high HBV DNA VL (≥ 5.3 Log_{10} IU/mL), showed that treatment with LdT and TDF significantly lowered maternal HBV DNA level at the time of delivery, with an average decrease of two Log_{10} IU/mL, and thus consequently reduced the MTCT rate. No adverse events were observed, thus both NA drugs are safe for mothers and newborns. In addition, pregnant women treated with TDF in the second trimester showed an even more significant reduction in HBV DNA level compared to those treated in the third trimester. This study showed that TDF has better efficacy than LdT in reducing maternal HBV DNA levels[69]. With regard to HBV genetic diversity, a 2022 Chinese study demonstrated that HBV genotype may be associated with response to antiviral treatment in pregnant women, as measured by changes in the HBV DNA level. HBV genotype has been identified as an independent factor related to the change in HBV DNA level, but not HBV RNA level, after antiviral treatment (either with LdT or TDF)[70].

Despite the good safety profile of TDF, its use has been correlated with kidney and bone loss problems. Therefore, another form of tenofovir, tenofovir alafenamide fumarate (TAF) is also currently used to manage HBV infection. However, there are still limited data on the effect of TAF treatment in pregnant women with CHB. A prospective observational study studied the effect of TAF treatment during pregnancy in 232 CHB women with high HBV DNA VL (≥ 5.3 Log_{10} IU/mL) in preventing MTCT. In comparison with TDF, TAF treatment was well tolerated with similar safety profiles in mothers and infants. Overall, both TAF and TDF treatment in the study cohort resulted in a reduced rate of MTCT to 0%[71]. These results indicate that TAF can also be safely used for antiviral prophylaxis in CHB pregnant women.

Additionally, a systematic review in 2023 found that no significant difference in the safety and efficacy of TDF and TAF treatment in highly viremic CHB pregnant women. Both NA drug treatments resulted in significant reductions in maternal HBV DNA levels during delivery and the MTCT rate reduced to 0%[72]. Another recent meta-analysis also concluded that TAF treatment showed good efficacy as antiviral prophylaxis in reducing MTCT rates with good safety profiles in both mothers and infants[73].

Infant immunoprophylaxis

Infants are more vulnerable to HBV infection. Infants who contract HBV infection during their first year of life are more likely to develop CHB. Several prevention strategies, such as active and passive immunoprophylaxis, can protect infants from HBV infection. Active immunoprophylaxis is given through HBV vaccination, while passive immunoprophylaxis is obtained through HBIG injection shortly after birth. Overall, the percentage of children under five years old with CHB has now decreased, from 5% in the pre-vaccination era (1980s–2000s) to around 1% in 2019[74]. These numbers represent a noteworthy progress in the global effort to reduce HBV infection in children.

Some studies have shown that infant immunization can effectively prevent MTCT. Regardless of the mother's HBV status, the WHO guideline advises that all newborns should receive HepB-BD within 24 hours after birth, followed by additional doses at one and six months of age. HBIG is also recommended for infants of HBsAg-positive mothers[46,47]. However, implementation of the guidelines may differ regionally, based on the HBV prevalence in that region. In China, which has a high prevalence of HBV infection, the implementation of three doses of the HepB vaccine for infants born to HBsAg-positive mothers is reported to result in a significant decrease in MTCT risk of up to 95%[75]. In addition, in low-prevalence HBV regions in Europe including Germany, Ireland, the Netherlands, and the United Kingdom, HepB-BD is typically administered to infants between the ages of six to nine weeks[76].

The effectiveness of the HepB-BD vaccine can be seen in the Americas and European countries. In the Americas, 57% of the countries have a national plan in place for viral hepatitis prevention, treatment, and control. They have also implemented infant vaccination programs for over 20 years. The region is considered successful in eliminating MTCT of HBV, as evidenced by the very low prevalence (< 0.1%) of hepatitis B in children under five years old. In addition, the three-dose vaccination coverage rate for children aged below one year was 87%, with birth dose vaccination coverage rate of 76% in 2017[77].

European countries also made significant progress toward hepatitis B control between 2016 and 2019. In Europe, the number of countries that have achieved the coverage targets for Hep-BD, all three doses of the HepB, and HBV screening in pregnant women are 35, 19, and 17, respectively. Italy and the Netherlands are the first two countries in Europe who

have been validated by the WHO as being successful in their hepatitis B control program, with HBsAg prevalence of 0.5% among the vaccinated cohort[77,78].

The efficacy of HBIG injection in preventing HBV infection in infants remains controversial. HBIG administration was shown to reduced MTCT in infants of HBsAg seropositive women compared to no intervention (6% *vs* 21%, risk ratio = 0.30, 95%CI: 0.20-0.52)[46,47,63]. However, several studies in infants born to HBsAg-positive/HBeAg-negative mothers have shown that the addition of HBIG injection to HBV vaccination dose did not provide an additional protective effect compared to HBV vaccination alone[79,80]. Another study has also corroborated these findings, and found that in infants born to HBeAg seronegative mothers, HBV vaccination alone is sufficient and effective in reducing the rate of MTCT without the addition of HBIG injection[58]. As HBeAg is not routinely tested in HBsAg seropositive pregnant women, HBIG injection would still be beneficial for infants born to these women, especially in high HBV prevalence areas, to reduce cases of infantile fulminant hepatitis[81]. In Taiwan, the government still encourages HBIG injection in infants of HBsAg seropositive mothers, regardless of the maternal HBeAg status, as the cost of HBIG is considered cheaper than HBeAg testing[81].

The protective effect of HBIG injection may also relate to the time of administration. A few studies had demonstrated that earlier immunoprophylaxis administration may provide greater protection against MTCT. A 2021 study in China where both HBIG and HepB-BD were injected in newborns within an hour after delivery, the overall MTCT rate was 0.9% [82]. Similarly, a recent study also confirmed the vital role of the administration time of combined immunoprophylaxis (both HBIG and HepB-BD) for infants born to CHB mothers. Infants that were administered combined immunoprophylaxis within two hours of birth had an MTCT rate of 0.32% (1/308) compared to 2.73% (8/293) in those who received combined immunoprophylaxis between two and 12 hours after birth[83].

A concern regarding the use of HBIG immunoprophylaxis in infants is the rate of OBI. OBI cases, defined as HBsAg seronegative but HBV DNA seropositive, were more commonly found in neonates who received both the HepB vaccination and HBIG doses, possibly due to the added immune pressure induced by the HBIG injection[79,80]. A study in China looking at the status of HBV infection in neonatal HepB- and HBIG-vaccinated infants born to HBsAg seropositive mothers found that 3.9% (3/77) of the infants were HBsAg-positive and 36.4% (28/77) were identified as OBIs. Furthermore, infants with OBI had lower levels of anti-HBs but higher levels of antibody against hepatitis B core antigen (anti-HBc). However, this high anti-HBc level was found to diminish after the 18-month follow-up period followed by HBV DNA seronegativity. This study concluded that the detected OBI in these vaccinated infants was not likely an established OBI, but a transient persistence of HBV DNA accompanied by passive transference of anti-HBc from the CHB mothers[84].

Passive transference of HBV antibodies from mothers to infants was also observed in a 2020 prospective cohort study in China. The authors found that despite the zero MTCT rate (shown as HBsAg seronegativity in infants) and anti-HBs seropositivity in the infants (with high antibody titers), a small proportion of the infants still expressed anti-HBc and anti-HBe (antibody to HBeAg) during the 7-month follow-up period after birth[85]. Thus, these findings indicated that even though perinatal transmission did not occur, HBV antibodies may still be able to enter the infants due to passive transfer from the mothers through intrauterine transmission[85].

PVST

The effectiveness of MTCT prevention can be determined by PVST. This test should be conducted after the last dose of the infants' HBV vaccine to determine the outcome of the given immunoprophylaxis.

The WHO recommend that PVST should be performed for all HBV-exposed HepB vaccinated infants around 1 to 2 months after the completion of the HepB vaccination series, when the antibody response is usually greatest. The infants will be tested for: (1) HBsAg, to determine chronic HBV infection; and (2) Anti-HBs, to detect immunity against HBV, where an anti-HBs titer of ≥ 10 mIU/mL indicates protective immunity[5]. The PVST is needed to monitor the outcomes and impact of MTCT intervention strategies, by determining the proportion of infants who are (1) Infected with HBV; (2) Uninfected and have protective immunity against HBV, and (3) Uninfected but not responding to HepB vaccination[6,7].

A 2017 retrospective study in China identified that, in 438 pairs of mothers and infants, 5.3% infants who had received complete HepB doses were HBsAg seropositive, with a PVST time around 1 to 8 months after the last HepB dose[86]. Similarly, in 2017 another PVST study in China reported that HBsAg positivity rate was 3.7% with an anti-HBs positivity rate of 90.9% in 1025 maternal-infant pairs. They also identified that maternal HBeAg status was correlated with the infant's positivity for both HBsAg and anti-HBs. Additionally, the highest anti-HBs levels in the infants were detected by PVST assessed at 1 to 2 months following the final HepB dose, and prolonged PVST intervals resulted in decreased anti-HBs geometric mean concentration[75].

The timing of PVST may influence the proportion of non-responders. Huang *et al*[87] showed that when PVST was assessed at an interval of 1, 2, 3, 4, 5, 6, and 7 to 8 months after the last HepB dose, the non-response rate was 1.6%, 1.1%, 0.9%, 0.7%, 1.1%, 0.7%, and 5.7%, respectively, with a significantly higher non-response rate in PVST at 7 to 8 months. In addition, anti-HBs titers also declined significantly in infants with medium anti-HBs responses when PVST is performed at a longer interval from the final HepB dose. These results indicate that the optimal PVST interval for infants born to HBsAg-positive mothers is at seven months of age or around one month after HepB vaccination series completion[87].

A more recent prospective observational PVST study in China confirmed the previous findings. Among 2120 mother and infant pairs, the HBsAg positive rate was 0.77%, anti-HBs positive was 96.84%, and both the HBsAg and anti-HBs seronegative rate was 2.39%. Among 34 infants with double seronegative results, 15 had received all three doses of HepB [88]. A recent study analyzing PVST results among at-risk babies born to HBV positive mothers from 2008 to 2022 in China showed that the MTCT rate between infants born to HBsAg-positive/HBeAg-negative and HBeAg-positive mothers was significantly different (0.75% *vs* 6.33%). In addition, the MTCT rate for infants born to HBeAg-positive mothers receiving antiviral prophylaxis was 1.72%[89].

The PVST is important for determining MTCT rate in at-risk infants born to HBV-infected mothers; however, there is also a risk of low compliance in PVST. In a retrospective cohort study in Fujian, China, only 4988 infants out of 8474 at-risk infants were eligible for PVST. Twenty-percent of infants (n = 994) were lost to follow-up in the testing cascade, with 55% of parents refusing venous blood sample collection or failure of field sample collection, 16% transferred out of the region, and 10% of parents chose to perform independent PVST without reporting the results. It was also identified that the high PVST noncompliance rates was associated with infants born to HBeAg positive mothers (OR = 1.2, 95%CI: 1.1-1.4)[90].

Vaccine non-responders may influence the effectiveness of HepB vaccination in HBV-exposed infants. For at-risk infants who were still negative for HBsAg and anti-HBs after receiving the complete HepB vaccine series, the WHO recommended that they received a revaccination followed by a repeat PVST 1 to 2 months after the last vaccination dose [6,7]. A 2012 study in China demonstrated the effect of the vaccine after revaccination in non-responding infants. From 1814 infants, 3.1% were identified as non-responders (anti-HBs titers < 10 mIU/mL) and 28.4% were low-responders (anti-HBs titers ≥ 10 and < 100 mIU/mL). After HepB revaccination, 14.7% became low-responders and 85.3% became responders in the 34 non-responding infants. On the other hand, in the 74 low-responding infants, 78.4% shifted to responders while 21.6% remained low-responders[91].

A recent retrospective study has shown that, for non-responders, an additional fourth vaccine booster dose may be beneficial to improve the anti-HBs response. It was found that a fourth dose of the vaccine resulted in detectable anti-HBs levels in 52.2% (105/201) of HepB vaccine non-responders[92]. Additionally, another study has shown that those with pre-booster anti-HBs levels of 2 to 9.9 mIU/mL had a higher possibility of responding to an additional vaccine booster dose compared to those with anti-HBs levels < 2 mIU/mL[93].

Another issue associated with HBV vaccination is vaccine escape mutations (VEMs). The appearance of VEMs has not been intensively studied; however, detecting the emergence, rate, and clinical significance of these variants is important for HBV management, especially in high HBV prevalence regions. There is a lack of viral genetic data on VEMs in the population, although current genomic data indicates a low prevalence of individual VEMs[47,94]. A phylogenetic analysis showed that VEMs may arise independently of antiviral treatment or HBV vaccine exposure, but these variants can be found across different HBV genotypes, with the highest prevalence identified in genotype C[94]. As such, the emergence of VEMs remains a potential challenge in association with infant HBV vaccination programs[46,47].

COMBINATION OF ANTIVIRAL AND IMMUNE PROPHYLAXIS TO REDUCE MTCT

Research shows that the combination of passive and active immunization in infants born to HBsAg-positive mothers reduced the rate of MTCT. However, this immunization approach cannot fully eradicate the risk of MTCT, as immunoprophylaxis failure has been reported in vaccinated infants[95]. Delayed vaccination and inadequate initial injections in infants, along with high maternal HBV DNA levels, have been associated with these immunoprophylaxis failures[96]. In addition, epidemiological and modeling studies have also shown that HBV immunization alone would not be sufficient to reduce the WHO's target of 0.1% HBsAg prevalence goal in children by the year 2030[7,45]. Therefore, additional approaches are needed to achieve a zero MTCT rate. Many studies in the last decade have shown the promising use of antiviral prophylaxis in CHB pregnant women, in combination with immunoprophylaxis for infants, to reduce the risk of MTCT, as antiviral treatment in pregnant women showed a good safety and efficacy profile in lowering HBV DNA level [95]. The mechanisms of both prophylaxes are described in Table 1.

A 2020 study compared the MTCT rate in neonatal HBV-vaccinated infants born to antiviral-treated and non-treated CHB pregnant women. Antiviral treatment (either with LdT, TDF, or 3TC) resulted in zero MTCT rate (n = 60) compared to 0.1% (3/30) in infants born to non-treated mothers and 39.2% (11/28) in the control group[97]. Accumulating evidence from randomized controlled trials (RCTs) has also shown the effectiveness of combined intervention to prevent HBV MTCT[46,47]. Data from 15 RCTs with 2706 infants from HBsAg seropositive mothers showed that reduced MTCT risk is higher in HBIG- and HepB-vaccinated infants born to highly viremic mothers (HBV DNA ≥ 5.3 Log_{10} IU/mL) who received antenatal NA prophylaxis [relative risk (RR) = 0.47, 95%CI: 0.29-0.75], compared to: (1) Vaccinated infants born to highly viremic but untreated mothers (RR = 0.31, 95%CI: 0.10-0.99); (2) Infants receiving combined HBIG and HepB vaccination (RR = 0.37, 95%CI: 0.210-0.67), and (3) Infants receiving only HepB vaccination (RR = 0.32, 95%CI: 0.21-0.50) [98].

A meta-analysis in 2022 analyzed 300 studies worldwide to determine the rate of HBV MTCT under different prophylaxis regimens. The overall MTCT incidence rate without prophylaxis was 31.3%, which varied in different regions. Infant vaccination reduced the MTCT risk in HBeAg seropositive mothers from 82.9% to 15.9% and from 10.3% to 2.3% in HBeAg seronegative mothers. A further reduction in MTCT rate to 0.3% (95%CI: 0.1%-0.5%) was achieved by combining maternal peripartum NA prophylaxis and infant vaccination. In addition, the risk of HBV transmission can be stratified based on the maternal HBV DNA VL, where MTCT incidence increased when the maternal HBV DNA level was higher than 4.29 Log_{10} IU/mL[46,47,99].

CHALLENGES AND EFFORTS

There are several challenges that may occur in implementing an antiviral and immune prophylaxis approach for MTCT prevention in HBsAg seropositive pregnant women. These include the costs and availability of antiviral and immune prophylaxis, ideal time to initiate antiviral prophylaxis, lack of access to HBV DNA tests, cases of immunoprophylaxis

Table 1 The mechanism of hepatitis B immune and antiviral prophylaxis to reduce mother-to-child transmission

MTCT Intervention	Mechanism	Ref.
Immunoprophylaxis		
Hepatitis B immunoglobulin	Blocks viral attachment and subsequent entry of HBV into hepatocytes; neutralizes circulating HBV and target infected cells *via* antibody-mediated immune response; has to be administered within 24 hours of birth	[56, 57]
Hepatitis B vaccine	Induces active immunity by producing antibodies that target the surface antigen of HBV; given in three doses, at 0, 1, and 6 months, with the first dose recommended within 24 hours after birth	[58]
Antiviral prophylaxis		
Lamivudine, Telbivudine	Deoxycytidine nucleoside analogues; acts as obligate DNA chain terminators; have low genetic barrier to resistance that can lead to drug resistance; not recommended as first-line antiviral therapy for pregnant women	[59, 60]
Entecavir	Deoxyguanosine nucleoside analogue; inhibits replication of HBV by inhibiting HBV polymerase; halts HBV DNA elongation after incorporating a few additional bases; has high genetic barrier to drug resistance	[61]
Tenofovir disoproxil fumarate, Tenofovir alafenamide fumarate	Nucleos(t)ide analogues; inhibits viral replication by inhibiting HBV polymerase; have a high genetic barrier to drug resistance; tenofovir disoproxil fumarate is the current recommended antiviral therapy for pregnant women to prevent MTCT	[62]

MTCT: Mother-to-child transmission; HBV: Hepatitis B virus.

failure in infants, in addition to the lack of trained health-care workers, lack of capacity, and limitations of infrastructure in some HBV endemic countries[7,45].

The cost and access to antiviral prophylaxis

The cost and access to antiviral prophylaxis could pose a serious issue for HBV MTCT prevention programs in pregnant women. TDF is now listed on the WHO list of essential medicines and is considered of low cost; however, access to TDF in some HBV prevalent regions remains poor[46,47]. In China, the lowered cost of TDF to less than USD 1.50 *per* month and ensuring its availability in most hospitals in the country, greatly supported the success of their SHIELD HBV MTCT prevention program[46]. In African countries, access to TDF for HBV patients may be possible through the existing human immunodeficiency virus (HIV) program, since TDF is also used for HIV treatment with already well-established supply chains[100]. In addition, it is estimated that 6.1% of women with HIV infection were also coinfected with HBV[7, 45], thus TDF treatment in pregnant women in Africa will be beneficial for the prevention of both HIV and HBV transmission in the region.

In the case of low accessibility to TDF in pregnant women, the use of combined antivirals (for example TDF with other anti-HBV agents) or more reliance on an immunoprophylaxis program may be considered for MTCT prevention[46,47, 101]. A 2018 clinical trial performed in 331 pregnant women in Thailand showed that the administration of neonatal HBIG and full doses of HBV vaccination in infants born to HBeAg seropositive women were sufficient to reduce the MTCT rate. Furthermore, it was found that additional TDF treatment did not result in a significantly lower rate of HBV transmission[101]. A 2023 retrospective study in Thailand performed cost-effectiveness analyses of the following different TDF-based intervention strategies: (1) TDF to eligible mothers and HBIG for all infants; (2) TDF to eligible mothers and HBIG for infants from HBeAg-positive mothers, and (3) TDF to eligible mothers without HBIG for infants. Their analyses showed that the HBIG-free strategy was the most cost-saving intervention, with 0 to 1.4% transmission rates, making it an ideal strategy for high HBsAg seropositive prevalence but resource-constrained populations[102].

The ideal time to initiate antiviral prophylaxis

Another issue related to antiviral prophylaxis in pregnant women is when to start treatment. The recommended initiation time for antiviral prophylaxis is after 24 weeks gestation. However, it is unclear whether antiviral treatment in CHB pregnant women can be initiated before 24 weeks gestation. A study has shown that the reduction in HBV DNA level was more significant in pregnant women who started treatment in the second trimester (< 27 weeks) compared to the third trimester (> 28 weeks)[69]. A significant reduction in HBV DNA level was also observed in pregnant women treated with TDF in the second trimester compared to those treated in the third trimester[69]. A network meta-analysis comparing the efficacy and safety of antiviral therapy in pregnant women also concluded that treatment administered during the early or middle pregnancy period had better efficacy in HBeAg seropositive pregnant women with a high HBV DNA VL (≥ 6 Log_{10} IU/mL). Furthermore, this particular effect was consistent, regardless of the NA drugs used[103].

A 2020 prospective cohort study in China enrolled 136 CHB women to assess the safety and efficacy of antiviral treatment before and during pregnancy. A small proportion of the women with active CHB was treated with either TDF or LdT prior to pregnancy to normalize their liver enzymes, and continued with TDF or LdT administration throughout the entire pregnancy. The study showed that these women showed no differences in obstetric-related complications compared to the other three groups of pregnant women who received antiviral therapy as follows: (1) In early pregnancy (< 24 weeks); (2) In late pregnancy (> 24 weeks), or (3) In late pregnancy, but with high HBV DNA VL (> 6 Log_{10} IU/mL). In addition, all their infants (who had received neonatal HBIG dose and a complete series of HepB vaccination) showed

negative HBsAg status after the 7-month follow-up period, with no differences in the infants' rates of congenital malformation and other growth indicators[85].

Current evidence on the use of antiviral prophylaxis in HBsAg seropositive mothers is positive, as there were no observed adverse events for the mothers or infants[69,85]. To date, no NA drugs have been associated with obstetric-related complications in mothers or congenital malformations in infants[67,69,85], thus all NA drugs are considered safe for mothers and newborns. However, there are still limited long-term safety data on the effect of antiviral prophylaxis use during pregnancy[103], and whether antiviral prophylaxis is safe to use in multiple pregnancies. Thus, more data are needed to fully understand the effect of this treatment on mothers and infants.

The costs of immunoprophylaxis

Despite the satisfactory effect of infant immunoprophylaxis, vaccine delivery including maintenance of the cold chain logistics, consistency and equity of supply, and timely administration may hinder MTCT elimination[46,47]. One strategy to improve feasible implementation of HBIG and HepB for infants of HBV-positive mothers within one hour of birth is to ensure the vaccine supply both in the delivery room and postnatal ward[82,104]. Current monovalent HBV vaccine is considered low cost; however, the total health care cost also includes the cost for the infrastructure and personnel. Therefore, the total cost for the patient might actually be higher than the cost of the vaccine dose itself[46,47]. Regardless, the rate of infants receiving the appropriate HepB dose at birth has increased in areas where local health care policies mandate for HBV vaccination shortly after birth with a constant supply of available vaccine in the labor and maternity wards[105,106].

Taiwan serves as a success story for infants' HBV vaccination program. As the first nation to implement an HBV vaccination program for infants in 1984, they gradually expanded their vaccination target to increase vaccination coverage, from only infants with high-risk mothers to all infants regardless of maternal HBsAg status. Now, they are targeting all infants who missed their vaccination as newborns and adolescents. After 30 years of implementation, Taiwan has successfully decreased the prevalence of HBsAg from 9.8% to 0.5% in individuals ≤ 30 years old and to ≤ 1% among 5-year-old children. Consequently, they also managed to reduce the incidence of fulminant hepatitis in infants and HCC in the population[81]. Similarly, the success of the HBV vaccination program in China is attributed to the government's commitment to reduce the MTCT rate. Early in the program, parents had to cover the cost of the infants' HBV vaccination, which affected the rate of vaccine coverage in the country. However, in 2002, the Chinese government successfully built a collaborative project with the Global Alliance for Vaccines and Immunization (GAVI) to ensure all children receive HBV vaccination, even in the poorer western provinces. This collaboration has increased the vaccination coverage rate in infants from 30% in 1992 to 93.4% in 2005, with a reduction of HBsAg prevalence from 2.1% to 1%[107]. Indeed, GAVI has provided support on vaccine-related financial and logistical costs since 2001, including vaccine cost reduction to below USD 1 *per* dose and the availability of pentavalent vaccines (DPT-HepB-Hib), has greatly increased the vaccine coverage rate in GAVI-supported countries[108].

Availability of HBIG supply is a costly investment, and as such HBIG is often not readily available in low-income countries. To try to overcome this shortcoming, a clinical trial was performed in Cambodia to assess the feasibility of the immunoglobulin-free strategy for MTCT in a limited-resource region. More than 1000 HBsAg-positive pregnant women were recruited in the TA-PROHM study from 2017 to 2020, and 28% (338/1194) of these women received TDF treatment. In infants not receiving HBIG injection, the MTCT rate was zero (n = 227) in those born to women who received TDF for more than four weeks before delivery. However, in those born to women who received TDF for less than four weeks, the MTCT rate was 8% (3/36). These results show that even without neonatal HBIG administration, TDF treatment was sufficient to reduce the MTCT rate[109]. In addition, determination of the mothers' eligibility for TDF treatment can be performed using the HBeAg rapid detection test and an ALT-based algorithm[109,110], which would be more feasible in a limited-resource setting with high HBV prevalence.

Cases of immunoprophylaxis failure in infants

Despite the protective effect of immunoprophylaxis administration in infants of HBsAg-positive mothers, cases of immunoprophylaxis failure have been reported. A 2021 Chinese study reported nine cases of immunoprophylaxis failure (n = 982) in newborns receiving both HBIG and HepB-BD within an hour after delivery, who were born to women with a high HBV DNA VL (> 6.4 Log_{10} IU/mL)[82]. In infants born to HBeAg-positive mothers, the frequency of immunoprophylaxis failure was higher at 5.2% (16/306) compared to those born to HBeAg-negative mothers. This immunoprophylaxis failure was associated with very high HBV DNA levels (≥ 8 Log_{10} IU/mL, OR = 4.53, 95%CI: 1.19-17.34), inadequate initial injections (OR = 7.69, 95%CI: 1.71-34.59), and delayed vaccination time (OR = 4.14, 95%CI: 1.00-17.18)[96].

A 2021 birth cohort study in central Vietnam discovered that in children born to HBsAg seropositive mothers, 13.1% (16/122) who received a complete series of HepB vaccination had HBV infection at the 2-year follow-up period, and 20.5% (9/44) who received incomplete HepB doses were also infected. In addition, in children born to HBeAg-positive mothers, 28.3% (15/53) who received a complete series of HepB vaccination had HBV infection, while 53.3% (8/15) who received incomplete HepB doses also became infected[111]. This study showed the importance of post-vaccination serological testing for vaccinated infants born to HBsAg seropositive mothers, to determine the seroprotective level of anti-HBs in these children. In addition, other vaccination strategies may be needed to reduce the rates of immunoprophylaxis failure in high HBV prevalent regions, by changing the dose of the HepB vaccine and/or HBIG for at-risk infants.

A multicenter study in China enrolled 955 pairs of infants and their HBsAg-positive mothers. The infants all received HBIG injection (at 0 and 1 month) and either 10 μg or 20 μg HepB (at 0, 1, and 6 months). The results showed that the immunoprophylaxis failure rate in the 20 μg HepB group was not significantly different compared to the 10 μg group, regardless of the maternal HBV DNA level, with both doses having good safety profiles in the infants. However, the

higher HepB dose was associated with a significant reduction in the low-response rate (anti-HBs titer ≥ 10–100 IU/L) and middle-response rate (anti-HBs titer 100–1000 IU/L), and increased the high-response rate (anti-HBs titer ≥ 1000 IU/L) in infants born to mothers with low HBV DNA levels (< 5 Log_{10} IU/mL)[112].

With regard to HBIG doses, there are currently two recommended HBIG doses used in several countries for newborn injection, 100 IU and 200 IU. Wei *et al*[113] has shown that for infants born to HBsAg-positive mothers, the infection rate in infants injected with either 100 or 200 IU HBIG did not significantly differ, with respective prevalences of 1.5% (8/545) and 1.9% (12/632). The respective anti-HBs positive rates in the two groups were 98.5% (529/537) and 98.2% (609/620), with comparable anti-HBs levels of 707.95 mIU/mL and 602.56 mIU/mL at the 7-month follow-up period. However, in the 200 IU HBIG group, one non-responding infant became HBsAg seropositive at the 12-month follow-up period[113]. A RCT of 331 pairs of infants and HBsAg- and HBeAg-positive mothers in China confirmed the previous finding. It was found that in vaccinated infants born to HBsAg- and HBeAg-positive mothers, the higher dosage of HBIG (200 IU) did not provide an additional protective effect against MTCT compared to the lower HBIG dose (100 IU). However, in relation to the cost of infant vaccination, the analysis demonstrated that the use of three-doses of HepB vaccine and a single dose of 100 IU HBIG was the more cost-effective approach in preventing HBV MTCT in the country[114].

Availability of HBV DNA testing

To successfully implement a prenatal intervention in HBsAg seropositive pregnant women, access to laboratory testing for HBV screening is crucial. Poor access to laboratory-based screening of HBV seromarkers (HBsAg and/or HBeAg) and HBV DNA VL quantification are often reported in resource-limited countries. Furthermore, in areas where the appropriate laboratory facilities are already available, the high cost of the tests might also be an issue[46,47].

HBV DNA VL quantification is considered the best method to determine whether a CHB pregnant woman is eligible for TDF treatment, as most of the antiviral prophylaxis guidelines use the maternal HBV DNA level of ≥ 5.3 Log_{10} IU/mL as the cut-off for antiviral treatment initiation. However, not all countries have the resources and facilities to perform routine HBV DNA quantification tests. As such a reliable surrogate marker for HBV DNA level is needed, especially in a limited-resource setting. HBeAg detection and a quantitative HBsAg level test have been identified as possible alternative cost-effective tests for HBV DNA level quantification[7,45].

A 2020 Cambodian study in 515 HBsAg-positive women demonstrated the potential use of a combined algorithm of the HBeAg rapid diagnostic test and ALT levels for identifying women eligible for TDF treatment. It was found that a positive HBeAg rapid test and ALT threshold level of 40 IU/L had a sensitivity and specificity of 79% and 93% for HBV DNA level > 5.3 Log_{10} IU/mL, and 88% and 93% for HBV DNA level > 7.3 Log_{10} IU/mL, respectively[110]. HBsAg levels have also been shown to positively correlate with HBV DNA levels in HBeAg seropositive pregnant women, with a correlation of low HBsAg levels of < 3 Log_{10} IU/mL with HBV DNA levels < 6 Log_{10} IU/mL. Thus, HBsAg quantitative level may serve as a good VL predictor in HBeAg-positive pregnant women[115]. Additionally, the detection of hepatitis B core-related antigen (HBcrAg) has also shown good correlation with HBV DNA level, where a value of 5.3 Log U/mL is equal to a HBV DNA level of ≥ 5.3 Log_{10} IU/mL. Thus, HBcrAg may also be used as an alternate serological marker to identify high viremia in treatment-naïve CHB patients[65,116].

The development of a rapid, affordable, and point of care test (POCT) or near-POCT for HBV DNA may also reduce the dependency on HBeAg testing[46,47]. Furthermore, the availability of an inexpensive POCT for HBV DNA may simplify the need for two separate tests for HBsAg and HBV DNA VL into a single HBV test for diagnostic and risk stratification purposes in pregnant women[46,47]. A widely available HBV DNA test would also allow for the identification of OBI in at-risk cases, although OBI is not considered a significant risk factor for MTCT as OBI cases typically have low HBV DNA levels[46,47]. In China, a multilevel step HBV MTCT prevention program was introduced to solve the issue of lack of trained healthcare workers and limitations of infrastructure. The multilevel approaches in their SHIELD program allows for proper training of healthcare workers and gradual improvement in healthcare infrastructure to ensure a good compliance rate (83.2%) in antiviral-treated pregnant women[46].

Another available approach to improve accessibility to HBV testing is by combining resources with the HIV infection elimination program. Established infrastructure for HIV MTCT prevention can be shared for the HBV MTCT prevention program due to their similar approaches, especially in resource-poor settings in Africa[117]. The use of dried blood spots for hepatitis B testing may also be considered, especially in low-resource settings with limited access to well-equipped health care facilities[100].

Lack of awareness of HBV screening

There are several identified barriers that may impede a successful HBV prevention MTCT program, which include lack of awareness of HBV screening and the benefit of HBV vaccination for at-risk infants and their caregivers. The WHO has identified that HBV immunoprophylaxis program expansion is greatly impacted by the local and diverse characteristics of the target population and the cultural beliefs and political acceptance in the country[46,47,108]. As such, enhancing health education and awareness of the risk factors associated with HBV infection, chronic liver disease progression, and effective screening and treatment regimens are crucial, along with advocacy and adequate representation for specific at-risk populations including rural communities, migrant populations, and marginalized groups[46,47].

In Africa, where some countries still have no dedicated HBV programs, expanding the existing HIV MTCT prevention program will enable HBV screening tests for pregnant women, resulting in improved identification and access to care for HBV mono infected pregnant women[100]. Meanwhile, in high-income countries, HBV undiagnosed individuals usually come from vulnerable populations, including intravenous drug users, homeless persons, and illegal immigrants[108]. These people are often difficult to reach, therefore community-based HBV screening and outreach program may be beneficial in identifying these at-risk individuals[46,47].

Increasing awareness of HBV screening in pregnant women may also be beneficial for MTCT prevention programs. In Nigeria, mothers with good knowledge of HBV (receiving tertiary education as an indicator) were more likely to vaccinate their newborns[105]. Similarly, in China, mothers with high education levels were the most likely to perform PVST follow-up after their infants' vaccination[88]. Overall, it is crucial to combine effective treatment and comprehensive policies in infection prevention, political commitment, financial structure, stakeholder engagement, and healthcare system integration[108].

CONCLUSION

Vast and significant efforts to reduce the risk and prevalence of HBV MTCT have been noted in many countries, including in HBV endemic regions in the Asia Pacific region. However, additional approaches are needed to achieve a zero MTCT rate, including HBV screening of pregnant women, interventions in HBV transmission during delivery, and infant immunoprophylaxis. These should also be accompanied by the availability and the affordability of HBV tests, HBIG, and antiviral therapies.

FOOTNOTES

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REVIEW

Oncometabolites in pancreatic cancer: Strategies and its implications

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Abstract

Pancreatic cancer (PanCa) is a catastrophic disease, being third lethal in both the genders around the globe. The possible reasons are extreme disease invasiveness, highly fibrotic and desmoplastic stroma, dearth of confirmatory diagnostic approaches and resistance to chemotherapeutics. This inimitable tumor microenvironment (TME) or desmoplasia with excessive extracellular matrix accumulation, create an extremely hypovascular, hypoxic and nutrient-deficient zone inside the tumor. To survive, grow and proliferate in such tough TME, pancreatic tumor and stromal cells transform their metabolism. Transformed glucose, glu-tamine, fat, nucleotide metabolism and inter-metabolite communication between tumor and TME in synergism, impart therapy resistance, and immunosuppression in PanCa. Thus, a finer knowledge of altered metabolism would uncover its metabolic susceptibilities. These unique metabolic targets may help to device novel diagnostic/prognostic markers and therapeutic strategies for better management of PanCa. In this review, we sum up reshaped metabolic pathways in PanCa to formulate detection and remedial strategies of this devastating disease.

Key Words: Metabolic reprogramming; Pancreatic cancer; Metabolic symbiosis; Therapy resistance; Anti-pancreatic cancer therapy

Core Tip: Pancreatic cancer (PanCa) is supported by reprogrammed metabolism. Cancer specific glucose, glutamine, fat, nucleotide metabolism and inter-metabolite communication between tumor and stroma, impart continual proliferation, therapy resistance, and immunosuppression. Key enzymes and intermediates of altered metabolic pathways would help to formulate disease specific markers and therapeutic approaches. This review sums up reformed metabolic pathways in PanCa to formulate detection and remedial strategies for better disease management.

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INTRODUCTION

Pancreatic cancer (PanCa) is one of the deadliest and highly aggressive malignancies worldwide, ranking third in mortality rate in both the genders in the United States. Despite recent advancements, prognosis for PanCa remains dismal, with a five-year survival rate of around 10%[1]. In 2020, globally, 495773 new cases and 466003 deaths due to PanCa with a ratio of male to female of 1.1:1, both at diagnosis and mortality are recorded[2]. GLOBOCAN 2020 points that, PanCa is 12th most common cancer accounting for 2.6%, among all cancer types and ranks 7th (4.7%) in cancer related deaths, globally[3,4]. Its lifestyle dependence is shown by the fact that countries with very high and high Human Development Index (HDI) exhibit nearly fivefold higher incidence and death rates due to PanCa as compared to low/medium HDI countries[5,6]. Insidious onset, metastatic nature, lack of specific symptoms and reliable biomarkers, ineffective screening methods, make PanCa almost undetectable at early stage[7]. Also, the repertoire of efficient therapeutic interventions remains constrained with chance of local recurrence over 80% in radical resection surgery[8].

Like other cancers, PanCa can also be detected through imaging techniques like, ultrasonography, magnetic resonance imaging, computerized tomography, and positron emission tomography (PET) scans, followed by biopsy. Elevated carbohydrate antigen 19-9 (CA19-9) in peripheral blood, specifically indicates PanCa; through false negative cases are recorded. PanCa is also associated with strong chemoresistance, possibly mediated through stromal diversity, cancer-stromal cross-talk and revised metabolism. Gemcitabine plus FOLFIRINOX (5-FU, leucovorin, irinotecan, and oxaliplatin) and nab-paclitaxel are regularly used, as the first-line of treatment, though associated with towering toxicity with insignificant improvement in survival[9]. Targeted therapy using small molecules like erlotinib in singular or in combination with gemcitabine hardly showed any benefit. Despite advanced chemotherapeutic regimens, disease progression is associated with chemoresistance and unfavorable outcome[10,11]. Consequently, development of innovative treatment modalities aimed at improving prognostic trajectories is need of the hour[12].

All malignant cells undergo metabolic alterations, encompassing glucose, lipid, amino acid, nucleotide, and energy metabolism, driven by both internal and external cues[13]. Intrinsically, PanCa exhibits gain of function mutations of *KRAS* gene (approximately 90% of cases) and loss of function mutations of tumor suppressor genes, like, *TP53*, *SMAD4*, and *CDKN2A*; which maps cancer progression with metabolic reprogramming[14]. This modified metabolism is not restricted to cancer cells only, but also, to various stromal cells, comprising of fibroblasts and an array of immune-cells. All these cells communicate with each other and with tumor cells through various metabolic intermediates to deal with nutrient, oxygen and chemotoxic stress, offering maximization of nutrient utilization, and immunosuppression, forming metabolic a "symbiosis"[15], aiding in immune-escape, sustained proliferation and therapy resistance. Moreover, this metabolically diverse disease can be classified, based to its metabolic dependencies, into more aggressive and therapy resistant glycolytic and lipogenic subtypes with superior prognosis[12]. Thus, a deep understanding of cancer-specific glucose, protein, lipid and nucleotide metabolisms in PanCa is crucial. Also, as per our previous published data, it is suggested that the architecture is different when Indian-origin patient data is compared with the Western world hence target therapeutic is required[16,17]. In this review, we summarize the current knowledge in the intricate metabolic alterations and its association with treatment-resistance in PanCa. Additionally, here, we tend to discuss, preclinical and clinical trial studies with antidotes of enzymes and intermediates of metabolic pathways to deduce diagnostic and therapeutic targets, which could be effective in devising innovative treatment strategies against the deadly disease.

PANCA SPECIFIC ALTERED CELLULAR METABOLISM

Alteration of carbohydrate metabolism

PanCa cells depend on less efficient cytosolic glycolysis for ATP production rather than oxidative phosphorylation irrespective of abundance of oxygen, known as Warburg effect, unlike, typical TCA cycle and oxidative phosphorylation in normal cells. Warburg effect facilitates rapid ATP production and avails ample intermediates for further use in other auxiliary pathways: PPP, HBP, lipid synthesis, proving building blocks for uninterrupted cell proliferation[18]. Oncogenic KRAS elevates glycolytic enzymes including hexokinase 1/2, phosphofructokinase 1, enolase-2, and lactate dehydrogenases (LDHA and LDHB) in PanCa leading to increased production of lactate, accumulation of which reduces cellular and extracellular pH, promoting invasiveness through destroying immune response, and facilitating metastasis, leading to poor overall survival (OS)[19,20].

Glucose is transported into cytoplasm through cell-membrane glucose transporters (GLUT), highly expressed in PanCa cells and associated with unfavorable prognosis[21]. Activation of oncogenes (*KRAS*, *c-MYC*), inhibition/loss of mutant *P53*, *PTEN*, epigenetic alterations, overexpression of hypoxia-inducible factor-1 (HIF-1) and GLUT1 activation promotes aerobic glycolysis[22]. Repression of GLUT-1 and LDHA mediate sodium-glucose co-transporter (SGLT2) inhibition and enhanced chemotherapeutic efficacy[23]. In PanCa, HIF-1α, activated by upstream oncogenic PI3K/AKT and MAPK/ERK pathways, upregulate the expression of several key glycolytic enzymes and glucose transporters to overcome oxidative stress to preserve mitochondrial redox potential and reactive oxygen species (ROS) generation, evade immune surveillance and promote invasion[22].

In PanCa cells, accumulated lactate produced by aerobic glycolysis, is neutralized by export through membrane-monocarboxylate transporters (MCT), which are often upregulated. Mitochondrial OXPHOS and NADPH oxidases regulated by mutant *KRAS* and *P53* genes alter redox metabolism and ROS levels in PanCa[24]. Additionally, mutant *KRAS* signalling induces mitochondrial translocation of phosphoglycerate kinase 1, resulting in phosphorylated PDHK1 and restricted OXPHOS[25]. Glucose deprivation further promotes *KRAS* mutations. However, refractory tumors including PanCa shows elevated OXPHOS and overexpression of mitochondrial respiratory complex I components at both RNA and protein level. Interestingly, targeting complex I coupled with usual chemotherapy showed hopeful results in PanCa[26]. Recently a hybrid glycolytic/OXPHOS phenotype in PanCa cells is proposed[22].

PanCa exhibit increased non-oxidative PPP, controlling cencer cell growth through the supply of ribose-5-phosphate for DNA/RNA synthesis and NADPH for cellular ROS detoxification, by upregulation of ribulose 5-phosphate isomerase and ribulose-5-phosphate-3-epimerase driven by mutant *KRAS*[27]. Chronic acidosis caused due to aerobic glycolysis significantly enhances PPP, promoting cell proliferation by activating Yes1 associated transcriptional regulator (YAP)/matrix metalloproteinase-1 axis in PanCa[28].

Alternatively, glucose in PanCa through hexosamine biosynthesis pathway (HBP), produces UDP-GlcNAc, a crucial substrate for protein and lipid glycosylation. Abnormal glycosylation by hyperactive HBP aids in tumor development and drug resistance in PanCa, mediated by altered O-GlcNAcylation. Additionally, elevated glutamine-fructose-6-phosphate amidotransferase-1, key enzyme of HBP, in PanCa cells is associated with poor survival[22].

Alteration of amino acid metabolism

Taken up by micropinocytosis, amino acids act as an alternative fuel of TCA cycle in glucose deprivation, and as a potential nitrogen source required for nucleotide synthesis in PanCa. Amino acids like, glutamine (Gln) and alanine support Krebs cycle after conversion into alpha-ketoglutarate (α-KG), and pyruvate respectively[18]. Driven by mutated KRAS, Gln is converted to glutamate (Glu) by mitochondrial glutaminase, which is then further converted to α-KG by transaminase [glutamate oxaloacetate transaminase 2 (GOT2)][29]. Gln also regulates cellular ROS through upregulation of reduced glutathione (GSH), thus showing the utility of Gln in hypoxic PanCa cell survival[30]. Asparagine, another essential amino acid, helps in biosynthesis of other amino acids, NO and polyamines in pancreatic ductal adenocarcinoma (PDAC)[31]. Gln also produces NADPH through non-canonical pathway. NO is associated with invasion and proliferation in PanCa. Aspartate derived from Gln and catalyzed by GOT2, also serves as TCA cycle intermediate. It is converted into oxaloacetate by glutamate-oxaloacetate transaminase (GOT1), often upregulated in PanCa with *KRAS* mutation to generate NADPH[32]. Oxaloacetate is converted to malate by GOT1 in cytoplasm, and then to pyruvate, both of which act as TCA cycle intermediates[33].

Non-essential amino acid (NEAA), arginine in PanCa cells is produced through urea cycle from aspartate and citrulline catalysed by argininosuccinate synthetase, following argininosuccinate lyase[31]. Another NEAA, proline, often converted to Glu through proline oxidase (POX) and Δ^1-pyrroline-5-carboxylate dehydrogenase and in reverse by Δ^1-pyrroline-5-carboxylate (P5C) mediated by P5C synthase (P5CS) and subsequent P5C reductase (PYCR) in PanCa cells. POX, contributing to collagen formation is overexpressed in PanCa cells[34]. Branched-chain amino acids (BCAAs) like, valine, leucine and isoleucine donate nitrogen α-KG to form glutamate and α-keto acids, mediated by branched-chain amino acid transaminase 1 (BCAT1 & 2). BCAAs are elevated in early-stage mutant *KRAS* driven PanCa[31]. BCAA also acts as a carbon source through acetyl CoA, for fatty acid synthesis and fuel TCA cycle in PanCa cells[33]. Piling up of ROS in PanCa cells, is dealt with the help of elevated GSH, produced from glutamate, cysteine, and glycine. First γ-glutamylcysteine is formed from glutamate and cysteine and finally GSH is formed from γ-glutamylcysteine and glycine [31].

Alteration of lipid metabolism

Lipids provide cellular building blocks, signalling molecules and energy to rapidly growing cancer cells. While normal cells depend upon dietary uptake of fatty acids (FAs) and cholesterol (approximately 93%), tumor cells synthesize lipids

from mitochondrial citrate, through *de novo* Lipogenesis[35]. However, recent findings suggest the utilization of food-derived FAs for phospholipid biosynthesis required in proliferation and signalling pathways[36]. Enzymes required for lipid/cholesterol synthesis, like citrate synthase, fatty acid synthase (FASN), *etc*., and their genes are elevated in PanCa cells[37].

Oncogenic KRAS, associated with elevated lipid droplet accumulation, regulates lipid storage and metabolism, influencing disease progression in PanCa. Lipid droplets are catabolized during PanCa cell invasion[38]. KRAS also upregulates transcription factor, regulatory element-binding protein 1 (SREBP1), which promotes palmitic acid and stearic acid synthesis leading to rapid cell proliferation in PanCa cell lines and animal models[39]. SREBP1, also upregulates SCD1 to promote unsaturated fatty acid synthesis in PanCa. Furthermore, KRAS boosts fatty acid synthesis and consequent energy metabolism mediated by elevated expression of phospholipase A2 group IIA. Saturated, monounsaturated, and ω-6 polyunsaturated FAs support carcinogenesis in PanCa[40], while ω-3 polyunsaturated FAs are anti-carcinogenic[41]. Unusual arachidonic acid (AA, a ω-6 polyunsaturated FA) metabolism favors epithelial malignancies, like PanCa through modulation of lipoxygenase pathway[33].

Cholesterol or cholesteryl esters, essential constituent of plasma membrane are required in PanCa progression. Aldo-keto reductase family 1B10 (AKR1B10), boosts cell survival and modulates cholesterol metabolism through its reduced products, FPP and geranylgeranyl pyrophosphate, is overexpressed in PanCa tumors[33]. SREBP2 regulating cholesterol synthesis is also upregulated in PanCa[42]. Increased cholesterol uptake through overexpressed LDLR in PanCa demonstrates elevated risk of recurrence[33].

Alteration of nucleotide metabolism

Amplified nitrogen need is a metabolic hallmark of cancerous cells. Tomour cells undergo uncontrolled growth and proliferation, supported by altered nucleotide metabolic pathways to enhance synthesis of extra nucleic acids through *de novo* as well as salvage path[43]. Hyperactivation of mTORC1 in proliferating cells promote pyrimidine synthesis, while extracellular signal-regulated kinase hyperactivation leads to long-term stimulation of *de novo* pyrimidine and purine biosynthesis[44]. Hyperactivated Myc, also boosts purine synthesis in PanCa. Oncogenic KRAS elevates intracellular nucleotides to promote non-oxidative pentose phosphate pathway (PPP), vital for *de novo* nucleotide biosynthesis[45]. Dihydropyrimidine dehydrogenase, helping in epithelial-mesenchymal transition and cancer progression, is upregulated in PanCa. PanCa with mutant p53 alleles also facilitate inosine monophosphate dehydrgenase and guanosine monophosphate synthase expressions[46].

In *de novo* pathway, purines are synthesized directly by combining pyrophosphate at C-1 of a ribose sugar following several more steps. First, ribose-5-phosphate is converted to phosphoribosyl pyrophosphate (PRPP). Next PRPP combines glutamine to form 5-phosphoribosylamine and pyrophosphate, catalyzed by PPAT. Then inosine monophosphate (IMP) is formed. It acts as a precursor of adenosine monophosphate and guanosine monophosphate, catalysed by adenylosuccinate synthetase and inosine monophosphate dehydrogenase through several kinetic intermediates. In case of *de novo* pyrimidine synthesis, its ring structure is combined following a 6-step process, where L-glutamine and L-aspartate acts as precursors. Carbamoyl phosphate synthetase, aspartyl transcarbamoylase, and dihydroorotase catalyzes the initial 3 steps, finally forming uridine-5-phosphate, serving as a building block in pyrimidine biosynthesis. In both purine and pyrimidine nucleotide biosynthesis, the homeostasis and conversion among nucleoside triphosphate and nucleoside monophosphate are facilitated by ecto-nucleoside triphosphate diphosphohydrolase-1 (ENTPD1/CD39) and ecto-5-nucleotidase (NT5E/CD73). CD73 transforms AMP to adenosine with phosphate, and CD39 can hydrolyzes nucleoside-5-triphosphates into nucleoside-5-monophosphate and its products. CD73 and CD39 catalyzes ribose-5-monophosphate and deoxyribose-5-monophosphate to form nucleosides and deoxynucleosides. Also, purine nucleoside phosphorylase (PNP), reversibly catalyzes phosphorolysis of nucleosides to produce purine and pyrimidine base and ribose 1-phosphate. Other than nucleotide metabolism, CD39 and CD73, frequently upregulated in human cancers, play immunomodulatory roles through furnishing anti-tumor activity of various immune-cells: T cells and macrophages[46].

In salvage pathway, free bases are derived from nucleic acid turnover or dietary intake. Ubiquitous PNPs catalyze hypoxanthine-guanine phosphoribosyltransferase to monophosphates of inosine and guanosine. Ribo- and deoxyribonucleosides are converted to ribonucleotides mediated by adenosine deaminase. Uridine-cytidine kinases, rate-limiting enzymes in pyrimidine biosynthesis salvage pathway, convert uridine and cytidine to their consequent monophosphates [41].

METABOLIC SYMBIOSIS IN PANCA

Rigid, nutrient-depleted, hypoxic and desmoplastic stroma is composed of collagen meshwork enclosing pancreatic tumor. Various cells like, fibroblasts (cancer-associated fibroblasts or CAFs), endothelial and immune cells like, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and few activated cytotoxic T (Tc) cells are present in highly heterogeneous PanCa microenvironment. All these cells converse with each other and with tumor cells through soluble factors, gap junctions and exosomes, ultimately resulting in symbiosis for unlimited proliferation, invasion, metastasis, immunosuppression and chemoresistance[47] (Figure 1).

Stromal collagen fuels cancer cells, through constant supply of nutrients. Misbalanced shear stress, due to thick stoma, upregulates PI3K/AKT pathway and ROS production resulting in boosted glycolysis in PanCa cells. In PanCa cells increased glycolytic flux, is coupled with overexpression of rate-limiting glycolytic enzymes like, PFK1 and LDHA and lactate amassing[48]. Lactate surplus in hypoxic tumor-central cells is exported to periphery, through gap junction connexin-43 channels and upregulated MCT4. While lactate in normoxic PanCa cells and TME is sensed by Gi-coupled

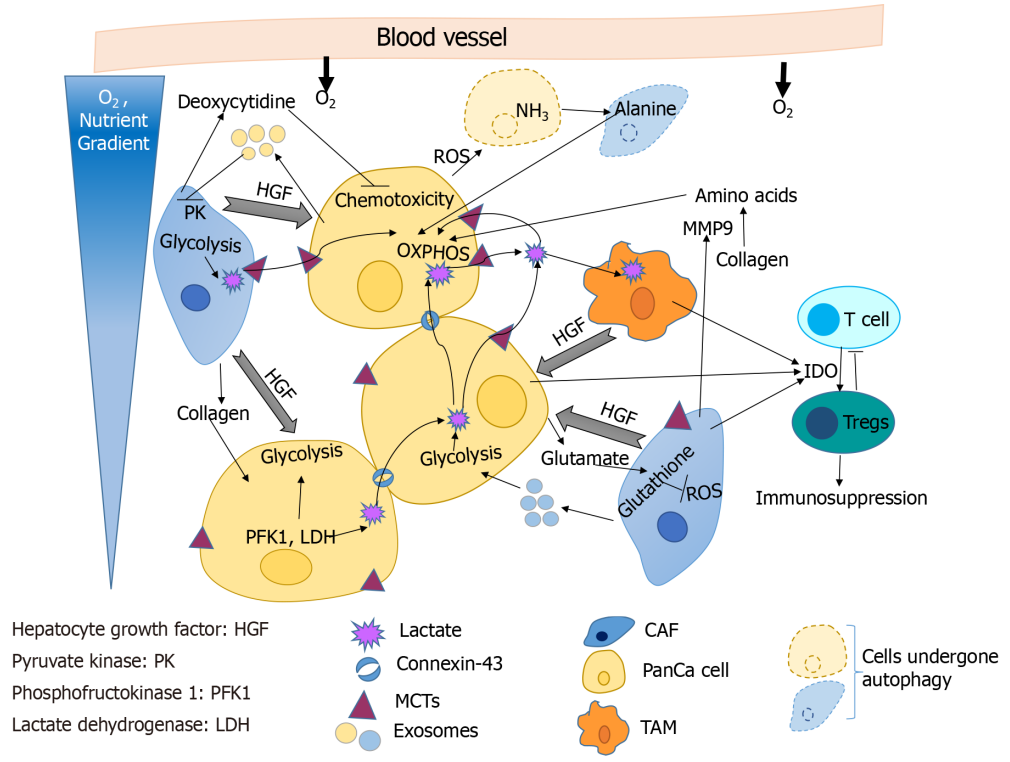

Figure 1 Metabolic crosstalk operating in pancreatic cancer and its microenvironment. HGF: Hepatocyte growth factor; ROS: Reactive oxygen species; IDO: Indoleamine 2,3-dioxygenase; CAF: Cancer-associated fibroblasts; TAM: Tumor-associated macrophage; MCTs: Monocarboxylate transporters.

receptor 81 (GPR81), overexpression of CD147, ultimately enhance MCT1 expression. Stimulated GPR81 also elevates peroxisome proliferator-activated receptor gamma coactivator-1α, which triggers mitogenesis and TCA cycle mediated respiration, using lactate as a fuel. Improved lactate absorption and utilization helps cell proliferation in glucose insufficiency and plays nonmetabolic roles like, promoting invasiveness, immunosuppression and angiogenesis in PanCa[44].

Constantly stimulated by adjacent tumor cells, CAFs show altered carbohydrate metabolism, shifting from TCA cycle/OXPHOS to aerobic glycolysis and generating various intermediate metabolites, including, pyruvate, lactate and ketone bodies. MCT-4, overexpressed in CAFs favour lactate export in TME, while MCT-1 overexpressed in normoxic tumor cells, facilitate lactate uptake from TME to fuel OXPHOS. This phenomenon of linking CAFs and neoplastic cells is known as 'reverse Warburg effect'. Stimulated CAFs also discharge exosomes, enclosing TCA cycle metabolites, lipids and amino acids, which are taken up by PanCa cells to exhibit low mitochondrial OXPHOS, while amplify glycolysis[49, 42]. Tumor cell derived exosomes on the other hand, suppress glucose uptake in stromal astrocytes and CAFs through inhibiting pyruvate kinase, thereby enhancing glucose availability for cancer cells[50]. CAFs produce a collagen-rich ECM, which acts as a reservoir of amino acids and lipids to support growth and metabolism of PDAC cells. Lipid uptake by tumor cells, support the formation of phospholipids aiding in biomembrane synthesis and lysophosphatidylcholine facilitating various regulatory pathways in PDAC[47]. Glutamate released by malignant cells encourages glutathione signalling in CAFs, restricting superoxide and ROS buildup[51]. Also, glutamine upregulate glutamine-transporters, stimulate mitogenesis while suppress mitophagy in PanCa cells[42]. CAFs expressing matrix metalloprotease 9 degrades ECM-collagens to amino acids, which are consumed by PanCa cells through macropinocytosis to fulfill their high amino acid demand[52]. Activated CAFs produce loads of type I collagen, inducing integrin-FAK signalling, leading to clonogenic amplification in PDAC cells[48]. They also secrete deoxycytidine to protect PDAC cells from chemo-toxicity [47,52]. Extreme stromal ROS results in genomic instability in tumor cells followed by their autophagy. On the other hand ammonia produced by tumor cells through glutaminolysis induces CAF-autophagy[48]. CAF-autophagy-derived alanine act as a substitute fuel of TCA cycle, generate lipids and non-essential amino acids (NEAA), in nutrient deprived PanCa cells[43]. Interestingly, PanCa cells harbouring oncogenic KRAS, activate neighboring PSCs major CAF precursors and secrete hepatocyte growth factor (HGF) which upregulate glycolytic metabolism of cancer cells[40]. TAMs, associated with tumor-immunity, favour glycolysis, in neighboring PanCa cells through HGF mediated paracrine cross-talk, promoting metastasis and angiogenesis. Consequently, microenvironmental lactate induces procancer M2-like polarization of TAMs, resulting in reduced immunity[40].

MDSCs and TAMs overexpress arginase and nitric oxide synthase, which deplete microenvironmental arginine and its metabolites (ornithine and citrulline), resulting in inhibition of T cell activation and associated immunesuppression[43]. Indoleamine2,3-dioxygenase (IDO) secreted by PanCa cells, Tregs and TAMs, also promote immunesuppression through suppression of T cell activation and NK cell activity[41]. IDO1upregulated in MDSCs promotes tryptophan breakdown, resulting in piling up of toxic guanosine, which represses immunity through enhanced conversion of T cells into Tregs [44]. Furthermore, lactic acid, accumulation in PanCa cells influence immune cells by blocking: (1) The conversion of monocytes to dendritic cells; (2) Monocyte migration; (3) Cytokine release from dendritic cells and cytotoxic T (Tc) cells;

and (4) Activity of Tc cell and antigen-presenting cells, ultimately leading to immunosuppression.

PanCa cells block Gln degradation in stromal aggressive adipocytes followed by their secretion, facilitating cancer cell proliferation in murine PanCa and adipocyte cell coculture. Also, adipocytes support malignancy through their cross-talk with PSCs and tumor-associated neutrophils. Other than these, many other stromal cells influence each other in PanCa TME[26,36].

ROLE OF ALTERED METABOLISM IN THERAPY RESISTANCE OF PANCA

Altered metabolism within PanCa cells, regulated by genetic and epigenetic factors, is a significant contributor of chemoresistance, based on which, alternate therapeutic strategies to overcome chemoresistance has been developed[53,54].

Oncogenic activation of KRAS, c-MYC, and HIF-1α often promotes upregulation of glucose transporters and several key enzymes of glycolysis. Activated GLUT1 further activates NF-κB and mTOR survival pathways thereby promote chemoresistance[33]. Glycolytic enzyme, hexokinase, often upregulated in metastatic PanCa supports chemoresistance through blocking apoptosis[55]. Rate-limiting glycolytic enzyme, pyruvate kinase M2 (PKM2), elevated in PanCa causes gemcitabine resistance[56]. Enhanced LDHA in PanCa cells block ROS production and apoptosis, resulting in uninterrupted proliferation and consequent drug (gemcitabine) resistance[54,57]. Similar effects are observed with Glyceraldehyde-3-phosphate dehydrogenase[58]. HIF-1α, associated with higher invasion and migration promotes enolase-1 (ENO-1) expression in PanCa[59] ENO-1 suppresses intracellular ROS and consequent apoptosis and gemcitabine resistance PanCa cells[53]. Pyruvate dehydrogenase kinase 3 (PDK3) catalyzes the first step of OXPHOS, is another crucial enzyme for drug resistance. HIF-1α induces PDK3 expression which switches mitochondrial respiration to glycolysis for energy production[60]. In PanCa cells, enhanced nonoxPPP, causes increased level of dCTP expression which competitively inhibits GEM activity. HBP pathway active in PanCa, confers chemoresistance with higher expression of Glutamine-fructose amidotransferase 1[7]. PanCa specifically overexpress Phosphoacetylglucosamine Mutase 3 (PGM3), a key enzyme of HBP pathway. Inhibition of PGM3 in PanCa cells exhibit gemcitabine sensitivity both *in vitro* and *in vivo* models[61].

Enolase (ENO1), a glycolytic enzyme also acting as a plasminogen receptor, is over-expressed in PanCa cells. A DNA vaccine targeting ENO1, is developed in PanCa mouse model. This ENO1 targeted DNA vaccine effectively destroys tumour cells mediated by antibodies and complement-dependent cytotoxicity, upregulation of effector T cells and suppression of MDSCs and Tregs[62].

PanCa cells, characterised by overexpression of lipid metabolism and lipid uptake often contribute resistance to conventional chemotherapy. A key enzyme of lipid biosynthesis, FASN offers gemcitabine resistance, possibly mediated by PKM2 and glycolysis[63,64]. Also, HMGCR, ACAT-1, SREBP2, CRABP-II and AKR1B10, involved in cholesterol biosynthesis, are elevated in PanCa. All these regulators furnish gemcitabine resistance, while only caveolin-1 showed NAB-paclitaxel resistance[65].

Gln is essential for PanCa cell survival. All the transporters like, ASCT2, SLC1A5, enzymes, like, GLS, glutamate dehydrogenase (GDH), alanine transaminase (ALT/GPT) or aspartate transaminase (AST/GOT) and metabolic intermediates associated with Gln metabolism offers chemoresistance, possibly regulated through EGFR signalling and downstream MAPK and AKT-mTOR pathway[66]. GOT1 specifically offers NAB-paclitaxel and gemcitabine resistance [67]. Argininosuccinate synthase 1, associated with arginine catabolism enhances cisplatin resistance in PanCa[68]. Both polyamine transport and biosynthesis upregulated in PanCa provide gemcitabine resistance in mice model[69]. Endoribonuclease or dicer elevated in PanCa also contributes gemcitabine resistance[70].

Radiotherapy is suggested as a neoadjuvant treatment for resectable or borderline disease, in locally advanced and recurrent tumor, and as palliative care in advanced and terminal PanCa. Enhanced carbohydrate and nucleotide metabolism regulated by oncodrivers of PanCa facilitates radioresistance. Glucose metabolism blocker, 2-DG enhances metabolic oxidative stress and radiosensitizes PanCa cells. Carbohydrate rich diet showed radioresistance while, ketogenic diet (high fat - low carbohydrate) amplified sensitivity to radiotherapy in xenograft mouse models with PanCa. Overexpressed FASN and several enzymes in cholesterol biosynthesis pathway like, farnesyl diphosphate synthase (FDS) possibly results in radioresistance in PanCa. Radioresistance of FDS can be mitigated by zoledronic acid (ZOL). A combination of ZOL and chemoradiotherapy after surgery is assessed in an ongoing phase II trial study in PanCa (NCT03073785)[40].

ALTERED METABOLISM BASED DETECTION OF PANCA

PanCa, known for early metastasis, can be detected by the technologies associated with metabolic imaging probes, including, PET and magnetic resonance spectroscopy (MRS). PET imaging is based on the elevated glucose uptake in PanCa cells monitored by, a glucose analog, 18F fluorodeoxyglucose (FDG). FDG is metabolized in PanCa cells like glucose by hexokinase and results in its cellular accumulation, proportional to hexokinase activity. Hexokinase activity is again proportional to tumour spread and thus can predict its pathological grade, distant metastasis, and survival. However, FDG-PET is unable to differentiate between PDAC and mass-forming pancreatitis, as it also shows enhanced FDG uptake. MRS, using the conversion of 1-^{13}C-pyruvate to lactate, distinguishes between normaland cancer tissue and the disease stage. 1-^{13}C-pyruvate imaging is used in mice models of PDAC[71].

Few other studies revealing metabolic signature of PanCa are as follows: (1) PanCa specific metabolomic signature consisting of 48 differentially expressed metabolites, including, intercept, phenylalanine, tryptophan, ethanolamine and carnitine in saliva samples of affected subjects have been identified[72]; (2) Altered expression of 14 biomarkers, including, intercept, proline, creatine and palmitic acid in tissue and serum samples of PanCa patients using UHPLC-Q-TOF/MS has been shown[73]; (3) Modified expression of a panel of 16 metabolites (including, xylitol, histidine, 1,5-anhydro-d-glucitol, and inositol) using GC/MS/MS with CA19-9, is able to differentiate early PanCa from benign tumor and CA19-9 negative PanCa patients from healthy individuals with high sensitivity and specificity[74]; (4) Another panel of 6metabolites, including, 5-hydroxytryptophan, lysophosphatidylethanolamine, phosphatidylcholine, phosphatidylethanolamine, phosphatidylcholine and sphingomyelin also exhibited high specificity and sensitivity, able to discriminate between early PanCa and benign tomours[75]; and (5) Differential lipid profiling in serum of PanCa patients, showed altered expression of sphingomyelin (41:1), sphingomyelin (42:1), sphingomyelin (39:1), ceramide (41:1), ceramide (42:1), lysoPC (18:2) and phosphocholine (O-36:3) compared to chronic pancreatitis and healthy subjects. Combination of these lipid biomarkers with CA19-9 showed better diagnostic performance compared to the metabolites or CA19-9 alone[76].

TARGETING ALTERED METABOLISM IN PANCA THERAPY

Altered metabolism, vital for carcinogenesis and therapy resistance, represents a prominent remedial target in PanCa.

Targeting glucose metabolism

Glucose metabolism suppressed by blocking GLUT1, CG-5, WZB117 and autophagy inhibitor hydroxychloroquine (HCQ) preclinically improved gemcitabine sensitivity and blocked PanCa cell proliferation (Table 1)[77]. After success in preclinical studies, HCQ was clinically assessed in a phase II monotherapy (NCT01273805) as well as in several phase I/II/II trials in combination with: (1) Gemcitabine (NCT01128296); (2) Gemcitabine + nabpaclitaxel (NCT01978184, NCT01506973); and (3) ERK inhibitor, temuterkib (NCT04386057) in advanced and metastatic PanCa. Few more phase I/II clinical evaluations of HCQ in amalgamation with: (1) Radiation + capecitabine (NCT01494155); (2) Chlorphenesin carbamate + mFOLFIRINOX (NCT05083780); (3) Kinase inhibitors (NCT03825289, NCT05518110, NCT04132505); (4) Paricalcitol + gemcitabine + nab-paclitaxel (NCT04524702); (5) Paricalcitol + losartan (NCT05365893); (6) Leflunomide or bevacizumab, (NCT06229340); (7) CPI-613 + 5-fluorouracil or Gemcitabine, (NCT05733000); and (8) paclitaxel protein bound + gemcitabine + cisplatin (NCT04669197) are in progress. Though HCQ in monotherapy furnished slight clinical effectiveness, HCQ + chemotherapy, diminished hypercoagulability, while failed to lower mortality, in advanced PanCa [77]. Interestingly, better prognosis was furnished with a pretreatment of HCQ, gemcitabine and nab-paclitaxel cocktail followed by surgery in PDAC (NCT01978184) (Table 2)[48,78].

In laboratory, glycolysis inhibited by (1) 2-Deoxy-D-glucose (2-DG)[79]; (2) Hexokinase blocker benitrobenrazide (BNBZ); (3) Phosphoglycerate mutase 1 blocker PGMI-004A and KH3[80]; and (4) Pyruvic acid analog, 3-bromopyruvate (3-BP) suppressed PanCa[81]. LDHA inhibitor, (1) N-hydroxyindole-NHI-1 singly or in combination with gemcitabine [82]; (2) Galloflavinin with anti-diabetic drug, metformin[83]; (3) Gossypol[84]; and (4) FX11 suppressed PanCa in cell-line/animal studies (Table 1). A phase I study with 2DG and/or docetaxel showed its safety and tolerance in advanced solid cancers including PanCa (NCT00096707)[85] (Table 2).

Mitochondrial enzyme, pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase blocker, lipoate analog, devimistat (CPI-613/CPI), blocks energy metabolism and proliferation in PanCa cell lines and mice. CPI combined with (1) LDH blocker, galloflavin; and (2) MCT suppresser, cyano-4-hydroxycinnamic acid, showed tumor contraction in PanCa mice models[86] (Table 1). CPI + mFOLFIRINOX showed encouraging results in a phase I (NCT01835041) and in a multicenter open label, randomized phase III trial in metastatic PDAC (NCT03504423)[48,78]. A phase 1 study (NCT03435289) with a blend of CPI-613 + gemcitabine + nab-paclitaxel in advanced or metastatic PanCa is in pipeline (Table 2).

OXPHOS (complex I, NADH dehydrogenase) blocker rotenone, metformin, phenformin and IACS-010759, lowered proliferation of PanCa cell lines[26] (Table 2). To test its clinical efficacy in gemcitabine resistant metastatic PanCa, metformin was administered singly in a phase II (NCT01971034) and in combination with (1) Gemcitabine (NCT01210911, NCT02005419); (2) MFOLFOX-6 (NCT01666730); (3) Stereotactic radiosurgery (NCT02153450); and (4) Rapamycin (NCT02048384) in phase Ib/II studies (Table 2). These cocktails showed well tolerance, while, only metformin +/- rapamycin remarkably prolonged survival[77,78]. A phase II trial with neoadjuvant metformin and few phase I/II trials with a blend of metformin with (1) Gemcitabine + paclitaxel albumin-stabilized nanoparticle formulation (NCT02336087); and (2) Everolimus + octreotide LAR (NCT02294006) are in progress. IACS-010759, in a phase I study in advanced PanCa (NCT03291938), showed slim therapeutic index with growing dose-limiting toxicities (Table2)[87]. An Food and Drug Administration (FDA)-approved anti-pneumonia and anti-malaria drug, atovaquone blocking mitochondrial ETC (bc1 complex), suppressed proliferation in PanCa cell lines and animal model[74] (Table 1).

Targeting amino acid metabolism

Glutamine (Gln) addicted PanCa cells, when treated with its antagonist, 6-diazo-5-oxo-L-norleucine (DON), diminished self-renewal and metastasis *in vitro*. Sirpiglenastat, a pro-drug of DON singly or in combination with trametinib, blocked PanCa tumor growth in *in vivo* models with prolonged survival[88]. Glutaminase (GLS) suppressors in preclinical studies with PanCa, like: (1) Ss-lapachone; (2) Thiazolidine-2,4-dione derivatives; (3) Bis2- (5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide (BPTES); and (4) BPTES derivatives, compound 968 and telaglenastat (CB-839) showed optimistic results [78]. BPTES encapsulated nanoparticles in singular therapy and in combination with: (1) Metforminand; and (2) ß-

Table 1 Overview of inhibitors of metabolism in pancreatic cancer

Agent	Target	Combined with	Ref.
CG-5	GLUT1	-	[1]
		Gemcitabine	
2-DG	Glycolysis	-	[2]
		Gemcitabine	
3-BP	Glycolysis	-	[3]
BNBZ	HK2	-	[4]
PGMI-004A	PGAM1	-	[4]
KH3	PGAM1	-	[4]
CPI-613	PDH	-	[5]
		Galloflavin	
		Cyano-4-hydroxycinnamic acid	
Rotenone	OXPHOS: Complex I	-	[6]
Metformin			
Phenformin			
IACS-010759			
Atovaquone	OXPHOS: Bc1 complex	-	[7]
N-hydroxyindoles	LDHA	-	[8]
N-hydroxyindole-NHI-1		Gemcitabine	[8]
Galloflavinin	LDHA	Metformin	[9]
Gossypol		-	[10]
FX11		-	[1]
Avasimibe	ACAT1	-	[11]
Luteolin	FASN	-	[12]
C75		-	[11]
Palbociclib		-	[13]
Paclitaxel nano-formulation		-	[14]
Orlistat	FASN	-	[15]
		Gemcitabine	[1]
EGCG	FASN, GLUD1 & PGAM1	-	[1,4]
Lansoprazole	FASN	-	[11]
Pantoprazole			
Rabeprazole			
Omeprazole			
DEAB	ALDH	-	[16]
		Gemcitabine	
Disulfiram	ALDH		[16]
A939572	SCD	-	[17]
CAY10566	SCD	-	[18]
		Gemcitabine	
SB-204990	ACLY	-	[11]
BAY ACC022		-	

TOFA	Fatty acid synthesis	-	[19]
Silibinin	Lipid metabolism	-	[20,21]
Atorvastatin,	HMG-CoA reductase	-	[22]
Lovastatin			
Pravastatin			
Rosuvastatin			
Simvastatin			
Etomoxir	FAO	Carnitine palmitoyl transferase I inhibitor	[11]
Artesunate	Lipid metabolism		[11]
Zalcitabine			
Avasimin	SOAT1		[11]
Opaganib	SK2		[23]
Vitamin D	Lipid metabolism		[24]
DON	Gln	-	[25]
Sirpiglenastat	Gln	-	[25]
		Trametinib	
ss-lapachone	GLS	-	[1]
Thiazolidine-2,4-dione derivatives			
BPTES	GLS	-	[26]
		ß-lapachone (ß-lap)	[27]
968	GLS	-	[26]
CB-839	GLS	-	[26]
		ß-lap	[27]
		BSO	
BPTES encapsulated nanoparticles	GLS	-	[26]
		Metformin	
GPNA	Gln transporter	-	[28]
ASNase	Asparagine	GCN2iA/B	[29]
		PD-325901	
Indoximod	IDO	-	[30]
Carbidopa		-	
Epacadostat	IDO	-	[27]
PEG-ADI	Arginine metabolism	-	[23]
		Radiotherapy	[27]
		Gemcitabine	
		Gemcitabine + docetaxel	
		Panobinostat	
SM-88	Mucin-1 synthesis	-	[23]
AG-270	MAT2A	-	[23]
		Taxane-based chemotherapy	

FASN: Fatty acid synthase; GLUT: Glucose transporter; OXPHOS: Oxidative phosphorylation; PEG-ADI: Pegylated arginine deiminase; SCD: Stearoyl-CoA desaturase; IDO: Indoleamine 2,3-dioxygenase.

Table 2 Overview of inhibitors of metabolism, evaluated through clinical trials in pancreatic cancer

Agent	Target	Combined with	NCT No.
HCQ	Glucose uptake/autophagy	-	NCT01273805
		Gemcitabine	NCT01128296
		Gemcitabine + nab paclitacel	NCT01506973, NCT01978184
		Temuterkib	NCT04386057
		Radiation + capecitabine	NCT01494155
		Chlorphenesin carbamate + mFOLFIRINOX	NCT05083780
		Kinase inhibitors	NCT03825289, NCT05518110, NCT04132505
		Paricalcitol + gemcitabine + nab-paclitaxel	NCT04524702
		Paricalcitol + losartan	NCT05365893
		Leflunomide/bevacizumab	NCT06229340
		CPI-613 + 5-fluorouracil or gemcitabine	NCT05733000
		Paclitaxel protein bound gemcitabine + cisplatin	NCT04669197
2-DG	Glycolysis	Docetaxel	NCT00096707
CPI-613	PDH	mFOLFIRINOX	NCT01835041, NCT03504423
		Gemcitabine + nab-paclitaxel	NCT03435289
Metformin	OXPHOS: Complex I	-	NCT01971034
		Gemcitabine	NCT01210911, NCT02005419
		mFOLFOX-6	NCT01666730
		Stereotactic radiosurgery	NCT02153450
		Rapamycin	NCT02048384
		Gemcitabine + paclitaxel albumin-stabilized nanoparticle formulation	NCT02336087
		Everolimus + octreotide LAR	NCT02294006
IACS-010759	OXPHOS: Complex I	-	NCT03291938
Omeprazole	FASN	-	NCT04930991
Disulfiram	ALDH	Gemcitabine	NCT02671890
Simvastatin	HMG-CoA reductase	Gemcitabine	NCT00944463
		Valproic acid + gemcitabine/nab-paclitaxel-based regimens	NCT05821556
		Metformin + Digoxin	NCT03889795
		Digoxin + Gemcitabine	NCT06030622
		Standard chemotherapy	NCT06241352
Atorvastatin	HMG-CoA reductase	Evolocumab + Ezetimibe	NCT04862260
Opaganib	SK2	-	NCT01488513
Paricalcitol	Lipid metabolism	Liposomal irinotecan + 5-FU/LV	NCT03883919
		Pembrolizumab	NCT03331562
		Gemcitabine + nab-paclitaxel	NCT03520790
ERY-ASP	Asparagine	-	NCT01523808
		Gemcitabine/FOLFOX	NCT02195180, NCT03665441
Indoximod	IDO	Chemotherapy	NCT02077881
Epacadostat		Immunotherapy, chemotherapy + GVAX	NCT03006302, NCT03085914

PEG-ADI	Arginine metabolism	Gemcitabine + nab-paclitaxel	NCT02101580
SM-88	Mucin-1 synthesis	-	NCT04229004
		MPS	NCT03512756

ALDH: Aldehyde dehydrogenase; FASN: Fatty acid synthase; OXPHOS: Oxidative phosphorylation; IDO: Indoleamine 2,3-dioxygenase; PEG-ADI: Pegylated arginine deiminase.

lapachone (ß-lap) suppressed tumor growth in PanCa, with enhanced effect in cocktail[31]. A cocktail of: (1) CB-839 + ß-lap; and (2) CB-839 + γ-glutamylcysteine blocker, L-Buthionine-(S,R)-sulfoximine suppressed tumor growth and increased survival in PDAC mice model[31]. Gln transporter blocker, GPNA also suppressed cell growth in PanCa[88]. Asparagine blocker L-asparaginase (ASNase), in combination with, GCN2iA/B or MEK inhibitor, PD-325901, repressed PanCa in preclinical studies (Table 1). RBC encapsulated ASNase (ERY-ASP) is tolerated well by metastatic PDAC subjects (Phase I, NCT01523808)[40]. ERY-ASP+gemcitabine/FOLFOX furnished enhanced OS and progression-free survival (PFS) in PanCa (phase IIb, NCT02195180 and phase III, NCT03665441) (Table 2). Suppression of metabolism of essential amino-acids like, tryptophan, tyrosine and methionine inhibit PanCa. IDO, associated with tryptophan metabolism, when blocked with indoximod, carbidopa and epacadostat suppress PanCa in animal/cell-line studies[89] (Table 1). Indoximod + chemotherapy is assessed clinically in a phase I/II study (NCT02077881), while, epacadostat, immunotherapy and chemotherapy + GVAX pancreas vaccine in PanCa is under evaluation (NCT03006302 and NCT03085914) (Table 2)[31]. Tyrosine mimetic, racemetyrosine (SM-88) blocks mucin-1 synthesis, resulting in enhanced oxidative stress, and cell-death in PanCa[90] (Table 1). SM-88 + MPS (methoxsalen, phenytoin, sirolimus) was evaluated in a randomized phase II/III multi-center study (NCT03512756) while SM-88 monotherapy in another phase III study (NCT04229004) in metastatic PanCa is underway. Preclinically, methionine adenosyltransferase 2α (MAT2A) blocker, AG-270 in monotherapy and in amalgamation with taxane-based chemotherapy, synergistically suppressed PanCa[90] (Table 2).

In laboratory, pegylated arginine deiminase (PEG-ADI) induced arginine deficiency. PEG-ADI singly or in combination with: (1) Radiotherapy; (2) Gemcitabine; (3) Gemcitabine + docetaxel; and (4) Histone deacetylase inhibitor, panobinostat showed synergistic lethality in ASS1-defficient PanCa cells (Table 1). PEG-ADI coupled with gemcitabine + nab-paclitaxel was endured well by advanced PanCa subjects in a phase I/Ib study (NCT02101580)[31] (Table 2).

Targeting lipid metabolism

Various small molecules like silibinin and ferroptosis inducer: artesunate and zalcitabine hamper lipid metabolism[91-93], and TOFA blocks fatty acid synthesis, ultimately suppressing PanCa cells proliferation and tumor shrinkage[94]. In preclinical studies in PanCa, several rate limiting enzymes of lipid metabolism are targeted: (1) Acetyl-CoA acyl transferase by avasimibe[93]; (2) Fatty acid synthase (FASN) by luteolin[95], palbociclib and, paclitaxel nano-formulation [96,97] FDA approved orlistat[98], and proton-pump blockers: Lansoprazole, pantoprazole omeprazole, and rabeprazole [93]; (3) Stearoyl-CoA desaturase by A939572 and CAY10566[99,100]; (4) ATP citrate lyase by SB-204990 and BAY ACC022 (oral)[93]; (5) Sterol-O-acyl transferase 1 (SOAT1) by avasimin[93]; (6) Sphingosine kinase-2 (SK2) by opaganib (ABC294640)[90]; (7) HMG-CoA reductase by atorvastatin, lovastatin, pravastatin, rosuvastatin, and simvastatin[101]; and (8) aldehyde dehydrogenase (ALDH) by N, N′-diethylaminobenzaldehyde (DEAB), and FDA-approved disulfiram with encouraging outcomes (Table 1). Some of these agents were clinically evaluated in PanCa, like: (1) FASN blocker, omeprazole in a phase I study (NCT04930991); (2) SK2 blocker, opaganib in an open-label, phase I study (NCT01488513) [90]; (3) Aldehyde dehydrogenase inhibitor disulfiram coupled with gemcitabine in a phase 1 trial (NCT02671890) and HMG-CoA reductase suppressor, simvastatin cocktailed with gemcitabine in a randomized double-blinded, phase II trial (NCT00944463)[93]. Few other phase I/Ib/II studies with simvastatin and (1) Valproic acid + gemcitabine/nab-paclitaxel-based regimens (NCT05821556); (2) Metformin + digoxin (NCT03889795); (3) Digoxin + simvastatin + gemcitabine (NCT06030622); and (4) Standard chemotherapy (NCT06241352) in metastatic PDAC are in progress. Another phase I study with atorvastatin + evolocumab + ezetimibe in advanced or metastatic PDAC (NCT04862260) is underway (Table 2).

Interestingly natural compounds like, green tea polyphenol, epigallocatechin-3 gallate (EGCG) targeting FASN, GLUD1 and PGAM1, inhibits tumor growth in PanCa mice model[80] (Table 1). Vitamin D, modifying lipid metabolism shows anti-carcinogenicity in PanCa. Few clinical trials with vitamin D analog paricalcitol, in combination with: (1) Liposomal irinotecan + 5-FU/LV (NCT03883919, phase I); and (2) PD1 inhibitor, pembrolizumab (NCT03331562, phase II) are completed. Additionally, a phase I/II study with paricalcitol + gemcitabine + nab-paclitaxel concoction in metastatic PanCa (NCT03520790) is in progress[102] (Table 2).

Targeting nucleotide metabolism

Uracil analogue, fluorouracil mimicks uracil and gets incorporated into nucleic acids. 5-FU has been approved by the FDA for the treatment of various solid tumors including PanCa. Antineoplastic and antimetabolite floxuridine, a pyrimidine analogue, is metabolized to 5-FU. Floxuridine is incorporated in DNA with high specificity, resulting in inhibition of cell proliferation. Pyrimidine antimetabolite, gemcitabine, a deoxycytidine analogue, after conversion into phosphate metabolites, disrupts DNA synthesis. Gemcitabine primarily kill dividing cells undergoing DNA synthesis (S-phase) and blocks cell cycle progression in G1/S-phase. Gemcitabine is approved by the FDA for PanCa treatment in clinics[103]. Floxuridine, a fluorodeoxyuridine analoge showed promising survival and improved prognosis in a phase II

single agent clinical trials in PanCa[9].

CONCLUSION

Oncogenic metabolic adjustments support quick and uninterrupted growth, proliferation, differentiation, invasion, metastasis, angiogenesis, immune-escape and therapy resistance in PanCa. Tumor cells, CAFs and different immune cells converse with each other mediated through various metabolic cues in extremely despomplastic TME of PanCa. This metabolic symbiosis is associated with therapy resistance Key enzymes of altered metabolic pathways and the intermediates render novel approaches for anti-malignant therapy. These drugs not only suppress malignancy, but also improve chemo- and radiosensitivity in combination therapy. FDA-approved drugs, metformin, statins, omeprazole and aspirin, with multifaceted metabolic roles, are being clinically evaluated for their therapeutic efficacy in PanCa. Glucose metabolism is targeted by 2-DG, CPI-693 in clinical trials. A blend of CPI-613 and mFOLFIRINOX displayed encouraging response (phase I, NCT01835041), though associated with slight enhancement of cytotoxicity and side effects, compared to only mFOLFIRINOX. Asparagine metabolism, targeted with ERY-ASP showed good tolerance and promising results in combination chemotherapy (NCT03665441). Targeting lipid metabolism with disulphirum and paricalcitol has also assessed with encouraging results in PanCa. FDA approved gemcitabine is used as a first line of treatment of PanCa. Regardless of the accomplishments on targeting PanCa metabolism, in some cases, applications are associated with serious side effects, as these drugs target basic metabolic processes, operative in all cell types. Thus, auxiliary components of metabolic pathways should also be targeted. Moreover, small molecules raised against these enzymes often fail to differentiate between various specific subtypes expressed in malignant and nonmalignant cells. Furthermore, specific inhibitors also show limited effectiveness due to extremely heterogeneity and flexibility in PanCa metabolism. Thus to crush PanCa, simultaneous targeting of two metabolic pathways could be a better strategy[50]. Another way-out could be the detection of metabolic checkpoints between healthy and cancerous cells with different genetic aberrations[40] Also, deeper understanding to detect metabolic-hub in PanCa would be of worth. Finer knowledge not only on altered cancer metabolism but also on metabolic cross-talk with various cells in the microenvironment leading to metabolic-symbiosis would provide novel approaches in precise anti-cancer therapy.

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FOOTNOTES

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REVIEW

Autologous blood in the management of ocular surface disorders

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Abstract

Autologous blood therapy has emerged as a promising modality in managing ocular surface disorders. This review provides a comprehensive overview of the current literature regarding the use of autologous blood in ocular surface disorders, encompassing its physiological basis, clinical applications, techniques, challenges, and future perspectives. The ocular surface, comprising the cornea, conjunctiva, and tear film, plays a critical role in maintaining visual function, and its disruption can lead to various pathological conditions. With its rich composition of growth factors, cytokines, and other bioactive molecules, autologous blood offers therapeutic potential in promoting corneal wound healing, reducing inflammation, and improving tear film stability. Clinical studies have demonstrated the efficacy and safety of autologous blood therapy in diverse ocular surface disorders, including persistent epithelial defects, neurotrophic keratopathy, and dry eye disease. However, challenges such as variability in treatment response, adverse effects, and optimal patient selection remain areas of concern. Further

research is needed to elucidate the underlying mechanisms of action, refine treatment protocols, and explore synergistic approaches with other therapeutic modalities. Despite these challenges, autologous blood therapy holds promise as a valuable adjunctive treatment option for ocular surface disorders, offering new avenues for improving patient outcomes and quality of life. This review examines the mechanisms underlying ocular surface disorders while discussing existing autologous blood-based therapies for managing these disorders. Current clinical trials are also summarized, and a comparison between autologous blood therapy and conventional eyedrops is attempted. Finally, safe techniques and protocols for autologous blood medicine are elucidated, and adverse effects and future perspectives of this novel therapy are reviewed.

Key Words: Autologous blood; Ocular surface disorder; Cytokines; Tear film; Dry eye

Core Tip: There are several reviews in the literature about autologous tissue therapy and its usefulness in eye care including stem cells and tissue transplants. Blood derivatives are playing a progressively significant role in management of ocular surface disorders. This paper reviews studies on autologous blood use in ocular surface disorders, its preparation and administration, patient selection and potential side effects. Recent clinical trials are also reviewed and future considerations are discussed.

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INTRODUCTION

Ocular surface diseases (OSD) are disorders plaguing the surface structures of the eyes; namely the conjunctiva, cornea, and tear glands[1]. The most common manifestations of OSDs are dry eyes and blepharoconjunctivitis. OSDs usually present with gritty sensations, redness, mucous or stringy discharge, occasional blurry vision, and excessive tearing. OSDs often lead to visual impairment and consequently reduced quality of living[2]. Although autologous blood medicine has been known for centuries, its use in the care of OSD is relatively novel and has not been extensively reviewed. The authors sought to review all publications on autologous blood used in ocular surface disorders.

Anatomy and physiology of the ocular surface

The ocular surface constitutes the boundary between the most anterior ocular tissues and the external surroundings. It is most anteriorly lined by squamous epithelial cells and protected from foreign objects and desiccation by the reflex shutting of the lids. The eyelids are a door-like structure whose action dictates what enters the eye. The lids cover the anterior eye and divide into the superior and inferior lids, which are continuous with each other and with the skin and muscles of the face at the nasal and lateral canthi. The eyelids are like a hinged double door entrance regulator with hair follicles for eyelashes arranged in two to three rows at the point on the eyelid margin just anterior to the mucocutaneous junction. The mucocutaneous junction can be described as the transition point where the stratified squamous epithelial of the skin is replaced by the cuboidal epithelial cells of the conjunctival[3]. The opening and shutting of the eyelids are enabled by the levator palpebral superioris, and the orbicularis oculi respectively. The orbicularis oculi inserts posterior to the subcutaneous tissue of the eyelid and anterior to the tarsal plate. The meibomian gland has its origin in the tarsal plate. It has its excretory ducts running through the tarsal plate in an imperfect straight line-like manner and end up as orifices at the lid margin *via* which its secretions (meibum) are discharged. The meibomian gland secretes the outermost layer of the tear film, and there are approximately 40 to 50 meibomian glands in the upper lid and 20 to 30 meibomian glands in the lower lid[4]. The glands of Moll and Zeis are both exocrine glands that maintain the health of the lids and lashes. The Zeis gland is a unilobar sebaceous gland, located at the anterior aspect of the eyelash base in the lid margin, while the Moll gland is a modified apocrine sweat gland located at the posterior aspect of the base of the cilia[5].

The conjunctiva is a translucent, well-vascularised mucous membrane that extends from the mucocutaneous junction at the lid margins and is continuous throughout the ocular surface until its epithelium becomes continuous with the cornea epithelium at the corneoscleral junction. As it extends from the mucocutaneous junction, it is named according to the part of the ocular surface it covers namely; the palpebral, fornix, and bulbar conjunctiva. The palpebral conjunctiva is firmly attached to the underlying tarsal plate while the bulbar conjunctival is loosely connected to the tenons capsule which links the conjunctival to the underlying sclera[5]. At the nasal canthus, the bulbar conjunctival makes a small crescent fold known as the plica semilunaris, and just nasal to it, is a small, mildly elevated, pink-yellowish nodule that consists of a mixture of tissues called the caruncle or caruncular lacrimalis. Histologically, the conjunctival epithelium is three to five cells thick and its basal cells are made up of cuboidal cells which subsequently become polyhedral flattened

cells as they ascend more superficially[5]. The conjunctival epithelium consists of non-keratinized stratified squamous epithelia and stratified columnar cells. Goblet cells are scattered throughout the epithelium, having their greatest density in the inferonasal aspect of the conjunctival epithelium[6]. The conjunctival stromal or subtantia propria which is the deeper part of the conjunctiva consists of a more superficial adenoid layer and a deep fibrous layer, and the accessory lacrimal glands of Krause and Wolfring are located in the deeper fibrous layer. Numerous immune cells such as monocytes, neutrophils, plasma cells, and T and B lymphocytes are present within the conjunctival. However, blood vessels and lymph channels that empty into the pre-auricular and submandibular lymph nodes are found in the epithelium. While the deeper layer of the conjunctival stromal contains nerve supply and deeper blood vessels.

The conjunctival epithelium becomes continuous with cornea epithelial cells at the limbus. Stellate symmetric ridges found at the limbus form the palisades of Vogt, which is considered a reservoir for cornea stem cells[7]. The cornea is a transparent, absolute avascular, curved refractive tissue. The avascularity of the cornea is maintained by several factors including the absence of blood vessels, the manner of stromal fibrillar arrangement, and the cornea endothelium pump action. This 'no-blood' vessel status is necessary to maintain its transparency and refractive status. The cornea can further be described as the refractive powerhouse of the eye. It possesses an average refractive power of 43.00 DS, constituting about 75% of the total refractive power of the eye[8]. The cornea is divided into five layers namely; the epithelium, bowman's membrane, stromal, descemet membrane, and endothelium.

The cornea epithelium is about 50 μm thick and consists of five to seven layers of cells. The outermost layer of the cornea epithelium is made up of non-keratinized squamous epithelial cells. The cells of the cornea epithelial surface regulate the movement of substances into the cornea through the tears. These cells are attached at their lateral walls, close to the surface of the cell facing the lumen by tight junctions also known as zonular occludens[9]. This barrier complex forms an effective semi-permeable membrane that allows certain substrates through into the cornea. Furthermore, the surface epithelial cells produce a thick glue-like transcellular barrier known as glycocalyx; which helps to adjoin the mucin layer of the tears to the cornea epithelial surface[10]. Moreover, the surface epithelial cells possess finger-like (microvilli) and ridge-like (microplicae) projections which aid in fastening the precorneal tear film to the cornea epithelial surface. The middle layer of the cornea epithelium consists of polyhedral-shaped wing cells which are attached each to other by gap junctions and desmosomes, and to the underlying germinal basal layer by desmosomes. The basal layer of the corneal epithelium consists of a single layer of columnar cells. The basal layer is where mitosis occurs, thus, older basal cells move up to the middle layer to become rhombic-like wing cells and subsequently to the epithelial surface where they are finally sloughed off due to aging or epithelial erosion.

The pre-cornea tear film is three-layered namely; the lipid layer, the aqueous layer, and the mucin layer. The tear film is coated anteriorly by the secretion from the meibomian gland. This layer of the tear film ensures that the integrity of the precorneal tear film remains intact. The aqueous aspect of the tear film is produced chiefly by the lacrimal gland with additions from the accessory lacrimal glands. The innermost layer which adjoins the precorneal tear film to the cornea surface epithelium is produced by the goblet cells scattered throughout the conjunctival epithelium.

The anatomical orientation of the ocular surface structures is designed in such a way that confers on the globe the ability to protect, regulate entrance, lubricate, and refract light rays. The eyelids shut against invading foreign objects consequently, fortifying the eye from foreign entities which could pose significant injury and health risks to the globe. The precorneal tear film is a mixture consisting of epitheliotrophic factors[11]. In patients with ocular surface disorders, the keratinocyte morphology and function are affected, making the use of tear substitutes inadequate in addressing the problem of OSDs[12,13].

Composition of blood components

Blood is a multifaceted fluid composed of several elements that collaborate to maintain homeostasis and support various physiological functions. The main components of blood are red blood cells, white blood cells, platelets, and plasma. Red blood cells, or erythrocytes, are the most prevalent cellular element, responsible for carrying oxygen from the lungs to body tissues and aiding in the removal of carbon dioxide. White blood cells, or leukocytes, are essential for the immune response, protecting the body against pathogens and foreign invaders. Platelets, or thrombocytes, are small cell fragments that facilitate blood clotting and wound healing. Plasma, the liquid part of blood, acts as a medium for transporting nutrients, hormones, and waste products, and helps maintain electrolyte balance and pH levels[14]. Each component of blood is essential for overall health and well-being, contributing to the body's ability to function properly and respond to various challenges. Numerous studies have extensively explored the functions and characteristics of these blood components, highlighting their importance in health and disease[15-17].

Mechanisms underlying ocular surface disorders

The various pathways through which the diseases of the ocular surface structures exert their pathological changes on the ocular surface are dependent on the type and nature of ocular morbidity, severity, and the ocular surface structure involved. However, these pathogenic processes are often initiated secondary to the breakdown of the ocular surface defense system offered by the tear film. The breakdown of the precorneal tear film integrity can result from cellular infiltration of the serous glands[18], fibrosis of the serous glands[19], prolonged and subsequently auto-sustained inflammatory reaction of the ocular surface from activated acquired immunity as well as conjunctival epithelia metaplasia[20,21]. Other etiologic factors include iatrogenic factors[22], graft *versus* host disease, chronic meibomian dysfunction, trauma, hormonal and nutritional deficiencies, and drugs.

This review investigates the mechanisms that cause ocular surface abnormalities and explores the current autologous blood-based therapies used to treat these problems. The present study provides a concise overview of ongoing clinical trials, aiming to compare the effectiveness of autologous blood therapy with traditional eyedrops. The text provides a comprehensive explanation of the safe processes and protocols used in autologous blood medicine. It also examines the

potential adverse effects and future prospects of this innovative therapy.

METHODOLOGY

Only full-length papers published in English language were used for this review. The PubMed (https://pubmed.ncbi.nlm.nih.gov) database was queried using the keywords "autologous blood ocular surface disease", generating the following search algorithm; ("autolog"[All Fields] OR "autologeous"[All Fields] OR "autologic"[All Fields] OR "autological"[All Fields] OR "autologous"[All Fields] OR "autologously"[All Fields]) AND ("blood"[MeSH Subheading] OR "blood"[All Fields] OR "blood"[MeSH Terms] OR "bloods"[All Fields] OR "haematology"[All Fields] OR "hematology"[MeSH Terms] OR "hematology"[All Fields] OR "haematoma"[All Fields] OR "hematoma"[MeSH Terms] OR "hematoma"[All Fields] OR "haemorrhage"[All Fields] OR "hemorrhage"[MeSH Terms] OR "hemorrhage"[All Fields] OR "haemorrhages"[All Fields] OR "hemorrhages"[All Fields] OR "haemorrhagic"[All Fields] OR "haemorrhaging"[All Fields] OR "hematologies"[All Fields] OR "haematomas"[All Fields] OR "hematomas"[All Fields] OR "hematoma s"[All Fields] OR "hematomae"[All Fields] OR "hemorrhaged"[All Fields] OR "hemorrhagic"[All Fields] OR "hemorrhagical"[All Fields] OR "hemorrhaging"[All Fields]) AND ("ocul surf"[Journal] OR ("ocular"[All Fields] AND "surface"[All Fields]) OR "ocular surface"[All Fields]) AND ("disease"[MeSH Terms] OR "disease"[All Fields] OR "diseases"[All Fields] OR "disease s"[All Fields] OR "diseased"[All Fields]). All publications retrieved were screened for relevance and eligibility by three authors (Suleman A, Aluyi-Osa G, and Musa M). A total of 169 publications were retrieved. 2 records were excluded for not having abstracts while 11 were out of scope.

Study selection

Three authors examined the extracted results for uniqueness, relevance to the topic, language, and content. Where one of the authors flagged a study as out of scope, the other reviewer was allowed to re-scrutinize before discarding the publication. There was no duration restriction on papers, but only papers in English language were included.

Results

Hence 153 manuscripts were included in this paper as shown in the PRISMA[23] diagram below. The guidelines of the PRISMA 2020 statement have been followed in this study (Figure 1).

MECHANISM OF ACTION

The mechanism of action of autologous blood is the promotion of cellular differentiation, proliferation, and migration of these cells due to the presence of very metabolically active substances such as growth factors, immunoglobulins, fibronectin, vitamins A, and anti-proteases[24-27]. Autologous blood therapy also supplies cytokines and growth factors that are found lacking in natural[28]. Autologous serum has been used in the development of cornea and oral epithelial cells making the case for its role in the proliferation of epithelial cells[29,30].

Autologous blood is usually used to treat persistent cornea defects and dry eyes. Still, it has also been shown to be effective in the management of superior limbic keratoconjunctivitis, recurrent erosion syndrome, aniridic keratopathy neurotrophic keratopathy, graft *versus* host disease, ocular cicatricial pemphigoid, Mooren's ulcer, filtering bleb post trabeculotomy and post-keratorefractive surgery[31,32]. In dry eyes the epitheliotrophic factors (fibronectin, growth factors, and vitamins) are reduced hence leaving the ocular surface at risk of infections, reduced optical properties, and persistent epithelial defect.

Shtein *et al*[33] in their review of 10 studies of the effect of autologous serum-based eyedrops on dry eyes and 4 studies of the effect on persistent epithelial defect showed that the majority of the studies reported a great improvement in symptoms, signs, or both. Unpreserved blood serum has shown similar properties to the tears hence the use of autologous blood serum in treating OSD[34,35]. The tears in basic terms is properly filtered blood[36]. In challenging and recalcitrant cases autologous blood has been used to reduce symptoms[37]. Thermally treated immunosafe autologous blood containing plasma rich in growth factors (PRGF) and eyedrops enriched in autologous growth factors have been shown to have promising results in treating cicatrizing conjunctivitis, and Sjogren syndrome[38-41].

Higuchi[42] showed a new serum protein selenoprotein which proved to be more efficacious in the treatment of dry eye by showing a better cornea fluorescein score and reduced cornea oxidative stress in eyes when it was applied compared to phosphate-buffered saline. Studies showed that when autologous blood is used as a therapy for dry eyes, there is a resulting improvement in tear stability, as seen by better staining scores under the slip lamp corneal examination[43,44]. Some reports showed that 20% autologous serum is effective in the management of epitheliopathy caused by antiglaucoma eyedrops[45,46]. Anitua *et al*[47] in their study, proved that PRGF improved the ocular surface wound healing and reduced scar formation better compared to autologous serum or insulin therapy. Autologous blood therapy has been shown to reduce the expression of mucin5AC (tumor-associated mucin) in the cornea amongst patients with limbal stem-cell deficiency and improve patient symptomatic perception, especially in those whose cornea was mildly affected before treatment initiation[48].

López-García *et al*[49] in a prospective study, showed improvement in ocular symptoms in patients with keratopathy associated with aniridia when autologous serum eyedrops were used. Autologous blood serum has also been found effective in treating neurotrophic keratopathy, resulting in epithelial healing. This led to the belief that autologous blood

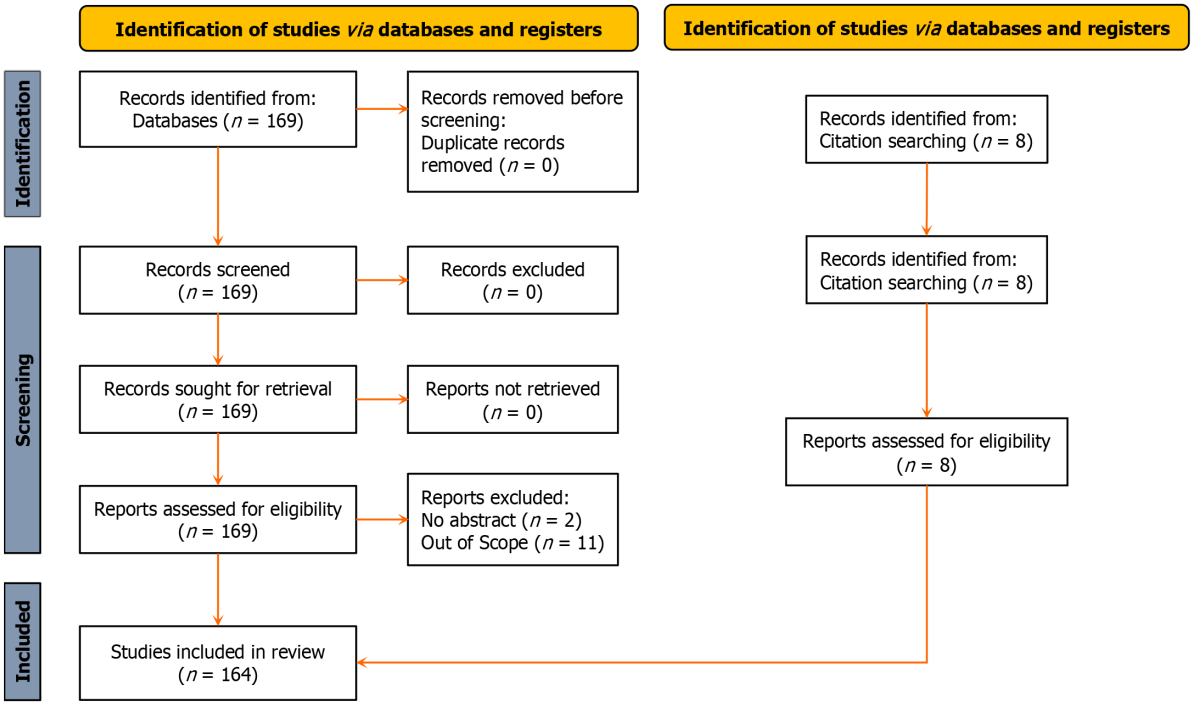

Figure 1 Selection of publications used for the review.

contains neurotrophic properties[50]. *In vivo* confocal microscopy has revealed early evidence that autologous plasma therapy can improve cornea nerve regeneration, this is beneficial in patients with neurotrophic keratopathy[51]. Mucin serum and retinoic acid are important components in the maintenance of healthy ocular surface[52]. In their work, Alio *et al*[22] found out that autologous serum eyedrops are effective management plan for post-lasik associated keratopathy. Patients who have undergone a corneal transplant are at risk of significant ocular morbidity in the healing phase[53-55]. They reported an amelioration of symptoms with this novel therapy. In patients with systemic autoimmune diseases that present with dry eye diseases, autologous serum therapy has been shown to improve both subjective and objective findings[56-59]. In patients with recurrent epithelial defects, autologous blood serum has been found to be useful when combined with contact lenses[60].

CLINICAL AND RESEARCH APPLICATIONS

Merayo-Lloves *et al*[61] and Anitua *et al*[62] in their retrospective cohort study, showed that PRGFs could be a safe and effective treatment of refractory OSD. The safety profile of autologous blood therapy in a hospital setting was proven true under strict protocol of storage and preparation[63]. In cases of ocular burn, autologous serum tears derived from the umbilical cord are more effective than artificial tears, as seen in the double-blind prospective randomized clinical control research by Kumar *et al*[64] and similar studies[65,66]. Edelmann *et al*[67] in a clinical trial carried out amongst dogs with spontaneous chronic cornea epithelial defects post epithelial debridement with diamond-burr showed no significant statistical difference in epithelization when using platelet-rich plasma or artificial tears. Mircheff *et al*[68] and Fea *et al*[69] in their clinical studies using *in vivo* confocal microscopy, confirmed that autologous platelet lysate eyedrops improved the ocular surface disease indices in patients with primary Sjogren syndrome. Other researchers have gone as far as recommending the autologous blood is adopted as a standard of care therapy for ocular surface wound healing[70]. Studies have been carried out to ascertain the effectiveness of platelet-rich fibrin derivative as compared to the human amniotic membrane, they found out that platelet-rich fibrin had more efficacy, probably due to its anatomical and chemical makeup[71-74]. Noble *et al*[75] in their randomized controlled trial, showed that autologous blood therapy (ABT) proved beneficial to patients with severe ocular surface disorders, as most patients reported improvement in symptoms.

Urzua *et al*[76] in a randomized double-blind clinical trial, proved that autologous serum in the short term provided better symptomatic relief in comparison to conventional artificial tears. Comparison of the autologous serum and plasma-rich protein in terms of epitheliotrophic properties showed negligible difference in baseline conditions. Platelet rich plasma is usually easier to prepare hence it is a viable substitute for autologous serum[77,78]. Rawat *et al*[79] showed that autologous platelet rich plasma was more effective in the management of moderate to severe OSDs. Platelet rich plasma lysate have also been indicated in the management of OSDs[80,81] In comparison with artificial tear substitutes, there is low evidence of their efficacy in managing dry eye diseases[82]. Randomized controlled trials have shown that there is a better improvement in OSDI scores when autologous blood therapy is used compared to artificial tears[83,84]. The effect of amniotic membrane suspension and autologous serum varies from individual to individual, depending on individual

growth factor concentration[85-88]. Semeraro *et al*[89] in their clinical trial, showed that 50% blood serum improved the signs and symptoms of patients formally treated by conventional therapy. Zheng and Zhu[90] also suggested autologous serum eyedrops to be more effective than artificial tears in treating complaints associated with moderate to very severe dry eyes, and might therefore be a better option.

Factors influencing patient selection include: (1) The severity of the ocular surface disorder: Patients with moderate to severe OSDs who didn't respond well to conventional therapy could benefit from autologous blood therapy; (2) Certain types of dry eye disease that other treatments may not have helped such as dry eyes secondary to meibomian gland dysfunction or aqueous deficiency; (3) Cornea epithelial damage, recurrent erosion of the cornea, or persistent defect of the epithelial despite the use of standard therapy; (4) Patients with an inflammatory component to OSD could benefit from ABT due to the anti-inflammatory properties present in autologous blood; (5) Patients with blood diseases who have contraindications to blood collection are not recommended for ABT; (6) The patient's willingness to undergo the procedure involved in ABT[91]; (7) Techniques and protocols for autologous blood preparation and administration; (8) Blood component differentiation and separation was pioneered in 1960[92]. While there are several ways blood can be collected for ocular therapy, two major routes are; (9) Fingerprick method; here blood is collected by pricking the fingers and the blood collected is applied on the lower fornix[93]. This method has been advocated by several authors to be efficacious especially in the absence of serum[94-96]; and (10) Blood collection *via* the arm; here the blood is collected directly from the arm of the patient usually consisting of plasma and platelet concentrates after preparation from the initial peripheral blood[97].

Routes of administration

Generally, the route of administration of ABT is topical, applying the serum directly on the ocular surface is usually done frequently to improve discomfort experienced by the patient[98]. The amount of times it would be applied depends on the degree of discomfort and symptoms experienced by patients[99,100]. In some studies, they confirmed that the sub-conjunctival surface can also be a route of application of autologous blood concentrate in the treatment of ocular burn, and this might even be a more economical and practical technique[101-103].

Adverse effects and complications

Success has been reported in most of the cases in published literature, with a few rare complications[104]. The limitations of ABT are the cost and inconvenience of obtaining blood-derived products[105,106], and the paucity of data with strong evidence from randomized double-masked clinical studies supporting its use[107-110]. Transmission of blood-borne diseases is possible if autologous serum is not aseptically acquired[111]. The lack of standardized protocol for blood collection, centrifugation, and other things needed makes it difficult to attain clinical use of autologous blood serum[112, 113]. One major challenge faced during outpatient therapy, is usually based on good manufacturing and delivery medium from the point of production to centers these autologous blood serums are finally utilized[114-116]. In line with this challenge of storage of autologous serum, studies suggest that autologous serum can be stored for about 6 months in freezer conditions, without alteration in potency, and still be used to attain re-epithelization in cases of ocular surface anomaly[117-120]. More studies have to be done on the effect of different levels of immunosuppressives on ocular surface integrity and symptomatic relief[121].

Variability in treatment response

There seems to be no significant difference in tear osmolality in patients with dry eye disease and those without dry eye disease who underwent autologous serum therapy[122]. Mesenchymal stem cell and limbal stem cell-derived autologous serum have been shown to have similar potency in treating OSD, as seen in the work carried out by Campbell *et al*[123]. Amniotic assisted conjunctival epithelial redirection, proves to be viable in reducing complications associated with the sequential sector conjunctiva epitheliopathy[124]. In some retrospective comparative case series, epithelial cells obtained from oral mucosa, are capable of staying on the cornea following transplantation, and maintain good corneal surface integrity[125]. Autologous serum eyedrops and allogenic serum eyedrops are almost equally effective in ameliorating symptoms and improving quality of life[126-129].

Integration with other treatment modalities

Umbilical cord blood or serum has been shown to contain a lot of biologically active chemicals that can nourish the ocular surface thus treating OSDs[130]. Hassan *et al*[94] in their feasibility studies, showed that autologous blood obtained from pricking the finger can be used as an adjunct to conventional medical therapy to improve the OSDI score.

Allogeneic blood therapy has also been shown to improve epitheliotrophic properties of the ocular surface in patients with OSDs similar to autologous blood therapy[131]. Calf blood extract gel has been shown to relieve dry eye symptoms in patients with chronic graft *versus* host disease due to its ability to promote healing and regeneration of epithelial cells [131,132]. Autologous blood therapy when used with punctal plugs has been shown to improve symptoms and signs in patients with chronic graft *versus* host disease refractory to artificial tears[133]. Autologous serum eyedrops obtained post-plasmapheresis and IV immunoglobulin infusion have been said to help manage patients with ocular complications secondary to toxic epidermal necrolysis[134].

Studies have isolated tumor necrosis factor as an inflammatory marker for dry eyes[135]. Tumor necrosis factor-inhibiting protein (a component of autologous blood lysate) has been found to help suppress signs related to Sjogren syndrome, by affecting histopathology of the lacrimal gland but having no effect on the conjunctiva tissues[136-139]. De-proteinized calf blood extract has also been reported to reduce the recovery time of patients who underwent pterygium excision, even when compared to the use of non-steroidal anti-inflammatory drugs, or even ocular lubricant[140]. The use

of autologous serum in cases of severe reduction in limbal stem cells secondary to contact lens used has been found to substitute for the need for surgical intervention, especially if attended to on time and aggressively[141,142]. It is important to note that prompt ophthalmic evaluation and an interdisciplinary approach to care are very important, especially in cases where transplantation of tissue is involved, in other to prevent progressive and subsequent vision deterioration[19].

FUTURE PERSPECTIVES AND DIRECTIONS

The use of autologous blood in the management of OSDs is affected by financial and logistic barriers[143]. The lack of proper regulation on the use of autologous blood serum in the eye care world makes its widespread use in the management of ocular surface disorders controversial[144]. There is limited information on the side effects of serum-based eyedrops, and modalities for monitoring need to be put in place if used should be to a large extent generalized for the treatment of OSD[145-147]. It is important to ensure strict monitoring of patients using autologous serum eyedrops that are not preserved, in other to avoid infection in the long run[148]. A standard protocol for the formulation should be adopted[149].

Potential advancements in autologous blood therapy and emerging research trends

Autologous serum-impregnated soft bandage contact lens shows promising results in the treatment of dye eyes stemming from autoimmune conditions like Sjogren syndrome[150]. Ang *et al*[151] showed that autologous serum-derived oral epithelial cells can be used for treating severe OSDs. Studies have also confirmed a dose-dependent effect of autologous serum on outcomes in dry eye diseases[152,153]. The use of isolated stem cells, through clonal analysis, has been found to also be effective in the treatment of corneal defects due to its ability to regenerate corneal tissue[154]. Some evidence also points to the effectiveness of complete autologous submandibular gland replacement, as it has been shown to have a good outcome, relieve symptoms, and improve quality of life[155-157]. Autologous tear serum has been found to affect the corneal nerve plexus as seen using *in-vivo* confocal microscopy[158,159]. Chiang *et al*[160] reported on a case series of patients treated with allogenic tear serum after suffering graft *versus* host disease. The use of corneal sheets mounted on platelet-poor plasma is an emerging trend in cases of limbal stem cell deficiency, and it helps in the treatment of bilateral ocular surface disorders[161-163]. Recent research has also been directed toward hemoderivatives including the platelets; associated with the platelets are the growth factors that are involved in the wound-healing process of the cornea and the conjunctiva[164].

The use of Xeno-feeder-free limbal stem cells in the treatment of ocular surface disorders is on its way, as it might be a potential substitute imminently, so more studies are being carried out in this regard[165]. Multiple research publications have found that a novel treatment (platelet-rich plasma) could ameliorate persistent epithelial defects and attributed it to the fact that it contains a lot of epidermal growth factor[166,167]. By the process of inducing the presence of anti-inflammatory mediators, dry eyes stemming from autoimmune conditions can be alleviated and this is a possible area of interest in the near future[168,169].

Considerations of long-term use of autologous blood therapy for ocular conditions

Wiącek *et al*[170] examined the long-term effects of autologous blood therapy in the management of retinitis pigmentosa by monitoring key indices at one month and then every quarter leading up to a year. They reported the therapy to be safe and efficacious over the one-year period. Lee and Chen[171] also evaluated the safety profile of long-term autologous blood therapy in managing chronic dry eye. They reported no significant complications even up to 17 months of constant use among a section of the cohort. Platelet-lysate drops, an autologous blood derivative, have also been reported to have long-term usefulness in the management of ocular graft *versus* host disease[172].

Limitations and challenges

While some smaller-scale data exists, the authors could not find studies on large-scale or multicenter experimental studies comparing autologous blood therapy to conventional drugs.

CONCLUSION

Autologous blood therapy in ocular surface disorder management presents an interesting proposition to the eye care industry. Autologous blood serum has successfully been used to manage many ocular surface disorders arising primarily secondary to other conditions and surgeries. The results of this review underscore the potential of autologous blood therapy to enhance patient outcomes in ocular surface disorders. Implementing this therapy in clinical practice could enhance healing, reduce inflammation, and improve the overall management of these disorders. It combines well with conventional medications and compares favorably with them. While it's widespread use is still hampered by regulations and the absence of a unanimous guideline for preparation and prescription.

FOOTNOTES

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REVIEW

Facing stress and inflammation: From the cell to the planet

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Abstract

As identified in 1936 by Hans Selye, stress is shaping diseases through the induction of inflammation. But inflammation display some yin yang properties. On one hand inflammation is merging with the innate immune response aimed to fight infectious or sterile insults, on the other hand inflammation favors chronic physical or psychological disorders. Nature has equipped the cells, the organs, and the individuals with mediators and mechanisms that allow them to deal with stress, and even a good stress (eustress) has been associated with homeostasis. Likewise, societies and the planet are exposed to stressful settings, but wars and global warming suggest that the regulatory mechanisms are poorly efficient. In this review we list some inducers of the physiological stress, psychologic stress, societal stress, and planetary stress, and mention some of the great number of parameters which affect and modulate the response to stress and render it different from an individual to another, from the cellular level to the societal one. The cell, the organ, the individual, the society, and the planet share many stressors of which the consequences are extremely interconnected ending in the domino effect and the butterfly effect.

Key Words: Climate change; Cytokines; Genetic diversity; Global health; Immunity; Microplastics; Resilience; Yin yang

Core Tip: Global health is dependent on healthy cells, healthy organs, healthy individuals, within a healthy society on a healthy planet. But all components are exposed to some specific stress that generates an inflammatory response, which affects physical and mental health of the planet inhabitants, while pollution and climate change affect the planet health.

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INTRODUCTION

Recently, a fascinating lead article and two accompanying papers[1-3] offered a contemporary reflection on the globalization of inflammation. While the word "inflammation" includes the word "flame", it is derived from Latin "*inflammatio*" meaning to set fire, and is used from the 14th century to address irritation and anger. The flames that wipe out millions of hectares of forests on all continents these recent years illustrate how the planet is suffering from the global warming and from deforestation achieved by humans. On the other hand, the human societies are also in great suffering. Wars, famines, pandemics while recurrent through the millenniums, have been particularly affecting this early 21st century. The stress linked to these events affects humans, their organs, their cells ending to an altered biological and psychological health. These last decades, a better understanding of diseases ended to link stress, chronic inflammatory insults and sicknesses. The aim of this paper is to offer an additional guise, including historical perspectives, to review most parameters that affect individual and global inflammation and to discuss the regulatory mechanisms.

FROM A PATHOLOGICAL TO A PHYSIOLOGICAL RESPONSE

Inflammation has accompanied humans since their first ancestors appeared on Earth. Aulus Cornelius Celsus [25 before Christ (BC) to 50 *Anno Domini* (AD)], a Roman encyclopedist, offered a still valid statement about inflammation: "Notae vero inflammationis sunt quatuor: Rubor et tumor cum calore et dolore", defining the four cardinal signs of inflammation as redness and swelling with heat and pain. A 5th element, the loss of organ function was later added. While it was claimed to have been conceived by Galen (129 AD to 216 AD), a careful analysis of his texts led to the conclusion that it was not the case[4]. Thus, either Thomas Sydenham (1624-1689), an English physician or Rudolf Virchow (1821-1902), a German physician could have proposed the concept. While inflammation has long been considered as a morbid phenomenon, John Hunter (1728-1793) a Scottish surgeon, and Elie Metchnikoff (1845-1916) a Russian polymath, understood that it was a natural and beneficial event that aims to address a sterile or an infectious insult[5]. In his "treatise on the blood, inflammation and gun-shot wounds" (1794), John Hunter stated "Inflammation in itself is not to be considered as a disease, but as a salutary operation, consequent either to some violence or some disease". One century later, Elie Metchnikoff in his "Lectures on the Comparative Pathology of Inflammation" (1891), considered that "Phagocytes are considered as participant of inflammation, and inflammation is no longer seen only as being deleterious"[6]. Not only did he define inflammation, but he also pioneered the study of the effects of microbiota on health and aging, coining the word gerontology.

Two more recent contributions were mentioned by Ghezzi[2]: (1) Rothman[7] who bridged causes and diseases, and most importantly evoked the concept of synergy, an event that is particularly relevant to the innate immune response and the inflammatory process; and (2) Goh *et al*[8] who analyzed the "diseasome" (a word coined by Barabási[9]), defined as a network of all known diseases associated with a network of gene mutations. Later in this review we will address the important contribution of genetic components in the sensitivity to stress and in the nature of the inflammatory response. Among the diseases with a genetic component[10] and an inflammatory link[11] is obesity of which a recent local pandemic has been recognized[12]. Obesity has been recognized as a comorbidity factor that contributed to higher risk of mortality during the corona virus disease 2019 (COVID-19) crisis[13].

A TRIBUTE TO HANS SELYE

Hans Selye (1907-1982), a Hungarian-Canadian medical endocrinologist, was born in Vienna. He got his degree in medicine in Prague and worked at John Hopkins University (Baltimore) before joining McGill University (Montréal). He pioneered the field of stress research in biology with his first article published in 1936 making a link between stress and disorders[14]. He also published numerous books including: "The physiology and pathology of exposure to stress-A treatise based on the concept of general-adaptation syndrome and the diseases of adaptation" (1950) and "The Stress of Life" (1956). In 1974, he introduced the terms distress and eustress to distinguish between bad and good stress. His famous statement "It's not stress that kills us, it is our reaction to it" remains fully relevant. This sentence can be related to that attributed to William Osler (1849-1919) a Canadian physician who had also worked at McGill University before joining the University of Pennsylvania in Philadelphia, and the John Hopkins hospital. About sepsis, William Osler would have claimed: "Except on few occasions, the patient appears to die from the body's response to infection rather than from it". But this statement is absent from his writings! Hans Selye precised: "What is the cause of our illness, the microbe or the stress? I think both are and equally so. In most instance diseases is due neither to the germ as such, nor to our adaptive reactions as such, but to the inadequacy of our reaction against the germ". To illustrate how Selye was a predecessor, let us quote Selye (from his book "The stress of life", 1956-1978): "If we compare inflammation with the defensive reactions of a whole human being, or even of a whole nation, we find striking similarities in the over-all pattern

everywhere. By recognizing these, we may gain more insight into the mechanism, and even philosophy, of defense in general, insight which penetrates far beyond the confines of medicine. In all these examples of reaction, whether we deal with the problems of a few cells, with those of a whole man, or of an entire nation-defense may bring salvation, or it may bring self-inflicted injuries" (Figure 1).

Among other pioneers, Androulakis[3] also appropriately cites the works of Walter Bradford Cannon (1871-1945), a physiologist from Harvard Medical School. Cannon further developed the idea of "milieu intérieur", initially introduced by Claude Bernard (1813-1878), as a pillar of homeostasis. Interestingly, he coined the term "fight or flight response" to which Vodovotz *et al*[1] are regularly referring to. Indeed, dealing with stress requires adjustments of homeostatic set points and adaptation of the milieu intérieur concept.

AN INFLAMMATORY WORLD … MILLIONS OF DEATHS

Cell death

In most severe cases of inflammation such as sepsis, the overwhelmed immune system may end to organ failure and death of the patient. The inflammatory cascade initiated by an infectious process, the release of pathogen associated molecular patterns and damage associated molecular patterns may end to cell death[15]. Many types of cell death have been recognized, such as netosis, a kind of suicide of neutrophils aimed to prevent bacterial infection. Pyroptosis, is a macrophage cell death following gasdermin activation being associated with the inflammasome activation aimed to release, interleukin (IL)-1β and IL-18, two key players in the fight against the microbial pathogens. Apoptosis of immune cells is a hallmark of sepsis and is responsible for lymphopenia and altered immune status. Finally, necrosis occurs in tissue like during necrotizing fasciitis after the action of cytotoxic toxins. It is accompanied by the release the cytoplasmic content of the cells into extracellular space causing inflammatory reactions in the surrounding tissue. In a recent investigation in a murine model of severe influenza A virus infection, it was elegantly demonstrated that preventing alveolar epithelial cell death by an inhibitor of receptor interacting protein kinase 3 prevented the lung injury and protected the animal from death[16].

21st century COVID-19 pandemic and many others

An estimated 14.83 million excess deaths have been attributed to the COVID-19 pandemic between 2020 and 2021[17]. Fortunately, the vaccines against the severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2) virus developed in record time are estimated to have averted 19.8 million additional deaths[18]. An estimation performed at the European region ended with more modest values[19]: COVID-19 vaccines ended to 1.6 million lives saved in those aged 25 years or older: 96% of lives saved were aged 60 years or older and 52% were aged 80 years or older. But pandemics have always accompanied humanity. The first known pandemic ended to the death of 70% of those who were living in Tel El-Amarna around 1350 BC in Egypt, and was due to malaria. The plague of Athens killed around 100000 people in 430 BC, probably because of typhus. The Justinian plague took the life of 25 million people around the Mediterranean Sea (541 AC). It was due to bubonic plague which had its greatest score in 1331-1353 with around 75-200 million victims during the black plague over Europe, Asia and Africa. Plague was regularly taking the life of inhabitants in major cities such as London (1664-1666), Vienna (1679), Marseille (1720), and Hong Kong (1894). Cholera also contributed to the deaths of many citizens in London (1849-1854), Paris (1876), Alexandria (1883), Calcutta (1884), Constantinople (1893), Constantine (1906). In 1918-1919, the Spanish flu affected 33% of the world population ending to the deaths of 25 to 100 million people, including the grand-father of the former president of United States, Donald Trump. Finally, acquired immunodeficiency syndrome pandemic which started in 1981 has been responsible for the death of 40.4 million people according to World Health Organization, more than half being African heterosexuals[20].

A complex interplay exists between natural events occurring on Earth and humans. An association between phases of climate change and episodes of acute health crisis has been recently proposed to explain the occurrence of the major pandemic events of Roman antiquity. The Antonine plague [onset approximately 165 common era (CE)], and the plague of cyprian (onset approximately 251 CE), are strongly associated with pronounced climate change. The Antonine Plague occurred during the cold pulse between approximately 160 and approximately 180 CE that followed several decades of trends toward cooling and aridity. The plague of cyprian coincides with a second phase of severe cooling, with even more arid conditions, after the brief warmer period between approximately 215 and approximately 245 CE. The pronounced cold phases are associated with pandemic disease, suggesting that climate stress interacted with social and biological variables[21]. Eruptions of Lipari volcanoes could have been the culprits of these cooling periods. This illustrates the inter connection between environmental stress and biological stress.

Climate changes

In ten years, the Earth lost 82 million hectares of forests in fires. Globally, boreal forests have the highest proportion of forest loss due to fire (69%-73%), followed by subtropical (19%-22%), temperate (17%-21%), and tropical forests (6%-9%) [22]. The death of all these trees has most severe potential impacts on biodiversity and animal survival. For example, in Amazonia, fires have comprised numerous species, including 83-85 bird species, 53-55 mammal species, 5-9 reptile species and 95-107 amphibian species[23]. Drought, storms and extreme temperatures also have major consequences on human health and are responsible for numerous deaths among the human population[24]. Most remarkably, human pathogenic diseases and transmission pathways are aggravated by climatic hazards. As pointed out by Mora *et al*[25], exposure to life-threatening conditions such as floods and hurricanes, extraneous conditions during heatwaves and depression from lost livelihood due to drought are a few examples in which climatic hazards are inducive to stress. Asso-

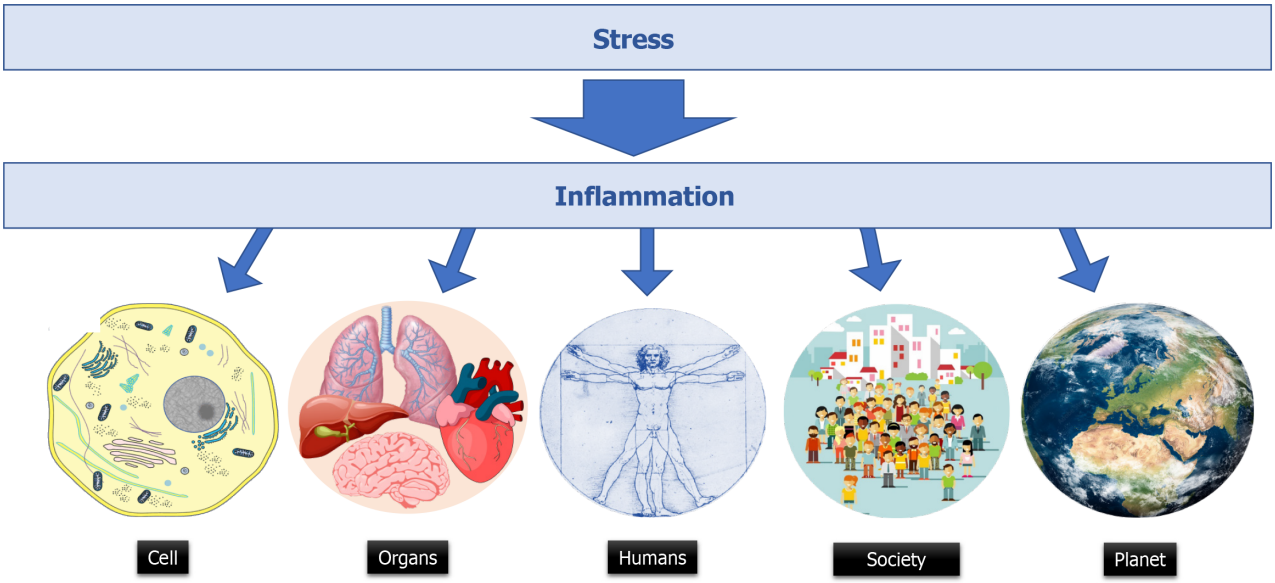

Figure 1 As pointed out by Hans Selye, stress and inflammation affect all human components as well as their own societal and planetary environment.

ciated cortisol variations are a mechanism by which climatic hazards reduce the body's capacity to deal with pathogens. In addition, drought, by reducing water availability, forced the use of unsafe drinking water, causing outbreaks of diarrhea, cholera and dysentery[25]. Weather changes, as seen in tornados, floods, and droughts have a disastrous impact on human societies. The climate changes associated with disasters create a psychopathological distress among the human populations. A recent report points out the negative impacts of climate change on human health, the delayed climate action of European countries, and the missed opportunities to protect or improve health with health-responsive climate action[26]. A meta-analysis led the authors to conclude that reducing greenhouse gas emissions, managing ecosystem health, and preventing biological invasions and biodiversity loss could help to reduce the burden of plant, animal and human diseases, especially when coupled with improvements to social and economic determinants of health[27].

Wars

The immune system is often explained to children as an army of specialized soldiers aimed to defend our body, entering in war against pathogens[28]. Interesting auto-immunity has been compared to a civil war[29]. Wars have always accompanied the story of humanity since its very beginning, being responsible for millions of deaths. For the almost 85 years following the end of World War II, the Western world considered that wars were from another age and would not anymore affect the European continent, although the civil war in ex-Yugoslavia had taken the life of some 100000 people. The war initiated on February 24, 2022, by Vladimir Putin, the president of the Russian Federation, invading Ukraine came as a surprise although revisiting his past speeches would have allowed the observers to anticipate this devastating event, likewise, the naivety of Europeans facing Hitler's speeches before world war II. On October 7, 2023, the terrorist attack by the Hamas on Israelis came as another surprise. The war initiated by Israel on the Gaza strip has led to tens of thousands of deaths. In all wars, one should not forget that many soldiers and even more civilians are wounded, initiating in their flesh their own inflammatory response and an inflammatory psychological insult to themselves and among their relatives and friends. Violent conflicts have a devastating effect on the physical and mental health of children [30]. Life under the bombs[31], displacement[32], separation from their parents[33] are all events that increases the likelihood of severe mental distress in adulthood and altered physical functioning in late adulthood. Furthermore, life expectancy can be altered as illustrated by a study which revealed that the mean loss of adult lifespan of orphans who had lost their father before birth during world war I was 2.4 year and was the result of increased mortality before age 65 years[34].

PHYSIOLOGICAL, PSYCHOLOGIC, SOCIETAL, AND PLANETARY STRESS INDUCERS

Stress and evolutionary process

Homeostatic life is associated with a bit of "physiological" stress. Eustress is beneficial for health through an optimization of homeostasis. Therefore, an ideal stress level is essential for building biological mechanisms needed for normal life processes[35]. Interestingly, wild mice have higher corticosterone levels than laboratory ones[36]. Indeed, laboratory mice having food ad libitum and not practicing any physical exercise are poorly replicating normal animals and are metabolically morbid[37]. Stress has existed with humans since the beginning of their presence on Earth. What evolution has selected to maintain life in an appropriate balance? The prehistoric humans were stressed by wild animals, by natural

events, by the need to find food, to keep the fire on, to defend their cave against their neighbors, to deal with wounds, to fight infectious bugs... Are the stresses associated with our contemporary way of life (see below) need or would generate other mediators, other pathways, other regulatory mechanisms than those initially selected by evolution? This is quite unlikely. On Earth, environmental stress could have shaped human traits. Drought, high or low temperatures are stressful situations for which adaptation could have been required for survival. Accordingly, stress-responsive mechanisms have facilitated evolution through plasticity of the genome and selection of the most adapted phenotype[38].

Cellular stress

Many conditions including hypoxia, starvation, infections lead to stress on animal physiology, on tissues and on cells (Table 1). Within the cells, the endoplasmic reticulum (ER) stress is a disturbance of the protein folding process. It is also associated with an up-regulation of death receptor expression that mediates cell death[39]. ER stress has recently been linked to inflammation in a variety of human pathologies including autoimmune, infectious, neurodegenerative, and metabolic disorders. In the cell, ER stress and inflammation share signaling regulators and effectors. These two signaling pathways have been shown to form a vicious cycle in exacerbating cellularity function and causing apoptosis in many cells and tissues[40]. Cell death and particularly apoptosis is accompanied by an increase of mitochondrial membrane permeability and the release into the cytosol of pro-apoptotic factors associated with their production of reactive oxygen species (ROS)[41]. The excessive production of ROS induces mitophagy to remove damaged mitochondria. The mitochondria maintain the balance of energy metabolism. Excessive energy stress can lead to mitochondrial dysfunction, which promotes metabolic inflammation, although moderate mitochondrial stress can have a beneficial effect[42].

Immune system as a social network

Within organs, through short or long-distance mediators-mediated cross-talks, individual cells of different natures display a highly interactive network that has been compared to a multi-layered social network[43]. This social network revealed by quantitative proteomics provides a framework for the orchestration of cellular interplay, and is a reference for altered communication associated with pathology[44]. The immune system is profoundly affected by its stressful environment, particularly because immune cells have specific receptors for stress hormones that shape the immune response.

Different experimental approaches have demonstrated the profound perturbation of the immune system following stressful events. For example, a cold-water stress augments the production of IL-1β, tumor necrosis factor (TNF), and IL-6 by lipopolysaccharide (LPS)-activated murine peritoneal macrophages[45]. With the use of specific inhibitors and antagonists, the authors identified substance P as the neuro mediator responsible for the effects in this model. Of note, epinephrine and norepinephrine display opposite effects on in vivo induction of TNF by LPS in mice[46]. Impressively, stress applied to pregnant rhesus monkeys diminishes the cytokine response of leukocytes to LPS stimulation in two-years old rhesus monkeys[47]. Stress also alters the interferon production in mice infected with viruses, impairing the ability of the host to control viral replication prolonging the infectious period[48]. Similarly, the severity of a cutaneous infection due to an intradermal injection of *Streptococcus pyogenes* is significantly increased by stress[49]. The authors showed that it was consecutive of the inhibitory effects of glucocorticoids on the expression of cutaneous antimicrobial peptides. In human chronic stress, it is a functional resistance to glucocorticoids in monocytes that has been observed, enabling activation of pro-inflammatory transcription control pathways[50]. Even moderate stress can modify the production of cytokines as observed in students after a major written exam[51]. Exposure to stress and violence is associated with altered immune status as illustrated by the higher frequency of asthma morbidity observed in children who had been exposed to physical or sexual abuse[52].

Response at the organ level

Immune cells (*e.g.*, macrophages, neutrophils, natural killer (NK) cells, and T-lymphocytes), as well as endothelial cells, differ from one compartment to another and contribute to specific organ responses to sterile and microbial insults[53]. Furthermore, tissue-specific microbiota influences the local and systemic response. At homeostasis gene expression and surface markers are different among spleen, lung and peritoneal, and microglial cells[54]. Furthermore, in tissue-resident macrophages environment in different organs governs diversity in the chromatin state of tissue-resident macrophages and epigenetic status[55]. Upon stimulation, cytokine production differs between blood monocytes and intestinal macrophages[56]. During an infectious insult, the same receptors or the same mediators can play different roles depending upon the stressed organ[57]. In an endotoxemia model, NK cells display organ-specific phenotypes and transfer experiments demonstrated that local microenvironmental factors overrun the intrinsic differences[58]. Upon LPS injection in mice, the cytokine production varies from organ to organ: The main production of IL-1 was observed in liver, spleen and lungs while the main production of IL-6 was found in heart, muscle and brain[59]. During systemic inflammatory response syndrome (SIRS) and sepsis, all tissues are concerned by the inflammatory process[60,61]. Adipose tissues in aged mice contribute to the cytokine storm[62]. In humans with septic shock high levels of inducible nitric oxide synthase are found in inflamed and putrescent fat tissue and artery[63]. In mice, gene expression patterns in leukocytes from blood and spleen are different depending upon the type of inflammation and injury (trauma/hemorrhage, burn, LPS injection) and are different between the two compartments[64]. The inflammatory process is perpetuated by a crosstalk between organs[65]. Similarly to their organ-specific properties, reprogramming of macrophages and NK cells after a primary stress, is also compartmentalized[66]. Most interestingly, a study revealed that commensal bacteria induce in the periportal zone, Marco$^+$ immunosuppressive macrophages, which consequently limit excessive inflammation at the gateway of the liver[67].

Table 1 Nature of the inflammatory stress on the different targets

Target	Inflammatory stress
Cell	PAMPs; DAMPs; Toxins; Inflammatory cytokines; Lipid mediators; Free radicals; Microplastics; Radiation
Organ	Inflammatory mediators; Ischemia; Hypoxia
Individuals	Physical stress: trauma; Burns; Surgery; Pathogens; Heat; Cold; Chemicals; Pollutants; UV; Allergens.
	Psychological stress: Harassment; Wars; Family disruption; Loneliness…
Society	Low or high incomes countries; Political regime
Planet	Loss of ecologic balance; Global warming; Natural disasters; Overpopulation

PAMPs: Pathogen associated molecular patterns; DAMPs: Damage associated molecular patterns; UV: Ultra-violet.

It was nicely demonstrated in models associating sepsis and trauma/hemorrhage that the *ex vivo* TNF production in response to LPS was reduced in peripheral blood mononuclear cells (PBMC) and spleen macrophages, while it was enhanced in alveolar macrophages and Kupffer cells[68]. Also, polymicrobial sepsis does not affect the function of skin cluster of differentiation (CD)8$^+$ T-cells in terms of interferon-gamma (IFN-γ) production[69] while the capacity of brain microglial cells to produce TNF was enhanced[70]. A similar discrepancy was observed in terms of macrophage reprogramming after LPS injection which was observed in peritoneal macrophages and blood monocytes but not in alveolar macrophages[71]. Most interestingly, a comparison of differentially expressed gene transcripts in different organs (lungs, heart, kidneys, liver, and spleen) from deceased meningococcal septic shock patients revealed a great number of modulated genes specific to each organ[72]. A similar study including transcriptomic analysis of the cortex and hippocampus of patients who died of septic shock, revealed that the brain also displays a specific pattern of activated and repressed genes[73].

Economical stress

Of course, higher education is associated with better professional positions and thus higher income. Furthermore, high-income countries ensure a high level of employment, better access to health system. Of course, the need for money, and monetary inflation are not affecting only low- and middle-income countries and the stress to offer enough financial support for oneself and one's family is universal. Most interestingly, a study revealed that among many potential confounders, high economical stress was associated with increased inflammatory activity, illustrated by higher IL-6, C-reactive protein (CRP) and IL-1 receptor antagonist levels in women with stable coronary heart disease[74].

Watching negative news on TV and exposure to fake news on social media

Watching negative news on TV is an obvious contemporary threat when violence, drug traffic, natural disasters, wars (…) enter the home[75]. During the COVID-19 pandemic, during the lockdown, when people were spending more time in front of their TV set, the French health director was announcing the number of deaths every day. Such news are common stressors. However, the results of a recent study (performed before the war in Ukraine and in Gaza) came as a surprise [76]. The study included half a million news headlines across 16 country over three weeks. These results demonstrate a dominance of positive news headlines (70.5%) over the negative ones (29.5%). Among the countries with the highest percent of positive news (> 85%) were some of the countries with the lowest press freedom index (PFI) (*e.g.*, Russia, China). However, there was no direct correlation between this index and the frequency of positive or negative bad news. Among the countries with the lowest percent of positive news (52%), Egypt and Lebanon have a significantly different PFI. Of course, different negative events such as the war in Ukraine would not be addressed the same way in different countries. While the Europeans are concerned, people living in China or in Kenya will not face so much the news about this war on European ground.

Fake news on social media are bad for society, and could be bad for health. During the COVID-19 pandemic, fake news have been associated with psychological disorders and panic, fear, depression, and fatigue[77]. Misinformation also resulted in vaccine hesitancy which was itself associated with an increased number of deaths due to COVID-19. An estimation of at least 232000 deaths could have been prevented among unvaccinated adults in the United States[78]. Misinformation concerning health has particularly severe consequences with regard to people's quality of life and even their risk of mortality[79].

Disinformation and propaganda are weapons in hybrid warfare. Historical revisionism-based information and influencing campaigns conducted by Russia is an example of how a country acts to influence the democratic countries and aims to alters their societies[80], while brain washing its own population with propaganda *via* its TV channels.

Professional and sexual harassment

Physical health is closely linked to mental health[81], and mental health is influenced by the numerous parameters mentioned in this chapter. Social and professional recognition is a key element for human welfare while unemployment or fear of unemployment can alter the well-being. The professional environment is a micro-society which is under the influence of its human components. Professional harassment can end in depression and even suicides. It has been reported that healthcare workers with workplace violence, especially emotional abuse, threat and verbal sexual

harassment, are more likely to experience burn-out[82]. Of course harassment also exists in the academic world and scientists have been unprecedently attacked during the covid-19 crisis[83]. Stress-induced chronic low-grade inflammation and a decline in immune surveillance are both implicated in cancer development and progression[84]. A meta-analysis revealed significant associations between work stress and the risk of colorectal, lung, and esophagus cancers were found. A statistically significant effect of work stress on colorectal cancer risk was observed in North America, but not significant in Europe. By contrast, a significant association between work stress and esophagus cancer was found in Europe, but not in North America. In contrast there was no association between work stress and the risk of prostate, breast, or ovarian cancers[85]. Moral harassment also exists at school with similar consequences on children than those observed in the adult professional environment. Born in 2006, the hashtag me too was created by African-American Tarana Burke (born 1973) to help women victims of sexual violence. But sexual harassment has been revealed to the public with the Weinstein affair in 2017. Since, in many countries new laws have been voted to better protect the rights of women. On March 8th, 2024, France was the very first country in the world to modify its constitution to guarantee freedom of women to resort to voluntary termination of pregnancy.

Domestic and family violence

In 2022, the number of women and girls killed globally was nearly 89000, and around 48800 women and girls worldwide were killed by their intimate partners or other family members[85,86]. This means that, on average, more than 133 women or girls were killed every day by someone in their own family. The rates of femicide differ depending on the specific country[87]. Among those with the highest rate are Central African Republic, Jamaica and South Africa. In contrast, Singapore, Japan and Belgium are countries with the lowest rate.

The COVID-19 pandemic has been responsible for an increased frequency of domestic violence: The home confinement led to constant contact between perpetrators and victims, resulting in increased violence and decreased reports[88].

Less violent but still with consequences on the mental health of parents and children is the family disruption. The impact of family structure on the health and well-being of children demonstrates that children living with their married, biological parents consistently have better physical, emotional, and academic well-being while there are clearly negative long-term consequences of divorce on children, parents, and society[89].

Access to food …not only a problem in developing countries

Famine is closely linked to climatic conditions and poverty while access to safe water is also key for the health of planet Earth inhabitants. The consequences of starvation of new born and young children on their physical and intellectual developments are very severe and sometimes lead to irreversible consequences in adulthood[90-92]. Similarly, the immune system is profoundly affected by under-nutrition. Of note, limited access to food is not restricted to low income countries. In rich countries such as France, the non-governmental organization “Les resto du coeur” served 8.5 million meals in 1985, while in 2022-2023, 171 million meals were served to 1.3 million people, including 126000 children under 3 years old[93].

Physical sterile and infectious stress

In 1994, Matzinger[94] proposed a new definition of the immune system and the nature of its triggering signal. Then, emerged the concept of danger and its associated damage associated molecular patterns released by damaged cells and injured tissues that initiate the immune response. The vision of Matzinger[94] completed that of Janeway[95] proposed five years earlier who defined innate Immunity system as able to recognize conserved patterns of pathogens using specific receptors. Indeed, similar receptors (the pattern recognition receptors) can sense the signal of sterile danger occurring after trauma (car crashes, surgery, intensive cares) or burns as they do for pathogen associated molecular patterns released during an infectious process[96]. Similar downstream signaling are initiated within the immune cells, and similar cytokines orchestrate the immune response and the whole-body information, the production of acute phase proteins by the liver, and the control of the response by the neuro-endocrine system. The discoveries of the toll molecule in drosophila, and its homologs in the animal kingdom (Toll-like receptors) offer an evolution perspective unifying through the different species the mechanism of defense against sterile and infectious insult[97].

Chronic stress

The incidence patterns of immune-mediated inflammatory diseases such as asthma, inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, psoriasis, and atopic dermatitis varies across the world[98]. Chronic inflammation favors the occurrence of these diseases as well as exposure to stress. Certain social, environmental and lifestyle factors can promote low-grade systemic chronic inflammation. It includes chronic infections, physical inactivity, obesity, intestinal dysbiosis, diet, social isolation, psychological stress, disturbed sleep and disrupted circadian rhythm, and exposure to xenobiotics such as air pollutants, hazardous waste products, industrial chemicals and tobacco smoking. These settings in turn, lead to several diseases that collectively represent the leading causes of disability and mortality worldwide, such as cardiovascular disease, cancer, type 2 diabetes, chronic kidney disease, non-alcoholic fatty liver disease and autoimmune and neurodegenerative disorders, sarcopenia, or osteoporosis[99].

A very interesting experiment was performed in 276 healthy adult volunteers who were challenged with two rhinoviruses, and followed for 5 days with nasal washes for viral isolation and assessment of signs/symptoms of a common cold. Those with recent exposure to a long-term threatening stressful experience demonstrated glucocorticoid receptor resistance, and were at higher risk of subsequently developing a cold. Greater glucocorticoid receptor resistance predicted the production of more local proinflammatory cytokines among infected subjects[100].

Post trauma distress syndrome

Post trauma distress syndrome (PTSD) is an altered mental health condition in certain people who have experienced or witnessed a traumatic event such as war[101], act of terrorism[102], natural disasters[103], rape[104], but it can also occur after hospitalization in intensive care unit[105]. Interestingly, PTSD was also observed among the healthcare professionals after the COVID-19 crisis[106]. People with PTSD may also experience physical symptoms, such as increased blood pressure and heart rate, fatigue, muscle tension, nausea, joint pain, headaches, back pain or other types of pain.

While PTSD is diagnosed based on classic psychological and behavioral symptoms, a link exists between this disorder and alterations in the immune and inflammatory systems. Compared to healthy controls, individuals with PTSD exhibit significantly elevated levels of proinflammatory cytokines, and CRP[107]. Glucocorticoids and adrenergic agents cause both immediate and late sequelae[108]. Traumatic stress disorder and immune disease share a common genetic basis at the gene expression level[109]. PTSD is associated with many epigenetic modifications including DNA methylation, histone modification and downregulation of certain miRNAs while an elevated expressions of IFN-γ and IL-12 in PBMC have been observed[110].

Global warming

The planetary insults consecutive to human activities have consequences on human health. Exposure to atmospheric pollutants and exposure to contaminating plastics have direct negative effects on the human and animal health. Global warming is modifying the geographic distribution of insects and as a consequence the exposures of herds and humans to vector borne diseases[111]. Of note, some of these new exposures may have already existed in the past. The name malaria is derived from mal aria ("bad air" in medieval Italian) and Giovanni Maria Lancisi (1654-1720), the physician of three consecutive popes, established a correlation between the presence of mosquitoes and the prevalence of malaria in certain areas of Italy.

Climate change is exacerbating mental disorders, which already affect almost one billion people and are among the world's biggest causes of ill health[112]. The climate anxiety (eco-anxiety) around the world particularly affects the youngest and in country as diverse as Philippines, India, Brazil, Portugal, France, and Australia, more than 50% of the people aged 16-25 years say they are extremely worried about it. Only around 3% of people with depression receive adequate treatment in low- and lower-middle-income countries, and 23% in high-income countries[112].

Air, water pollution and microplastics

Activities of human societies contribute to an alteration of their environment. Air pollution is by far the most dominant environmental risk factor for health in general, and is responsible for over 9 million annual deaths globally, most of which are due to cardiovascular causes. The major sources of modern-day air pollution in high-income countries include fossil fuel combustion, while other sources such as wildfires, volcanic ashes and wars play a major role in air pollution. The association between air pollution and metabolic and cardiovascular disorders has been recognized, including diabetes [113]. At the organ (*e.g.* lungs) and cell level, oxidative stress, and ER stress play an important role[114].

Since 1950, plastic has revolutionized the way humans do shopping and their food containers. But, we are now facing a huge plastic waste crisis with major environmental consequences. Their degradation leads to microplastics (MPs) and nano plastics present in air and water. They have been detected in many marine species and organisms, but also in drinking water and in numerous foods, such as honey and salt (Figure 2). For instance, the MPs concentration in table salt in Indonesia, is around 100 times higher than that in the United States. The *per* capita daily MP dietary and inhalation uptake rates is particularly important in South Asian countries such as Indonesia, Malaysia, the Philippines, and Vietnam, with more than 50% by aquatic sources *via* fish consumption[115]. Once absorbed in the lungs after inhalation, or in the gut barrier after ingestion, plastic micro- and nanoparticles can distribute to the liver, spleen, heart, thymus, reproductive organs, kidneys and even cross the blood-brain barrier[116]. The remarkable increase during the COVID-19 pandemic in use of face masks, which mainly contain polypropylene, and poor waste management have led to worsening MPs pollution. Ultra-violet (UV) light and wind break down and modify MPs in the environment into smaller particles which display increased toxicity[117]. MPs can be taken up by cells, altering the immune homeostasis and finally causing damage to tissues and organs. Once attached to the plasma membrane, MPs can be taken up through endocytosis disrupting the intracellular signaling pathways. MPs can induce intracellular ROS and oxidative stress by affecting the mitochondria function. The MPs could be released out from cells after cell death[118]. When human gingival fibroblasts are *in vitro* exposed to MPs, their inflammatory response is initiated ending to the up-regulation of gene and protein expression of NF-κB, MyD88 and NLRP3[119]. These results illustrate that the inflammation process is stimulated by MPs at the cellular level. *In vivo* short-term exposure of mice to MPs in drinking water led to the detection of MPs in liver, kidney, gastrointestinal tract, lung, spleen, heart, and brain, and an increase in TNF mRNA expression was observed in the liver. Finally, the investigators noted behavioral changes in terms of exploratory comportment and spontaneous locomotion. These changes differed depending on age, indicating a possible age-dependent effect[120].

In human patients who underwent carotid endarterectomy for asymptomatic carotid artery disease, polyethylene was detected in plaques of 58.4% (n = 150) of them. In this study, patients with carotid artery plaque in which MPs were detected had a higher risk of a composite of myocardial infarction, stroke, or death from any cause at 34 months of follow-up than those in whom MPs were not detected[121].

Political stress and inflammatory statements

Societies used to a democratic leadership are not immune to totalitarianism. The fear of totalitarianism is like a homeostatic stress for those societies. We should not forget that Hitler came to power democratically. Politicians regularly give inflammatory statements or inflammatory speeches. Some recent events illustrate this fact. For example, far-right Israeli

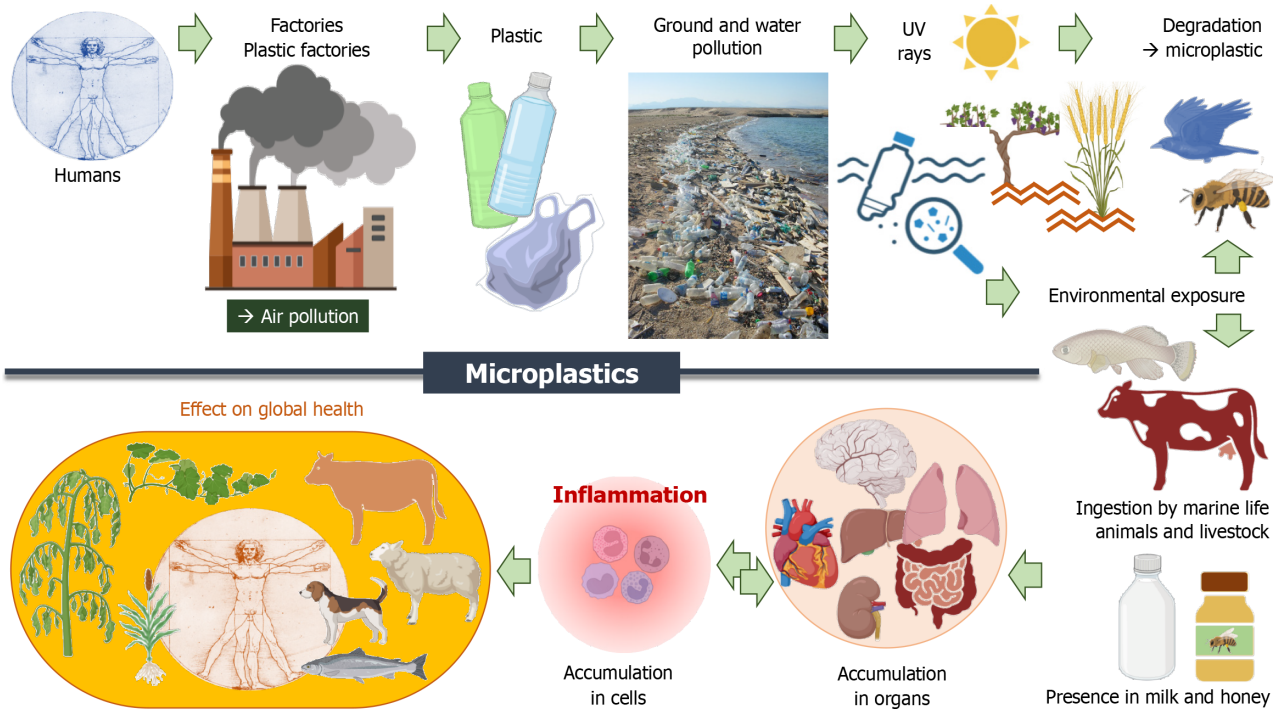

Figure 2 A domino effect, from the production of plastics by human industries to the environmental pollution to their degradation into microplastics that contaminate wild life animals and livestock, and the products derived therefrom. The ingestion and inhalation of microplastics by humans generate an inflammatory reaction in cells and organs. UV: Ultra-violet.

Jerusalem Affairs and Heritage Minister, Amichai Eliyahu calls for ways more painful than death' for Palestinians. Speaking on the sidelines of the human rights Council in Geneva, French foreign minister Catherine Colonna dismissed former Russian president, Medvedev's comments as "inflammatory rhetoric". In a major joint operation, Mumbai police in collaboration with Gujarat's anti-terrorism squad arrested Islamic preacher Mufti Salman Azhari. He was accused of delivering an inflammatory speech that has since grown viral. Former United States president Trump criticized the judge presiding over his 2020 election case, just days after she warned him against making any "inflammatory statements" that could intimidate witnesses or prejudice the jury pool. His inflammatory language to demonize immigrants during his election campaigning New Hampshire echoed those of Adolf Hitler. An inflammatory speech by the Houthi leader targets Baha'is in Yemen with genocidal intent was propagated on social media. Indeed, hate speech on social media threatens the mental health of its victims and poses severe safety risks to our modern societies[122]. The parallelism between this human behavior and activities of cells was proposed by Schuster *et al*[123] who offered a provocative title for their review: "The inflammatory speech of fibroblasts". The authors refer to activated fibroblasts which can contribute to inflammatory process, pathological repair and fibrosis as seen in articular synovium during rheumatoid arthritis and osteoarthritis.

Stress of the future: Travel to Mars

There are very few humans who are concerned by the stress associated with spaceflight. But who knows whether in the future more people will undertake a long travel to the moon or to Mars. Astronauts are exposed to various stressors including radiation, dietary restrictions, microgravity, isolation, confinement, noise, circadian rhythm disturbance. The complicated space environment does not only affect their physical functions but may also induce psychological problems, such as anxiety, depression, and cognitive decline[124]. The human body is intrinsically adapted to Earth's gravity, thus exposure to conditions of microgravity can lead to a plethora of complications in normal body functions. Particularly, bone loss has been regularly observed. Osteoclasts have been demonstrated to display an increased resorptive activity in response to microgravity[125]. Since IL-1 was initially identified as the "osteoclast activating factor"[126], because the production of IL-1 is enhanced by spaceflight[127], one could hypothesize that IL-1 is an inflammatory cytokine that activates the osteoclasts ending to an alteration of the bone status during spaceflight. Various immune parameters such as the maturation of the immune cells, and the distribution of leukocytes are altered. Neutrophils are elevated and eosinophils are reduced in the peripheral blood of astronauts. Moreover, *in vitro* activation of T-cells is significantly reduced, granulocyte and monocyte function, and NK cell function are also modified[128]. Accordingly, acquired immune responses are disturbed by gravitational fluctuation, stressors, and space radiation both directly and in a stress hormone-dependent manner.

THE PARAMETERS INFLUENCING THE RESPONSE TO STRESS ARE MANY

To illustrate the heterogeneity of humans in terms of response to stress and inflammation, let us quote the bright medical investigator, Antoine Béchamp (1816-1908). He was the unfortunate competitor of Louis Pasteur, having understood before him the process of fermentation and the infectious origin of the diseases of silkworms[129]. His statement adapted from his view "the pathogen is nothing, the terrain is everything" has been regularly erroneously attributed to either Louis Pasteur or Claude Bernard. In this chapter we will review the different parameters which influence the response to stress (Figure 3).

Education

The immune system has to be educated not to attack one's own tissues and organs to prevent autoimmune disease. The behavior, function and life of immune cells are fully dependent upon their education associated with early life microbial exposure imprinting long-lasting fate of the immune system[130]. Among immune cells, T-lymphocytes follow a specific educational program within the thymus during which both non-functional and self-reactive T cell clones are eliminated by means of positive and negative selection[131,132]. Another educational program is needed during pregnancy which presents a unique challenge, since the fetus expresses proteins genetically distinct from the mother[133].

In a study on social determinants of human health it was revealed that length of life expectancy was associated with education levels[134]. Adults with higher educational attainment have better health and lifespans compared to their less-educated peers[135]. A study of a cohort of adults born 1906-1915 revealed that one additional year of education was associated with approximately 0.4 more years of life[136].

Socioeconomic status

Socioeconomic status mentioned earlier as a stress factor can indirectly affect the health of the individuals. This was particularly well illustrated during the lockdown consecutive to the COVID-19 pandemic which has disproportionately impacted the most vulnerable and widened the health disparity gap in both physical and mental well-being. The impact of the COVID-19 pandemic on unhealthy lifestyle patterns encompasses reduced physical activity, increased sedentary behavior, augmented screentime, disturbed work and sleep schedules, more smoking and alcohol consumption. These altered behaviors were associated with mental health disorders, such as anxiety and depression[137]. Individuals with lower social resources, lower economic resources, and greater exposure to stressors (*e.g.*, job loss) reported a greater burden of depression symptoms[138]. Parent-reported mental health problems were more likely to affect children with low socioeconomic status, with complex chronic disease and those whose parents screened positive for depression[139]. Individuals of racial minority groups and lower socio-economic status experienced the worst economic outcomes of employment losses[140]. Altogether, the lockdown was not egalitarian: the costs fell on the most economically disadvantaged within the societies.

Age

Aging is responsible for a great disparity among individuals and among societies. The percent of the population younger than 20 represent more than 50% in the African countries. The older populations are found in Japan (30% of the population is over 65 years old), and in the European countries (around 20%). Major modifications of the immune system occur with aging ending to the so-called immunosenescence which combines decreases in innate and adaptive immune responses in addition to the exacerbated production of inflammatory cytokines[141]. COVID-19 has been particularly deadly among the oldest population. At the beginning of the pandemic, according to the Chinese Centre for Disease Control and prevention, the mortality rate was 14.8% among those older than 80 years, 8% among those 70%-79%, and less that 0.5% among the younger than 49[142]. In Italy, the case-fatality rate increased from 4.2% to 14.0% between March 9 and June 30, 2020, and more than 90% of the change was due to increasing age specific case-fatality rates[143]. Although there was a certain discrepancy in terms of mortality from one country to another, the influence of age remains a common feature of mortality[144]. In 2021, the highest COVID-19 mortality rates continued to be observed at ages 75 +, despite vaccinations having specifically targeted those ages[145]. A coordinated $CD4^+$ T cell, $CD8^+$ T cell, and antibody responses are protective, but uncoordinated responses frequently fail to control disease, with a connection between aging and impaired adaptive immune responses to SARS-CoV-2[146]. Trauma is another setting which illustrates the altered responsiveness among aged people. After trauma, the discharge to home or rehabilitation (59% *vs* 30%), the discharge to long term care facilities (28% *vs* 47%) and the 28 days mortality (9% *vs* 18%) was significantly different among people aged below or above 55[147]. An experimental study performed in young and aged mice combining polytrauma and pneumonia ended to a greater mortality among old mice, and, most interestingly analysis of bone marrow gene expression revealed a specific expression of 386 genes in young mice, and 666 in old mice, with only 46 genes in common[148]. In a model of mouse poxviral infection, young mice are fully resistance whereas old mice succumb to the infection. The investigators showed that it was associated with a decreased number of total and mature NK cells in the blood and the spleen and an intrinsic impairment of NK cells functions since infected old mice could be partially rescued by the injection of NK cells from young mice[149]. In a murine sepsis model (cecal ligation and puncture model), 70% old mice (24 months) succumbed while all young mice (4 months) survived[150]. It was accompanied by higher levels of circulating IL-6, and higher TNF mRNA expression in lungs and hearts of old mice. Similarly, the mortality of aged mice is higher than that of young mice upon LPS injection, and IL-6 production was higher in the heart, the kidney, the spleen and the adipose tissues of old mice[151]. The greater capacity of human PBMC to produce TNF was also observed in human elderly individuals as compare to younger ones[152].

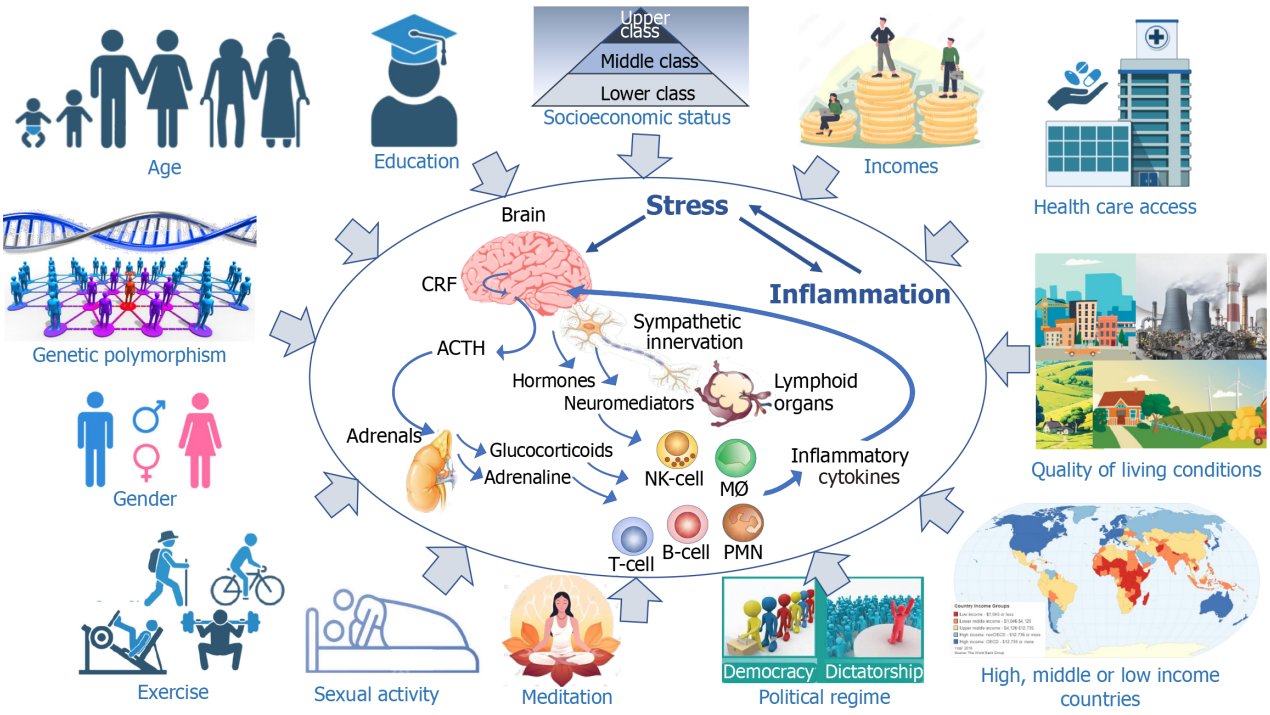

Figure 3 Stress favors an inflammatory response which is regulated by the neuro-endocrine axis. Many parameters modulate positively or negatively this response. CRF: Corticotropin releasing factor; ACTH: Adrenocorticopic hormone; MØ: Macrophage; PMN: Polymorphonuclear cell (Neutrophil); NK cell: Natural killer cell.

Gender

Despite the constitutions of many countries claim the equality between male and female for their rights, and despite the fact that Women's Lib has been fighting to obtain the societal equality, many biological parameters differ between genders. Associated with this heterogeneity, differences exist in the male and female response to sterile and infectious stress, not only at the physiological level but also at the psychological one. For example, gender is one factor among many that affected the frequency of suicides associated with the stress generated by the COVID-19 crisis and its associated lockdown. It has also been shown to vary as a function of age, economic level of countries, and political regime. In most countries there was evidence of lower-than-expected numbers of suicide during the COVID-19 crisis[153]. However, a greater level of COVID-19 associated suicides has been reported among women in Japan[154], in United Kingdom[155], in Mexico[156], and in South Korea[157]. Furthermore, the confinement has led to an increase in the number of domestic violence, particularly against women[88].

Regarding the influence of gender on the sensitivity to infection, there are numerous reports in animal models that exemplified it[158]. Of note, depending on the pathogen, male or female are the most sensitive[159]. A fascinating study revealed that upon *Coxiella burnetii* infection in mice (Q fever), 1857 genes were modulated in the liver of male animals *vs* 1290 genes in females with only 398 in common. Upon castration of both genders, the number of modulated genes fell to 774 in males and 912 in females, with 396 in common[160]. Differences also exist in humans. For example, the early acquisition of mucoid *Pseudomonas aeruginosa* contribute to a poorer survival of female cystic fibrosis patients as compared to male[161]. In contrast, male gender is associated with an increased risk for postinjury pneumonia as revealed in a study on 20288 trauma patients[162]. Regarding sepsis, females appear less susceptible to sepsis and seem to recover more effectively than males; however, a great disparity exists between studies in terms of incidence and mortality [163]. In murine model of sepsis post-hemorrhage proestrus female survived better than male[164]. The key role played by female sexual hormones was demonstrated with the disappearance of the protective effect of gender in ovariectomized mice[165]. In male, sterile and infectious stressful insults result in an alteration of the immune status as illustrated by the reduced IL-2 release by *ex vivo* activated splenocytes[165,166]. Such an alteration disappears after male castration and is evident in ovariectomized mice. In the later as well as in male mice, *in vivo* 17 β-estradiol administration prevents the alteration. In normal female mice, the treatment with 5 α-dihydrotestosterone will allow the observation of an altered immune status after trauma-hemorrhage[166,167]. Altogether, a number of studies have reported gender dimorphism in terms of response to trauma, hemorrhage, shock and sepsis. The advantageous outcome in females in the proestrus stage is due to the prevailing hormonal milieu, *i.e.*, high estrogen levels. In this respect, various experimental and clinical studies have demonstrated beneficial effects of estrogen for the central nervous system, the cardiopulmonary system, the liver, the kidneys, the immune system, and for the overall survival of the host[168]. Estrogen have been regularly reported to dampen the inflammatory response. For example, 17 α-ethinylestradiol sulfate, which acts through the estrogen receptor can blunt multiple harmful outcomes arising from hypoxia and hypovolemia. In a rat model of hemorrhagic shock, ethinyl estradiol sulfate dramatically reduces damage by apoptosis, proinflammatory activity, and nitric oxide production and improved heart performance even in the absence of any fluid resuscitation following

hemorrhage[169]. In a swine model of combined traumatic brain injury and hemorrhagic shock, this estrogen was efficacious in promoting survival and more rapidly restoring cardiovascular homeostasis following polytraumatic injuries in pre-hospital environments (*i.e.*, rural and military) in the absence of standard therapies[170]. In another swine model of multiple injuries and hemorrhagic shock, the administration of this estrogen, even in the absence of fluid resuscitation reduced mortality and improved cardiac inotropy[171]. The role of sexual hormones may, however, vary depending on the inflammatory settings. In experimental acute cholangitis, testosterone was shown to be protective against liver inflammation[172].

Quality of food and microbiota

Julius Strasburger (1871-1934) claimed in 1902 that the human gut was hosting 1.28×10^{14} bacteria[173]. One century later, the number has only been slightly adjusted to 3×10^{13}, very close to the number of human cells[174]. A strong symbiosis exists between the bacterial flora and its host[175]. Not only the gut microbiota, but also the skin microbiota shapes the immune system[176]. Gut dysbiosis has adverse health effects on the human body that lead to a variety of chronic diseases[177]. This is supported by studies which showed that diet has a profound impact on the gut microbiota[178]. Among the many nutritional elements which favorably modify the quality of the immune and inflammatory response, let us mention vitamin D[179], fish oils[180] and probiotics[181]. For example, dietary supplementation with long-chain n-3 fatty acids in human volunteers suppressed the *ex vivo* production of inflammatory cytokines such as IL-1α, IL-1β and TNF[182] and inhibits tachycardia and attenuated maximal increases in oral temperature and metabolic rate following typhoid vaccine[183]. After listening Stamen Grigorov (1878-1945) who discovered the *Lactobacillus bulgaricus*, Elie Metchnikoff suggested the use of probiotic to limit degeneration associated with aging and to prevent the occurrence of various inflammatory disorders such as atherosclerosis consecutive to bacterial gut metabolites[184]. One century later Metchnikoff's view was confirmed[184]. Interestingly, in 1910, the treatment of patients with melancholia (depression) with lactic acid bacillus allowed eleven out of eighteen to recover from their depression and accompanying delusions[185, 186]. It was recently confirmed that gut bacteria influence behavior, and both depression and anxiety symptoms are directly associated with alterations in the microbiota, while psychobiotics, are defined as probiotics that confer mental health benefits to the host[187].

Obesity

The epidemic of obesity has not affected the world similarly. The prevalence of obesity in 2015 was above 25% in Turkey, Mexico, South Africa, Iraq, United States, and Egypt and below 2% in Vietnam, Bangladesh, China, Ethiopia, Indonesia, Myanmar, Nigeria, India, Japan, and South Korea[188]. Nowadays, only Viet Nam remains below 2%. Obesity is associated with chronic low-grade inflammation which contributes to systemic metabolic dysfunction. Adipose tissues of obese people are infiltrated with activated macrophages that release inflammatory cytokines[189]. However, transcriptome analyses performed on highly-purified adipocytes from lean and obese women, revealed that nucleotide-binding oligomerization domain-signaling, and NLR-inflammasome-related genes are activated in obese women[190]. Obesity has been a comorbidity factor during the COVID-19 pandemic. SARS-CoV-2 infection could potentiate the status of systemic inflammatory disease in obese people, *via* the activation of the *NLRP3* inflammasome and the release of pro-inflammatory cytokines[191]. Indeed, active *NLRP3* inflammasome activation was detected postmortem in the lungs of COVID-19 patients upon autopsy[192].

Genetic polymorphism

Many genetic elements govern the individual inflammatory response. Genetic predisposal to produce high or low levels of corticotropin releasing factor (CRF), adrenocorticotropic hormone (ACTH), cortisol and to respond to these mediators *via* genetic diversity of the respective receptors govern the individual response to inflammation and thus the threshold of resilience. The genetic heterogeneity of the human population, the genetic predisposal to diseases and the genetic diversity of the inflammatory response govern the great disparity of the response to any stress and insult. For example, cortisol or corticosterone levels and cortico sensitivity are under genetic influence[193,194]. One given genotype is associated with greater cortisol reactivity to social threats[195]. A polymorphism within the promoter of the serotonin transporter gene has been associated with differential psychological sensitivity to stressful experiences. Furthermore, variants have been found in the genes of the mineralocorticoid and glucocorticoid receptors that operate in balance and coordinate behavioral, autonomic, and neuroendocrine response patterns involved in homeostasis and health[196]. These variants contribute to individual differences in resilience and vulnerability to stressors. Similarly, the hypothalamic-pituitary-adrenocortical axis is influenced by gene polymorphism[197] and levels of CRF, CRF binding protein and ACTH can vary from individuals to individuals. Good examples of these genetic heterogeneity to sense stress and to respond to it, are the genetic polymorphisms associated with the PTSD which results in many epigenetic modifications [198]. PTSD does not affect similarly all individuals exposed to a stressful situation. Using a genome-wide association study, eight distinct significant regions were identified in patients with PTSD[199]. The glucocorticoid receptor gene (*NR3C1*) single nucleotide polymorphism (SNP)[200], a SNP in Tolloid-Like 1 gene[201], the retinoid-related orphan receptor alpha gene[202], the genes *INTS8* and *TP53INP1*[203], and genetic variability in the CRP gene[204] are associated with PTSD. Polymorphism of genes, such as those of catechol-O-methyltransferase, serotonin transporter, and neuropeptide Y[205] have an impact on resilience. Finally, genetic factors also influence the occurrence of depression following exposure to stress. Such association has been particularly reported for genes coding for galanin receptor 2, brain-derived neurotrophic factor, purinergic receptor P2X7, and 5-hydroxytryptamine (serotonin) receptor 1A[206].

Current over population, scheduled declining fertility rate and global stress

The global population was 1 billion in 1800, estimated to be about 8 billion in 2020 and anticipated to be 10.4 billion in 2100. This growth is expected to be concentrated in sub-Saharan Africa where there are already resource problems. This overpopulation is associated with global stress for humans and for the planet. Overpopulation is associated with a rise in food demands which has an impact on water consumption by agriculture. Humans exceeded the natural carrying capacity of the planet. "Earth overshoot day" is the calculated illustrative calendar date on which humanity's resource consumption for the year exceeds Earth's capacity to regenerate those resources that year. In 2023, the non-governmental organization "Global Footprint Network" considered that it happened on August 2nd. Overpopulation is responsible for a precarious situation on Earth[207]. All forms of natural resources are consumed for human needs and in turn producing waste, pollution, depletion of natural resources such as petroleum and metals, loss of biodiversity and ecosystems, environmental degradation, emission of carbon dioxide produced from burning fossil fuels and greenhouse gases causing global warming and climate change. In turns these modifications affect human physical and mental health. Population control measures are needed despite religious and cultural taboos, and confusion about rights. They should not be coercive and always voluntary and would then be highly beneficial for women's health and rights, for economic betterment and for the planet[208,209].

However, on other continents than Africa, societies will have to deal with another stressful situation: The net reproduction rate fell or will fall below the replacement level. During the period from 1950 to 2021, global total fecundity rate more than halved, from 4.84 to 2.23. Global annual live births peaked in 2016 at 142 million declining to 129 million in 2021. Twenty-three countries in the reference scenario proposed by the investigators[210] are forecasted to have population declines greater than 50% from 2017 to 2100. A sustained total fertility rate lower than there placement level in many countries, including China and India, would have economic, social, environmental, and geopolitical consequences. In a more recently published prospective study, only Niger, Chad, Somalia, Samoa, Tonga, Tajikistan, Egypt, and Israel are scheduled to maintain a fertility equal or above 2.1 in 2100[211]. The economic and societal consequences will be due to ageing populations and declining workforces in higher-income countries, combined with an increasing share of livebirths among the already poorest regions of the world.

THE YIN YANG ASPECT OF STRESS AND INFLAMMATION

Good, moderate or bad stress

Hans Selye himself approached the yin yang aspect of stress and inflammation. He showed in a rat air pouch model that a restraint stress induced after a weak irritant (diluted croton oil) has been added earlier in the pouch, ends to cure of the inflammatory reaction. In contrast, the restraint stress following the addition of a strong irritant in the pouch (more concentrated croton oil) ended in a strong inflammatory reaction and to damaged tissues[212]. Nowadays, similar demonstration can be achieved with the two-hit model. For example, in a murine model, a mild systemic inflammatory response (a pancreatitis induced by cerulein injection) fully protected against a subsequent peritonitis whereas a severe systemic inflammatory reaction (induced by cerulein + injection of LPS) ended to a more severe response to sepsis (up to 100% mortality)[213].

The concept of yin yang also illustrates the detrimental and beneficial effects of different infectious disease–influencing alleles in the human genome. Certain mutations serve as a fortress against one infection while conferring susceptibility to another[214]. The yin yang phenomenon is particularly significant in the immune response to infectious insult. For example, the same cytokines can be either beneficial or deleterious during sepsis and severe infections depending upon the investigations. This was shown for IL-17[215,216], IL-33[217,218], Granulocyte/macrophage-colony-stimulating factor [219,220]. Similarly, Treg[221,222] and apoptotic cells can protect or be detrimental[223,224]. Indeed, a given cytokine may behave as a pro- as well as an anti-inflammatory cytokine. The cytokine amount, the nature of the target cell, the nature of the action and even the experimental model are parameters which influence cytokine properties[225]. Similarly, prostaglandin E2 regulates the immune response in opposite fashion depending on the target cells[226].

Stress can simultaneously favor both fear and the induction of analgesia because of a double-edged sword of stress hormones. It was observed that exposure of mice and rats to male but not female experimenters produces pain inhibition. Male-related stimuli (androstenone and androstadienone pheromones) induced a robust physiological stress response that results in stress-induced analgesia. Experimenter gender can thus affect apparent baseline responses in behavioral testing[227].

Fever

"Saturday night fever" was a movie in which the characters take part in gang fights as well as racist and sexist behavior, and there is a disturbing gang rape scene in the back of a car. So that fever in the city was clearly one with negative effects. However, good stress (eustress) *vs* bad one (distress) is illustrated by the occurrence of fever in humans, one of the cardinal signs of the response to an inflammatory insult. There are good and bad fevers which may be different depending on whether the inflammatory insult is microbial or not. Intermediate fever is associated with better outcome in patients with sepsis whereas high fever is associated with poorer outcome in both septic patients and non-septic patients [228,229].

The most appropriate definition of fever was proposed by Welch[230] (1850-1934), one of the founding professors at the Johns Hopkins Hospital. He wrote: "The real enemy in most fevers is the noxious substance which invades the body, and there is nothing to prevent us from believing that fever is a weapon employed by nature to combat assaults of this enemy. According to this view, the fever-producing agents light the fire which consumes them. It is not incompatible

with this conception of fever to suppose that the fire may prove injurious also to the patients and may require the controlling hand of the physician".

Exercise

Exercise is given as offering a protective effect *vs* sedentary lifestyle. However, while this is the case of moderate exercise, heavy exertion favors inflammation and increases the risk of infection[231]. Heavy exercisers reported higher mental health issues, and more stress, but also higher mental toughness scores and less sleep disturbances. As such, the association between exercise frequency, intensity, and duration and psychological well-being might be related to an optimum point[232]. Many features of immunesenescence may bedriven by reduced physical activity with age while exercise can ameliorate some aspect of the immune status in aged people[233].

In a very convincing report, it was demonstrated in animal model that regular exercise reduces the risk of cancer and disease recurrence. In this study, tumor-bearing mice randomized to voluntary wheel running showed over 60% reduction in tumor incidence. Training induces upregulation of pathways associated with NK cell immune function. Infiltration was significantly increased in tumors from running mice. NK cells were mobilized by epinephrine, which induces a selective mobilization of IL-6-sensitive NK cells, controlling tumor growth[234]. Insufficient physical activity increases the risk of non-communicable diseases, poor physical and cognitive function, weight gain, and mental ill-health. If one draws a relationship between all-cause mortality and incident cardiovascular diseases as a function of exercise (inactive, optimal active, most active), one gets a reverse J-curve suggesting that to improve life expectancy, a regular regimen of moderate activity is adequate[235].

An estimation of the prevalence of insufficient physical activity for 163 countries from 2000 to 2022 was recently published. The global age-standardised prevalence of insufficient physical activity was 31.3% in 2022, an increase from 23.4% in 2000 and 26.4% in 2010. Prevalence was higher among female (33.8%) than male (28.7%) individuals. Insufficient physical activity increased in people aged 60 years and older in all regions and both sexes[236].

THE NATURAL ANTI-INFLAMMATORY REGULATORY MECHANISMS

Compensatory anti-inflammatory response syndrome

An appropriate response to an inflammatory insult associates a pro-inflammatory response aimed to control the aggression, and an anti-inflammatory response aimed to control the pro-inflammatory arm and to allow a return to homeostasis. There are evidences that inflammation is normally compartmentalized within the body[237,238]. Host defenses are acting at sites of injury or microbial invasion while a systemic inflammatory response can accompany the process. Importantly, the intensity of both responses should be proportional allowing the body's normal responses to stress to prevent systemic inflammation[239]. However, despite appropriate balance, the anti-inflammatory response [compensatory anti-inflammatory response (CARS)] can lead to an alteration of the immune status[240]. We postulate that SIRS and CARS should not be opposed, but most probably occur concomitantly in different compartments: SIRS predominates within the inflamed tissues while blood leukocytes show hyperactivity.

Anti-inflammatory regulators

As mentioned above, the anti-inflammatory players enter into action concomitantly with the pro-inflammatory mediators in response to the same stressful insult. There are numerous cytokines that display anti-inflammatory properties (Figure 4) while the two conductors of the orchestra (namely IL-1 and TNF) have their own specific inhibitors. Many investigators have shown in sepsis that there was a correlation between the levels of IL-10, a key anti-inflammatory cytokine, and the pro-inflammatory cytokines[241-243]. Indeed, as with any pro-inflammatory cytokines, IL-10 is a marker of severity in sepsis[244] or in sterile SIRS such as in patients resuscitated after cardiac arrest[245] and can be predictive of outcome. Among the anti-inflammatory mediators let us mention the resolving lipidic mediators[246] and the acute phase proteins[247],the latest being induced by the inflammatory cytokines, particularly IL-6 within a regulatory loop (Table 2).

Neuro-endocrine regulation

Hans Selye has been a pioneer in our understanding of how stress activates the hypothalamus-pituitary-adrenal axis, which results in the development of the "general adaptation syndrome". CRF produced by the hypothalamus was identified in 1981 by Vale *et al*[248]. It acts on the pituitary gland that in turn produce the ACTH which was characterized in 1933 by Collip *et al*[249]. ACTH acts on the adrenals ending the production of glucocorticoids. They were discovered in 1936-1941 by three scientists [Philip Showalter Hench (1896-1965), Edward Calvin Kendall (1886-1972), and Tadeusz Reichstein (1897-1996)] who were awarded the Nobel prize in 1950 for their discoveries related to the hormones of the adrenal cortex, their purification, their structure, and their biological effects.

When added to epithelial cells, some 2766 different genes are modulated (1436 up-regulated; 1330 down-regulated). Most interestingly, the addition of glucocorticoid to TNF activated cells further induced or repressed additional genes [250]. The glucocorticoid receptors are within the nucleus of the responding cells. On the cell surface many other receptors and cell surface molecules contribute to the negative signaling (*e.g.*, anti-inflammatory receptors, α-7 nicotinic receptor, resolving receptor, CD39, programmed death 1/programmed cell death-ligand 1). Within the cell, many negative signaling molecules (*e.g.*, myeloid differentiation factor 88, IL-1 receptor associated kinase, suppressor of cytokine-1, tumor necrosis factor alpha-induced protein 3…), miRNA, NF-κB inhibitors and epigenetic modification

Table 2 Nature of the anti-inflammatory stress process among the different targets

Target	Anti-inflammatory stress processes
Cell	Anti-inflammatory cytokines; Resolution lipid mediators; Acetylcholine
Organ	Anti-inflammatory drugs; Glucocorticoids
Individuals	Yoga; Meditation; Psychologist; Anti-depressant drugs
Society	United Nations; NATO; Non-governmental organizations; OECD
Planet	Climate change conferences (COP)

NATO: North Atlantic Treaty Organization; OECD: Organization for Economic Co-operation and Development; COP: Conference of the parties.

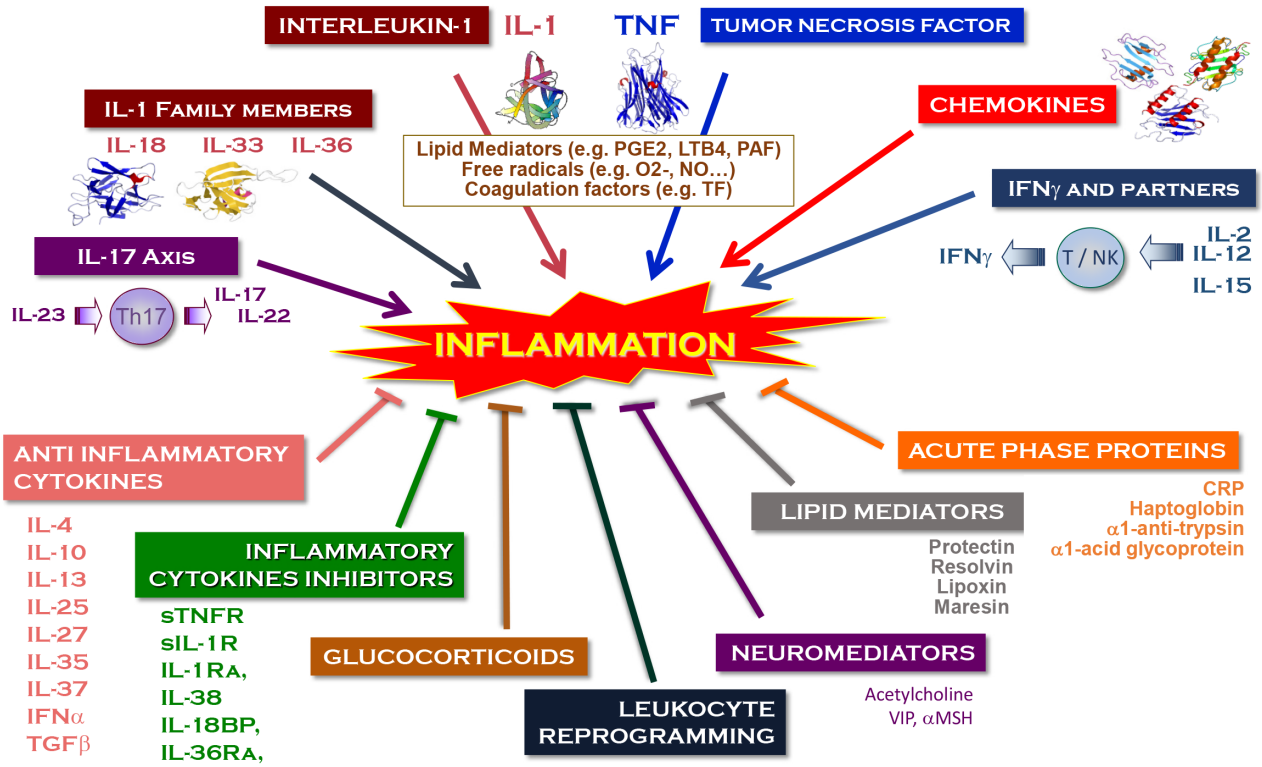

Figure 4 Inflammation involved many pro-inflammatory molecular players while many others allow a controlled response and a return towards homeostasis. IL: Interleukin; TNF: Tumor necrosis factor; CRP: C-reactive protein; IFN: Interferon; NK cell: Natural killer cell. Citation: Cavaillon J. Molecular Mediators: Cytokines. *Reviews in Cell Biology and Molecular Medicine* 2015. Copyright © 2015 John Wiley and Sons, Inc. All rights reserved. Published by *Reviews in Cell Biology and Molecular Medicine*[358].

participate in the negative regulation[251].

The cholinergic anti-inflammatory pathway

The team of Kevin Tracey has brilliantly demonstrated how the vagus nerve was controlling the inflammatory process. At the site of insult, cytokines trigger the afferent vagus nerve till the brain. Then the efferent vagus nerve induce the release of acetylcholine, which induces in the spleen the release of norepinephrine by adrenergic splenic neurons. Locally, T-cells bearing a β-2 adrenergic receptor release on their turn acetylcholine that shuts down the production of inflammatory cytokines by macrophages[252,253]. An implantable vagus nerve-stimulating device was shown to inhibit peripheral blood production of TNF, IL-1β, and IL-6. Vagus nerve stimulation in rheumatoid arthritis patients significantly improved the clinical scores and reduced disease severity[254]. A vagus nerve stimulator implanted in 16 biologic drug-refractory patients with moderately to severely active Crohn's disease, led to clinically meaningful reduction of the disease activity[255]. Noninvasive transcutaneous auricular vagus nerve stimulation in patients with mild to moderate pediatric-onset Crohn's disease or ulcerative colitis (UC), led to clinical remission in 3/6 (50%) with Crohn's disease and 2/6 (33%) with UC at week 16[256].

Tolerance/Trained immunity

No stress suffered by the cell or the individual leaves them unharmed. Whether the first encounter with a given insult will end to a tolerance/desensitization status or a priming effect depends on the nature of the inflammatory insult. Endotoxin tolerance was first reported as a decreased fever to a subsequent injection of endotoxin following a former one [257]. The phenomenon is associated with a reduction of certain inflammatory cytokines but not all[258]. It protects against the inflammatory consequences of trauma and hemorrhagic shock[259] and of ischemia/reperfusion[260]. Furthermore, endotoxin tolerance protects against fungal[261], bacterial[262], polymicrobial[263], viral[264] and parasitic [265] infections. Proposed in 1993[266], the term reprogramming has been associated with the clinical status of patients after sepsis[267] and has been considered as an appropriate terminology to define as well trained innate immunity[268]. While memory was only assigned to adaptive immunity, it becomes clear that innate immunity displays some kind of memory as well[269]. Among the primary signals that end to innate memory, β-glucan and bacillus Calmette-Guérin (BCG) have been widely studied. Indeed, it was shown a long time ago that BCG could lead to non-specific protection against bacteria! infection[270] and cancer[271].

Yoga and meditation

Stress can induce depression and modify immune status in some individuals, illustrating that some external insults can alter the mental well-being and the health of individuals. It makes sense that in some individuals the reverse could be true. In other words, psychological therapy, yoga and meditation could have beneficial effects on mental and physical status and immunological defense mechanisms.

For example, a review of literature identified 16 studies among 124 trials which met rigorous criteria to establish the beneficial effect of yoga in treating depression and sleep complaints, and having adjunctive value in schizophrenia and attention-deficit hyperactivity disorder[272]. Ten trial totaling 840 patients with rheumatoid arthritis included in a meta-analysis revealed a statistically significant overall effect in favor of yoga for physical function, disease activity and grip strength but no effects were found for pain, tender joints, swollen joints count or inflammatory cytokines[273]. A review of 28 randomized controlled trials involving 1789 participants with inflammatory bowel disease was indicative of beneficial effects on generic inflammation as well as disease-specific biomarkers (fecal calprotectin and CRP)[274]. In adults with chronic inflammatory–related disorders, the most common biomarkers measured have been IL-6 and CRP. Most studies (n = 11/15) reported positive effects on inflammatory biomarkers from baseline to post-yoga intervention [275]. Other approaches such as brain education-based meditation, a modernized method of traditional mind-body training in Korea performed in patients with type-2 diabetes/hypertension led to reduce low density lipoproteins cholesterol and inflammatory gene expression, and improved some elements of the investigated physical and mental health of the patients[276]. Exposure to natural environmental stimuli to induce physiological relaxation, potentially enhancing immune functions and aiding in disease prevention is also proposed. However, the current body of evidence offers limited support for advocating nature immersion therapies as a primary approach to reducing stress, depression, and anxiety[277].

Sexual activities

Not surprisingly higher self-reported stress in daily life of women was associated with lower levels of sexual activity and a decrease in relationship satisfaction[278]. Despite there are few studies to consider whether sexual activity is a player of eustress, there are few examples to suggest it. Sexual intercourse relieved stress for both men and women in satisfying relationships, but not in unsatisfying relationships[279]. A study on 425 German women (mean age 26.6 years) revealed that 92% of them indicated having masturbated in the past 12 months, once or several times a day (10%), once to two or three times a week (53.1%). For many women, masturbation does not represent "a partner substitute" to seek sexual pleasure, but rather is a stress coping and relaxation strategy[280]. During the COVID-19 pandemic, pornography consumption and solo sex activities offered an alternative to conventional sexual behavior during a highly stressful period and were found to have positive effects of relieving psychosocial stress otherwise induced by the pandemic. Those who maintained an active sexual life experienced less anxiety and depression, and greater relational health than those who were not sexually active[281]. Results from cross-lagged models suggest that high frequency of sex is positively related to later risk of cardiovascular events for men but not women, whereas good sexual quality seems to protect women but not men from cardiovascular risk in later life[282]. The demonstration that in mole rats sexual activity is favoring longevity, through the putative release of dehydroepiandrosterone, an anti-aging molecule, remains to be transposed to humans[283]. Sexual activity triggers the release of dopamine, endorphins, and oxytocin, which help promote relaxation and well-being and reduce the stress hormone cortisol. Oxytocin can impact stress, anxiety, and the processing of negative emotional stimuli[284]. It has been shown that oxytocin maintains homeostasis, shifts the set point for adaptation to a changing environment (allostasis) and contributes to recovery from the shifted set point by inducing active coping responses to stressful stimuli (resilience)[285]. Indeed, oxytocin has a beneficial therapeutic effect for the treatment of stress-related neuropsychiatric disorders (anxiety, depression and post-traumatic stress disorder)[286].

An interesting study performed in male on the effect of orgasm on the immune system failed to demonstrate any effects on T cell and B cell or on the production of IL-6 and TNF. In contrast, sexual arousal and orgasm increased the absolute number of leukocytes, in particular NK cells ($CD3^-$, $CD16^+$, $CD56^+$), in the peripheral blood[287]. Whether orgasm would boost the immunity against sexually transmissible infection remains to be fully established.

Drugs

Steroids and nonsteroidal anti-inflammatory drugs are medications aimed to reduce or eliminate symptoms related to an inflammatory disorder. They have direct inhibitory properties in the inflammatory process. Of note, anti-depressant

drugs have also different actions on immune cells and inflammation[288,289].

Most chemotherapeutic drugs employed to treat cancer patients display side effects such as nausea, vomiting and depressed food intake[290]. Indeed, cancer cachexia is responsible for up to 30% of cancer deaths. Recently, it has been reported that a liver stress pathway ends to an increased hepatic expression and circulating levels of growth differentiation factor 15, which plays a crucial role in regulating body weight in response to cisplatin and doxorubicin. Its increase follows a selective activation by chemotherapy of inositol-requiring transmembrane kinase endoribonuclease-1α, an ER stress sensor within the liver[291].

Nutrients

Some foods or some excesses (red meat, salt, sugars, lipids) can favor inflammatory disorders. For example, the consumption of ultra-processed foods was associated with a 5% increased risk of cardiovascular diseases and a 12% higher mortality[292]. The inflammatory status can be modulated by the intake of food containing anti-inflammatory constituents such as omega-3 fatty acid in fish oil, vitamin D, or probiotics (see above). Other natural constituents can be taken on purpose as additive such as red ginseng very popular in South Korean[293], or resveratrol, a molecule found in red wine, which, like red ginseng, inhibits the inflammasome activation[294].

Healthy environment

Just like bad air mentioned above, there is also good air, a healthy air that helps diseased humans to regain their health. During the 19th century, Madeira was a place to be when one was suffering tuberculosis[295]. However, this was not an absolute remedy. Princess Maria Amélia (1831-1853), the daughter of the Brazilian Emperor Dom Pedro I, developed a persistent cough, the onset of tuberculosis. The princess traveled to the island of Madeira where she passed away at the age of 21. Thomas Wakley (1795-1862) an English surgeon, and the founding editor of *The Lancet* had been in declining health for about ten years because of tuberculosis. He joined Madeira where he died in May 1862. Two famous Pasteurians lost their wife in Madeira. It was the case of Rose A. Shedlock who married Emile Roux and who passed away in Funchal on October 10th, 1879. She was 30 years old[296]. Few years earlier, Élie Metchnikoff accompanied his first wife in Madeira. Ludmila Vassilievna Feodorovna passed away on April 20th, 1873. She was 26 years old. Paul Langerhans (1947-1988), the discover of the Langerhans cells of the skin, and the islets of Langerhans in the pancreas, after he contracted tuberculosis in Freiburg, and after the failure of several treatments, left Germany for the island of Madeira in the hope of finding a climate there conducive to curing his illness. Unfortunately, he passed away in Funchal on July 20th, 1888. He was 41 years old.

Hermann Brehmer (1826-1889) when he was a student was diagnosed with tuberculosis. He went to the Himalayas, and came back cured. Back in Germany he authored a dissertation titled "Tuberculosis is a Curable Disease". In 1854, he founded the first sanatorium in Görbersdorf/Sokołowsko, offering to his patients fresh air, and good nutrition. In 1876, John Harvey Kellogg (1852-1943), in his sanitarium, introduced vegetarianism and invented for his patients the famous cornflakes. Kellogg strongly discouraged sexual relations which he condemned. Even more than sexual relations, it was onanism that Dr. Kellogg abhorred. To combat what he considered absolute evil in humans, Kellogg even recommended various forms of genital mutilation!

In addition to the quality of environmental air, and a healthy food, the quality of water does also play a role on health. Two epidemiological studies conducted in France and Quebec established an increased frequency of Alzheimer cases among the population living in areas where water is prepared by aluminum precipitation[297,298].

The sun is another environmental parameter which illustrates the yin yang concept while influencing our lives. On one hand, it contributes to the skin production of the beneficial vitamin D, and a limited exposure to sun like in the Inuit population is associated with a prevalence of vitamin D insufficiency[299]. On another hand, exposure to sunlight and spending leisure time in greenspaces have a positive impact on people's mental health, including depression, anxiety, and stress states[300]. This is also illustrated by the regional distribution of suicides and the relationship with sunlight duration[301]. The rate of production of serotonin by the brain is directly related to the prevailing duration of bright sunlight. Serotonin is a neurotransmitter involved not only in mood, but also in cognition, regulation of feeding behavior, anxiety, aggression, pain, sexual activity, and sleep[302]. There are substantial amounts of evidence linking variants of genes coding for tryptophan hydroxylase-1 (the enzyme involved in the synthesis of serotonin), the serotonin transporter and the serotonin receptor 2A gene with suicidal behavior[303]. In addition, serotonin has immunomodulatory properties *via* the serotonin receptor 2A expressed by human PBMCs, including inhibition of TNF[304]. On the other hand, the beneficial effects of sun exposure are opposed to the increased frequency of skin cancer upon exposure to UV[305] in genetically predisposed people[306].

Failure of united nations

While Nature has set up efficient mechanisms to prevent propagation of inflammation at the level of the cells, the organs and the individuals, unfortunately no efficient mechanism exists at the societal level. After the horrors of world word II, with the idea of "never again" in mind, on 25 June 1945, fifty nations adopted the charter of United Nations (UN) with aim to maintain international peace, to protect the human rights, and to deliver humanitarian aid. The most recent events (*i.e.*, war in Ukraine, war in Gaza, war in Ethiopia) are a sad illustration of the failure of a regulatory mechanism. In 2022, fatalities from organized violence increased by staggering 97%, compared to the previous year, from 120000 in 2021 to 237000 in 2022, making 2022 the deadliest year since the genocide in 1994 during which some 800000 people were slaughtered in Rwanda by ethnic Hutu extremists[307]. But deaths are not the only consequence of wars. The number of people displaced by war, persecution, violence and human rights violations worldwide is likely to have exceeded 114 million by the end of September 2023, according to the UN[308].

Conference of the Parties

Planet Earth has its own regulatory process to avoid perpetuating the insults made to the fragile balance of its climate: The UN climate change conferences (Conference of the parties) meet every year since 1995. Thirty years of awareness that the planet is suffering from natural and human driven outrages. Global warming has never been so obvious and 2023 has been the warmest year in centuries. Despite an ambitious and utopist goal limiting warming to 1.5 °C by the reduction of greenhouse gas emissions, and the transition from energy fossils to renewable energy, the Intergovernmental Panel on climate change expects the 20-year average global temperature to exceed + 1.5 °C in the early 2030s[309].

The effects of climate change on societies is a source of chronic environmental stress. Climate change makes people uncertain and stressed, with a sense of powerlessness. Mental health outcomes of climate change range from minimal stress and distress symptoms to clinical disorders, ranging from anxiety and sleep disturbances to depression, post-traumatic stress, and suicidal thoughts[310]. A large number of people exposed to climate or weather-related natural disasters experience distress and serious mental health consequences. In late December 2022, more than 190 parties adopted the 30 × 30 target, *i.e.* to protect at least 30% of the world's lands, oceans, and inland waters by 2030[311]. Because climate change is contributing to an increase in immune-mediated diseases, such as asthma and other allergic diseases, autoimmune diseases, and cancer, there are urgently needs to adapt to and mitigate the effects of climate change. To favor planetary health and human health, key actions include reducing emissions and improving air quality (through reduced fossil fuel use), providing safe housing (*e.g.*, improving weatherization), improving diets (*i.e.*, quality and diversity) and agricultural practices, and increasing environmental biodiversity and green spaces[312].

THE RESILIENCE

Psychological resilience

Psychological resilience is the ability to cope with adversity and to adapt to stressful life events. It varies widely from person to person and depends on environmental as well as personal factors[313]. We are not born resilient, we become resilient. But not surprising, genetic markers are associated with the low or high reactivity to environmental stress[314]. In the case of a disability linked to a life accident, the implementation of adaptive strategies first allows one to survive, in society, with one's new body. Once this objective is achieved, we are then equipped to embark on another path, that of resilience. The athletes with disabilities are an example of individuals whose strength to fight against adversity has a positive impact on their levels of well-being, personal development, quality of life and integration into society[315]. The concept of psychological resilience applies to both the individual and the society. Depending upon the country, the population may not sense similarly a stressful situation. In other words, the perception of a similar stressful situation can be fairly different from countries to countries. Brain washing, lies and propaganda, can modify the perception of reality by a society, and the use of certain words (*e.g.*, special military operation) instead of others (*e.g.*, war) has boosted the resilience of the Russian populations as it happened during the first years of the war Russia initiated in invading Ukraine. Different is the resilience of the Ukrainian population which is directly facing the horrors perpetuated by the Russian army on its territory and the threat for loss of independence and sovereignty. The Ukrainian population under risk, despite a lower sense of wellbeing and higher levels of distress, sense of danger, and perceived threats has developed an enhanced societal resilience and hope[316]. A recent study investigating 30000 years of human history established that resistance and resilience to disturbances was key in terms of adaptation[317].

Biological resilience

Lifespan exhibits a significant, positive correlation with resistance to stress. Genetic pathways and molecular mechanisms which enhance resistance to exogenous stressors participate in extending longevity[318,319].

Insults such as severe infection, trauma or ischemia-reperfusion can result in multiple organ dysfunction and death. A lack of sufficient adenosine triphosphate provision to fuel normal metabolic processes drives downregulation of metabolism, and thus cellular functionality. In turn, a decrease in metabolism will provide negative feedback to the mitochondria, inducing a bioenergetic shutdown. These processes may offer protection against a prolonged inflammatory hit by sparing the cell from initiation of death pathways, allowing a later recovery phase. This adaptive process has been called "hibernation" by Singer[320]. Immune resilience is defined as the capacity to preserve and/or rapidly restore immune functions that promote disease resistance (immunocompetence) and control inflammation in infectious diseases as well as other causes of inflammatory stress[321]. Immune resilience despite inflammatory stress promotes longevity and favorable health.

Most interestingly resilience can also be observed within the vegetal world. Forests exhibit complex drought responses, indicating both resilience (photosynthetic greening) and vulnerability (browning and tree mortality). Notably, the resilience of shallow-water-table forest weakened as drought lengthened. By contrast, lower-fertility northern Amazonia, with slower-growing but hardier trees (or, alternatively, tall forests, with deep-rooted water access), supported more-drought-resilient forests independent of water-table depth[322].

Species specific resilience

Vertebrates vary in resistance and resilience to infectious diseases. For example, mice are 10^5 more resistant to LPS than humans[323]. Variability in the sensitivity of species to the induction of damaging inflammation in response to equivalent pathogen loads complicates the use of animal models that reflect human disease. It has been reported that the induction of proinflammatory cytokines from macrophages in response to inflammatory stimuli *in vitro* is regulated by proteins in

the sera of species in inverse proportion to their *in vivo* resilience to lethal doses of bacterial LPS over a range of 10000-fold. This finding suggests that proteins in serum rather than intrinsic cellular differences may play a role in regulating variations in resilience to microbe-associated molecular patterns between species[324]. Zoonosis is illustrating how animals can host pathogens which remain healthy while transferring the infectious disease to humans. The human deficiency virus (HIV) has been spreading since a hunter in Kinshasa in 1920 was bitten by a monkey, itself infected with a simian virus (SIV) close to HIV[325]. In striking contrast to HIV infection, natural SIV infection of African nonhuman primates is asymptomatic and usually does not induce significant $CD4^+$ T cell depletion despite high levels of virus replication[326]. Migratory birds can also be zoonotic reservoirs and can contribute to the dispersion of microorganisms as biological carriers transmitted initially to domestic birds. Avian species do not always have symptomatic influenza infection. It has been suggested that the recognition and elimination of invading pathogens (resistance) or the control of the infection associated tissue damage (tolerance) may explain asymptomatic or minimally symptomatic infections[327].

Bats occupy all regions of the planet, except the Arctic and Antarctic poles. Bats harbor many viruses and share human and domestic animal environments. They have a long-life expectancy and their diversity and winged locomotion favors the emergence and dispersal of new viral species. It has been estimated that at least 3200 different coronaviruses exist in all the different bats[328]. Bats display an altered inflammasome, leading to a reduced ability to produce IL-1. They have dampened transcriptional priming, reduced protein function, loss of one player (*AIM2*), and reduced caspase-1 activity which leads to an overall reduction in inflammation[329,330].

Of course, resilience is also a mechanism displayed by bacteria to address soil environmental changes and exposure to antibiotics. Climate change is responsible for longer period of drought that select bacterial community more resistant and resilient to drought[331]. The ability of bacteria to recover after being perturbed by an antibiotic corresponds to resilience [332]. For example, a resilience regulator has been identified in *Mycobacterium tuberculosis* of which mutants shorten the post-antibiotic effect, meaning that they enable the bacteria to resume growth after drug exposure faster than wild-type strains[333]. Interestingly, in *Pseudomonas aeruginosa*, pyocyanin, a self-produced antibiotic activates defenses that confer collateral tolerance specifically to structurally similar synthetic clinical antibiotics[334].

Disease tolerance

Disease tolerance is a mechanism that allow the host to maintain tissue integrity and to limit organ damage[335]. During inflammation, following microbial or sterile insult, different physiological mechanisms are turned on allowing a return to homeostasis. We previously mentioned the mitochondrial metabolism shutdown that allows a further recovery of the organs during systemic inflammation[320]. Interestingly, during this process, the same players can display a yin yang behavior. For example, IL-6 is a paradigm of ambiguity[225]. It can protect the lungs during a viral infection[336], but it favors coagulation[337]; IL-6 can promote the regeneration of tracheal epithelium[338], but it can also contribute to cachexia[339]. Furthermore, IL-6 is the dominant cytokine inducible upon acute stress and is a key regulator of the acute psychological response to stress. Stress-inducible IL-6 is produced from brown adipocytes in a beta-3-adrenergic-receptor-dependent fashion. During stress, endocrine IL-6 is the required instructive signal for mediating hyperglycemia through hepatic gluconeogenesis, which is necessary for anticipating and fueling "fight or flight" responses[340].

FROM MATHEMATICAL MODELS TO THE USE OF ARTIFICIAL INTELLIGENCE

Hans Selye was the first one to propose a very simplistic and preliminary mathematical model: "Yet the visible degree of stress in a man is proportionate to the sum of everything that is going on him at the time. How could the body calculate the sum of, say, that much contraction plus that vision plus that much inflammation?" Many authors have offered more sophisticated models[1,341-345]. It is not sure that any of the mathematical models can integrate all the elements listed in this review that affect individual response to stress and inflammatory processes. Particularly, the genetic diversity and individual responsiveness to stress could complexify the mathematical models. Ghezzi[2] challenged the fact that theoretical and mathematical models need to be validated in a relevant population. Indeed, it is not sure that the mathematical models could easily fit globally knowing the genetic heterogeneity of the human population, the genetic predisposal to diseases and the genetic diversity of the inflammatory response to stress.

Personalized medicine (or precision medicine) will revolutionize the 21st century medicine. Whether mathematical modeling will offer the ground for personalized medicine remains an open question. Of note, personalized medicine may in part require individual's genetic profiling to guide decisions made regarding the prevention, diagnosis, and treatment of diseases[346].

The aim of precision medicine is to design and improve diagnosis, therapeutics and prognostication through the use of large complex datasets that incorporate individual genes, function, and environmental variations. There is also a large heterogeneity among the patient with psychiatric disorders with respect to symptoms, treatment responses, causal role of immune mechanisms in the pathogenesis of depression and the nature of the psychological or environmental stressors such as childhood maltreatment. Personalizing clinical phenotyping and definition of subgroups of patients become a challenge in order to offer the most appropriate treatment[347,348]. Artificial intelligence (AI) is expected to interact constructively with precision medicine. Genetic, epigenetic, metabolomic, microbiotic and proteomic analyses are generating a huge amount of information that AI will consider for further individual stratification[349]. The complex pathophysiology involving multiple proinflammatory pathways and molecular dysregulation across different inflammatory disorders can be modeled by AI and used to design drug candidates *in silico*, and predict drug efficacy in virtual patients[350].

CONCLUSION

As pointed out by Androulakis[3]: "Inflammatory stress-related non-communicable conditions are a major challenge for 21st century medicine". As we tried to illustrate in this review, the cell, the organ, the individual, the society, and the planet share many stressors of which the consequences are extremely interconnected ending to the domino effect and the butterfly effect[351-353]. These effects are exemplified by the consequences of climate change on wine production[354], or on decline of bees[355], both events having an economic impact. In addition, increased melting of ice in Greenland and Antarctica has decreased the angular velocity of Earth more rapidly than before, with effect on Coordinated Universal time which closely follows the rotation of Earth[356]. Similarly, external distress suffered by an individual will lead to the release of mediators that will affect its mood, its organs and its cells. Nature has set up a complex mechanism aimed to counteract the inflammatory response to stress, and the brightness of scientists can further help nature to address the inflammatory insults and to favor global health. However, those stressful events which depend on the human activity (war, climate change …) remain weakly controlled, with an increased alteration by societies and the planet. To conclude, let us quote Dr Tedros Adhanom Ghebreyesus, World Health Organization director-general: "If our planet was a patient, it would be admitted to intensive care. Its vital signs are alarming. It is running a fever, with each of the last nine months the hottest on record, as we hurtle towards the 1.5 °C threshold. Its lung capacity is compromised, with the destruction of forests that absorb carbon dioxide and produce oxygen. And many of the earth's water sources - its lifeblood - are contaminated. Most concerning of all, its condition is deteriorating rapidly. Is it any wonder, then, that human health is suffering, when the health of the planet on which we depend is in peril? The health of humans, animals and our environment are woven together in a bond that is inextricable, yet fragile. We belong to the same unique, finely balanced ecosystem. This is not a new realization. Hippocrates, the father of medicine, wrote in the 5th century BC that, 'The physician treats, but nature heals'[357]".

FOOTNOTES

Author contributions: Cavaillon JM proposed the topic; Chaudry IH added additional ideas and amended the English language; Both authors wrote the manuscript.

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MINIREVIEWS

Perspectives on the extracellular matrix in inflammatory bowel disease and bowel decellularization protocols

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Abstract

The extracellular matrix (ECM) is a non-cellular three-dimensional structure present in all tissues that is essential for the intestinal maintenance, function and structure, as well as for providing physical support for tissue integrity and elasticity. ECM enables the regulation of various processes involved in tissue homeostasis, being vital for healing, growth, migration and cell differentiation. Structurally, ECM is composed of water, polysaccharides and proteins, such as collagen fibers and proteoglycans, which are specifically arranged for each tissue. In pathological scenarios, such as inflammatory bowel disease (IBD), the deposition and remodeling of the ECM can be altered in relation to the homeostatic composition. IBD, such as Ulcerative colitis and Crohn's disease, can be differentiated according to ECM alterations, such as circulating levels of collagen, laminin and vimentin neoepitopes. In this context, ECM presents parti-cularities in both physiological and pathological processes, however, exploring methods of tissue decellularization is emerging as a promising frontier for new therapeutic interventions and clinical protocols, promoting the development of new approaches to intestinal diseases.

Key Words: Extracellular matrix; Inflammatory bowel disease; Decellularization protocols; Regenerative medicine; Intestine

Core Tip: Regenerative medicine provides a promising perspective in the treatment of inflammatory bowel diseases (IBD) by targeting tissue repair. The development of decellularization techniques to produce scaffolds that mimic the native environment of the intestine is crucial to the advances in this field. This article addresses how research focused on the extracellular matrix in IBD tissues and studies ways to improve regenerative therapies that represent fundamental steps towards furthering the efficacy and safety of IBD treatments.

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INTRODUCTION

The extracellular matrix (ECM) plays a fundamental role in intestinal maintenance, function and structure[1]. It has a non-cellular three-dimensional conformation and is present in all tissues, with each tissue having a unique composition. Structurally, the ECM is composed of water, polysaccharides and proteins as well as collagen fibers and proteoglycans[2].

The ECM has several functions, including providing physical support for cell integrity, tissue elasticity and remodeling to control tissue homeostasis[3-5]. The molecular interaction between ECM and cells is complex and reciprocal, allowing the regulation of various processes involved in tissue homeostasis and in the wound healing process, as well as taking part in cell growth, migration, differentiation and in the production of neo-ECM[6]. Despite this, it is often neglected in relation to both its physiological functions and complications. Regarding injury and disease processes, matrix deposition and remodeling can be strongly altered according to the homeostasis composition. The pathological formation of the matrix may be associated with changes in the expression and activation of enzymes along with other factors that alter the matrix[7].

Inflammatory bowel diseases (IBD) are conditions characterized by an inadequate immune response, presenting chronic inflammation with symptomatic moments and clinical remission. The most prevalent forms of IBD are Crohn's disease (CD) and Ulcerative colitis (UC)[8]. UC affects the superficial mucosa, starting in the rectum and being limited to the colon, while CD causes transmural inflammation and can affect any region of the gastrointestinal tract[9].

Regenerative medicine seeks to develop approaches to replace or regenerate tissues and organs that have lost their functional effectiveness, as well as to treat tissue injuries. Scaffolds are therefore responsible for providing a support structure for cells, growth factors, signaling molecules and offering structural, biochemical and biomechanical properties capable of guiding as well as regulating cell behavior and tissue development[10]. This brief review proposes tissue bioengineering as a relevant technique in regenerative medicine for IBD, considering the aspects of the ECM in tissues presenting IBD as well as different protocols used to obtain a scaffold in order to properly remove cells, DNA and cell contents.

ECM AND COMPONENTS

ECM is recognized by its role as a support for delicate cells of the body, as well as for providing mechanical properties for each function[11]. In addition, it supplies the chemical and mechanical signals necessary for the regulation of cell proliferation, migration, survival and differentiation, crucial to maintain homeostasis and proper tissue function[12,13]. The ECM can be classified into two types, according to its components and structure: The basement membrane and the interstitial matrix surrounding the cells, and the pericellular matrix, which is in close contact with the cells[14]. The interstitial matrix is located in a thin layer below the basement membrane, consisting of four main components: Collagen IV, laminin, nidogen and perlecan[15]. The matrisome, proteins associated with the matrix, is made up of the main ECM constituents (collagens, glycoproteins, proteoglycans and polysaccharides), associated regulators and secreted factors. It is produced by unique combinations of resident cells found in different tissues[16].

Studies indicate that the mesenchyme is responsible for tissue differentiation, the matrisome being dynamically maintained and associated with various responses, such as cell growth and differentiation, through direct interactions between ligands and receptors. It is also responsible for the biomechanical properties, being able to sequester and regulate the availability of cytokines along with growth factors. Embryonic precursor cells grown on complex ECM structures from decellularized tissues are capable of differentiating into epithelia of the same type of tissue from which the structure was derived[16].

Fibroblasts are fundamental in parenchymal organs and are responsible for providing structure, regulation, survival, differentiation as well as migration of cells. Thus, fibroblasts support the architecture of the mucosal crypt, the remodeling of the ECM and the efficiency of the immune system in the intestine[17-19]. In general terms, ECM components, such as collagens, represent the most abundant family of proteins found in the ECM. Collagens are proteins that form a triple helix of three polypeptide chains, forming supramolecular structures in the ECM that vary in size, function and distribution throughout the tissues[20].

The elastic fibre is a specialized structure of connective tissue and its main component is elastin, a structural glycoprotein. However, different proportions of elastin and microfibrils mediate different functions adaptable to each local tissue demand. Mature elastic fibers are responsible for the reversible distension of many elastic tissues and ligaments, being synthesized by fibroblasts, chondrocytes and hydrolyzed by elastases[21]. ECM regulators such as family members are classified into subgroups of mainly collagenases, stromelysins, matrilysins, membrane-type metalloproteinases and gelatinases[22].

Proteoglycans are among the structural and functional biomacromolecules of the tissue and are made up of a protein core covalently linked to one or more glycosaminoglycan chains of either the same type or different. In addition, glycosaminoglycans are long, negatively charged heteropolysaccharides containing disaccharides comprising: N-acetylated hexosamines (N-acetyl-D-galactosamine or N-acetyl-D-glucosamine) and D-/L-hexuronic acid (D-glycuronic acid or L-iduronic acid). Six types of glycosaminoglycans are recognized: The chondroitin sulphate/dermatan sulphate galactosaminoglycans, and the heparan sulphate, heparin, keratan sulphate and hyaluronic acid glycosaminoglycans[23].

Metalloproteinases are zinc-dependent[24-27] composed of 24 families grouped by domain structure and substrate preference, being able to degrade matrix components such as cytokines, chemokines, adhesion molecules, growth factors and their receptors, which is why they are important for the homeostasis processes of ECM remodeling. They also contribute to angiogenesis, cell migration, tissue repair and inflammation[24]. The family of metalloproteinases have common structural and functional elements, with substrate specificity and affinity, being divided into different classes such as collagenases [matrix metalloproteinase (MMP)-1, -8 and -13], gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, -11 and -19), matrilysins (MMP-7 and -26), membrane-type MMPs (MT-MMPs), macrophage elastase (MMP-12), among others[24].

In normal tissues, the expression of collagenases is low and they are upregulated in inflammation processes linked to tissue destruction. Since they are potentially dangerous to tissues, their activity and inhibition are highly regulated by tissue inhibitors of metalloproteinases (TIMPs). MMPs are synthesized and secreted rapidly, and may be stored in granules of inflammatory cells with release upon stimulation[24]. MMPs are controlled by three different pathways: Regulation of transcription, inhibition by specific inhibitors or proenzyme activation[26].

ECM COMPONENTS IN THE GASTROINTESTINAL TRACT

Fibroblasts are present in the intestinal compartment and are responsible for producing ECM components, while myofibroblasts are fibroblasts with smooth muscle cell properties, such as the expression of alpha-smooth muscle actin, which plays a role in smooth muscle contraction[28]. They may be present and support the architecture of mucosal crypts, ECM remodeling and immune fitness. They provide support to the architecture of the crypts in the colon by creating discrete anatomical zones that maintain epithelial stem cell niches in defined areas, while differentiating epithelial cells and inhibiting cell proliferation in others[29]. Fibroblasts are recognized as the main source of MMPs in the intestine, furthermore, interleukin (IL)-21 is part of the regulation of intestinal fibroblasts synthesis and the production of MMPs increases as a result of the cells' cytokine production[30].

MMPs and TIMPs (MMPs tissue inhibitors) are expressed by different cell types in all layers of the intestine and are important in the remodeling process of the ECM in the intestine[31,32]. In normal tissue, epithelial cells express mainly MMP-1, -3, -7, -10 and -12, while mononuclear cells express MMP-2, -3, -9 and TIMP-1. Polymorphonuclear cells express only MMP-9 and fibroblasts just express MMP-2[33].

Type IV collagen, described as a basement membrane collagen, performs the structural function of the intestine and is the most abundant basement membrane collagen. The α1, α2, α5, and α6 chains of collagen IV are the most abundant in the intestine, while the α3 and α4 chains present in the basement membrane of the intestinal epithelium are only found on the surface of the mucosa[34,35]. Type IV collagen is found at the interface between the basement membrane and the interstitial matrix, separating the two layers. It binds to various proteins in the ECM, the basement membrane and the interstitial matrix, such as type IV collagen, perlecan, decorin and fibronectin[36].

In one study, the expression of collagen chains was examined in both human and animal small intestines[37]. The classic α1 and α2 (IV) chains were found to be individually distributed in the basement membrane throughout the epithelium, as well as in cellular elements present in the fetal and adult small intestine's lamina propria, and α5 was found in the mucosa of the fetal small intestine. However, the α3 and α4 (IV) chains were not identified in the intestine, which is consistent with the restricted distribution of these chains in tissues[34].

ECM IN IBD

Cells are not able to grow in solutions and need to be anchored to a solid matrix. This way, cells can bind to receptors such as integrins, which are responsible for the cells' perception of the ECM rigidity and thus alter the intracellular state, hence verifying that the ECM is capable of altering the behavior of cells, including differentiation[28]. During chronic inflammation, the excessive degradation and inadequate deposition of ECM by proteases makes it impossible to restore the matrix, a process that is called fibrosis, and may be responsible for the development of cancer. Therefore, ECM has a great influence on cancer development and ECM remodeling is fundamental to the development of cancer as a consequence of chronic inflammation[38-42].

IBD are chronic and relapsing diseases. In CD, inflammation can occur throughout the gastrointestinal tract in all layers of the intestine, but is most present in the ileum and colon[43]. In UC, there is a greater severity of inflammation and pathological alterations in the mucosa and submucosa. However, it is possible to observe that in intestinal diseases, an increased remodeling of the ECM is common due to the excessive and prolonged inflammatory response, interfering in the structure and functioning of the intestinal tissue's ECM[44].

Fibrosis is a pathophysiological mechanism that promotes the deposition of connective tissue in the ECM as a consequence of injury[45], and is a serious complication in the intestine[46] (Figure 1). Seen mainly in CD, intestinal fibrosis occurs through the activation of fibroblasts. Excessive deposition of ECM impairs the gastrointestinal motility and can lead to intestinal stenosis and obstruction, which is a cause of morbidity and mortality in IBD. Collagen deposited in intestinal tissue can cause chronic hypoxia and stimulates neoangiogenesis through the positive regulation of the vascular endothelial growth factor, allowing more fibrosis to be deposited, which cannot be reversed by controlling inflammation [45].

In general, mucosal inflammation influences *MMP* gene variation and deregulation promotes the development of fibrosis. The deposition of collagen fibres can also be observed, especially type I and type III, due to the high activity of fibroblasts and myofibroblasts. Biglican proteoglycan, which is involved in the assembly of collagen fibres, is also abnormally degraded and contributes to the fibrotic process. In addition, an accumulation of ECM fragments can be observed, which are released during the remodelling process and cause deterioration of intestinal function in IBD patients[47].

Furthermore, it is possible to observe that, during inflammatory processes, alterations in the composition of the ECM lead to a greater affinity for leukocyte binding and a greater retention of immune cells in the inflammation, despite the possibility of the matrix metalloproteinases production by immune cells and the consequent ECM degradation, however, the deregulated activity and inhibition of metalloproteinases by their inhibitors can lead to serious complications such as fistulas and fibrosis[24].

In IBD, it is possible to observe regulatory molecular mechanisms in the maintenance of the ECM integrity, such as hyaluronan, a non-sulfated branched glycosaminoglycan that participates in wound healing processes, in the migration and proliferation of cells and in the modulation of the inflammatory process, which means that there is a glycosaminoglycan increase during IBD[25]. Overexpression of tenascin-C, a protein that mediates the inflammatory process, is also observed in IBDs. Studies with tenascin-C knockout animals have shown that the absence of this protein can trigger an anti-inflammatory and tissue regenerative process[48]. Tenascin-C has already been described in other studies demonstrating its expression in the adult intestine, which is one of the few organs in which tenascin remains at maturity [37,49], as well as being present in IBD such as UC[49]. Other alterations in the ECM may be correlated with the circulating levels of collagen, laminin and vimentin neoepitopes, which correlate with IBD subtypes and may act as a differentiation mechanism between UC and CD[7].

Treatment with dextran sulfate sodium, an inducer of IBD, causes an increase in the levels of collagen I, integrins and focal adhesion kinases which may impair intestinal repair after treatment with dextran sulfate sodium. It is suggested that Yes-associated protein (YAP) is essential for the detection of ECM properties during the regenerative process, since focal adhesion kinases inhibition is responsible for the YAP decrease. It can be seen that ECM detection is fundamental for modulating the intestinal regeneration response[28].

The main proteases in intestinal tissue responsible for remodeling the matrix are neutrophil elastases and meprins. The expression of periostin is higher when compared to normal tissue, and it may be deposited in the stromal component in IBD as well as in tumors, in addition, YAP/TAZ has also been shown to be significantly increased in colorectal tumors and weakly increased in inflammatory diseases[50].

In CD, the inflamed mucosa presents a lower gene expression of MMP-2, MMP-9[51] and MMP-3[24] than in UC. In another publication, there was an overexpression of MMP-1, -3, -9, and -12 in UC when compared to CD[52]. Some studies have indicated the role in the induction of MMPs in IBD, based on this, IL-17A and IL-17F may be associated with an increased secretion of MMP-1 and -3 in the subepithelial myofibroblasts, it may also increase the actions of IL-1 and β and tumor necrosis factor-α on MMPs being mediated by mitogen-activated protein kinases[27].

TISSUE BIOENGINEERING

When a tissue suffers an injury, it can be said that the ECM is also damaged, which impairs the functional support of the tissue repair process and results in the formation of scar tissue[53], which can become an aggravating factor for an effective treatment. In this context, tissue bioengineering has been seen as an alternative to free patients from these exhausting treatments, offering new perspectives for tissue repair[54].

A challenge in this process is to obtain an ECM with suitable physicochemical properties and the complex biological characteristics native to ECM for functional tissue production. These necessary physicochemical properties consist of surface structure, pore size, mechanical properties, biocompatibility, biodegradability and cell adhesion[6]. The main product of the decellularization process is the ECM, so it must act as the physical structure in which the cells are integrated, as well as regulate processes such as growth, differentiation, migration and morphogenesis[12]. In order to decellularize tissues, there are enzymatic, chemical and/or physical processes for removing cells from the ECM[2]. The complete removal of DNA, the preservation of the tissue's specific mechanical properties, along with the maintenance of the structural and functional proteins of the ECM are of fundamental importance for the process effectiveness. In this process, a large number of cells and immunogenic molecules must be removed, while proteins such as collagen, elastin, fibronectin and macromolecules such as proteoglycans and glycosaminoglycans must be maintained[2,55].

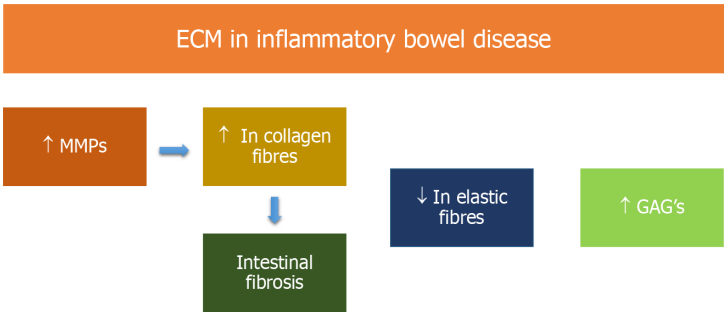

Figure 1 Summary of changes in the extracellular matrix in inflammatory bowel disease. There is an increase in collagen fibres influenced by the action of metalloproteinases, contributing to the development of fibrosis. There is a reduction in elastic fibres and a change in glycosaminoglycans, affecting the elasticity and hydration of the tissue. ECM: Extracellular matrix; MMP: Matrix metalloproteinase; GAG's: Glycosaminoglycans.

In the bioengineering of ECM biomaterials for the gastrointestinal tract, there is the challenge of mimicking the mucosal and muscular layers, as well as restoring motor functions so that proper gastrointestinal peristalsis occurs. It is therefore necessary to try to modulate chronic inflammation and stimulate the deposition of neo-ECM in the gastrointestinal tract[6]. Furthermore, in order to produce collagen, the cells need to have sufficient adhesion to the ECM for cell proliferation. Thus, a biomaterial that resembles the structure and functions of ECM is required[56].

Biomaterials produced from decellularized ECM are used in different reconstructive surgical applications and are increasingly applied in regenerative medicine strategies[57]. These biomaterials are capable of supporting specialized cell types, promoting a regenerative process and providing a microenvironment similar to the target tissue. For example, some biomaterials such as the submucosa of the small intestine and the matrix of the urinary bladder have been approved by the Food and Drug Administration for the manufacture of regenerative biomaterials with evidence in skin, muscle and gastrointestinal tissues[6].

Scaffolds are classified as either natural or synthetic. Natural scaffolds include natural polymers such as collagen, silk fibroin and hyaluronic acid, while synthetic scaffolds include synthetic polymers such as polyglycolic acid, polylactic acid, polylactic acid-co-glycolic acid and polycaprolactone. The natural ECM is an ideal biomaterial for tissue scaffolding. ECM provides support for the cells structure, regulates cell growth, proliferation and differentiation through the action of bioactive substances, growth factors and cytokines. Hence, natural ECM is composed of a variety of complex components such as proteins and glycosaminoglycans with a highly complex spatial structure and proportion of components, making therefore difficult to reconstitute with the same composition using conventional physicochemical methods[58]. Thus, decellularization is an effective method for the preparation of biomimetic ECM structures, and studies have shown that decellularized ECM scaffolds can remodel various tissues, such as for the regeneration of the heart, valves, blood vessels, skin, nerves, lungs, cornea, esophagus, as well as of the submucosa of the small intestine and various other types of tissues[58].

TECHNIQUES FOR DECELLULARIZING INTESTINAL TISSUE

There are different agents that act to aid the decellularization of each tissue, and it is necessary to investigate various factors such as cellularity, density and lipid content in order to choose the best agent. Ionic detergents, such as sodium dodecyl sulfate (SDS), solubilize cell membranes and dissociate proteins from the ECM, being highly effective at removing cells, although they may damage the ECM. Non-ionic detergents, such as Triton X-100, are less damaging to the ECM and can remove cell debris from thicker tissues; however, it may be necessary to combine detergents for better results[57]. In general, other agents take part in the decellularization process, such as deionized water, which is capable of causing hyposmotic shock in the cells[2].

Studies published by Kim *et al*[59] adapted decellularization techniques in the small intestine and stomachs of pigs based on the type (non-ionic or ionic) and time of treatment. In a first optimized protocol, they used Triton X-100, a non-ionic detergent, and observed the removal of cellular components along with the preserved components of the ECM. In a second protocol using sodium deoxycholate, an ionic detergent, the effective complete removal of cellular components was verified, however, it was not possible to preserve the structure of the ECM due to the glycosaminoglycans decrease. However, they observed that ionic detergents may be responsible for damaging the ECM and impairing the bioactivity and properties of ECM hydrogels[59].

On the other hand, other studies have indicated that the decellularization process used sodium deoxycholate in the distal neck of mice allowed the tissue to become opaque and translucent and completely removed the DNA, so there was no cell growth after the tissue was immersed in cell culture medium. However, the nature of the decellularization agents such as deionized water, sodium deoxycholate and DNAse used in the protocol were ideal for removing the cells as well as for preserving the ECM structure[2,60].

Another publication defining the protocol with 1% (v/v) Triton X-100 with 0.1% (v/v) ammonium hydroxide overnight in rat ileum showed that decellularization was successful, given the minimal amount of DNA present after the perfusion decellularization process, the tissue was translucent, as well as preserved villous structures on the luminal side

of the tissue[61]. Another study in the small intestine of rats also using 1% (v/v) Triton X-100 with 0.1% (v/v) ammonium hydroxide in dH_2O for 24 hours followed by immersion in peracetic acid to sterilize the tissue found translucent tissue, preservation of the mesentery and macroscopic visualization and permeability of the vascular channels. There was almost total removal of cells and 97% removal of DNA after the decellularization process[62].

Decellularization by serial perfusion using SDS, triton X-100 and deionized water reduced the total DNA content per unit length of original small intestine to less than 3%, immunofluorescence confirmed the presence of major ECM proteins such as fibronectin, laminin and collagen I, also maintaining about 40% of the intestinal collagen, while reducing sulfated glycosaminoglycans and elastin[63]. Another study used 0.5% SDS in human fetal intestines for 12 hours in which it was possible to observe the preservation of the ECM structure and removal of cells, with no degradation of collagen and no nuclear content observed after decellularization[64].

In order to define a suitable protocol for the decellularization of submucosal tissue from the small intestine of goats, Singh *et al*[65] used four different types of protocols: In the first protocol, they used the ionic detergent 0.05% SDS and the non-ionic detergent 0.1% Triton-X 100, while the second used a degreaser (methanol/chloroform, 1:1 v/v), enzymatic digestion with 0.05% trypsin/0.05% EDTA and was incubated with 0.5% SDS in a 0.9% sodium chloride solution. The third protocol used 1% SDS, then was incubated in 1% triton-X 100 solution, however, the last one used incubation with a 1.0 M KI solution, followed by treatment with 0.1% TX-100. Although all the protocols decellularized the caprine SIS, the DP4 method stood out for preserving the ECM, presenting a greater viability and cell proliferation compared to the other methods[65]. Another publication used 2% SDS for 24 hours to decellularize the small intestine of goats. This way, they were able to observe a rapid and effective decellularization, as well as biocompatibility and cytocompatibility[54]. Despite the various techniques described (Table 1), it can be said that there are major challenges for the application of regenerative medicine procedures in clinical treatments, as well as the source of cells, meaning that further in-depth studies on the subject are needed[66].

FUTURE PROSPECTS FOR TISSUE BIOENGINEERING IN IBD

In general, regenerative medicine and tissue bioengineering aim to develop functional organs and overcome immunological and transplantation limits. Methods such as decellularisation and recellularisation have been seen as effective in traditional organ transplantation, which integrates a cell-free framework (ECM)[67]. Many studies have elucidated the behaviour of cells in a two-dimensional model based on cell culture on a flat surface with the interaction between the cells and the substrate, which are effective for an initial study. While the three-dimensional model favours the simulation of various cell behaviours such as migration, proliferation, differentiation and morphogenesis, and the relationship between the cells and the ECM[68].

Future research into three-dimensional intestinal models aims to develop *in vitro* models with a greater capacity to reproduce a more immunocompetent physiological environment, further investigating microbial interactions, immunological responses, pathogenicity mechanisms and cellular alterations mediated by dysfunctions in microbial composition. Examples of immunocompetent three-dimensional intestinal models using organ-on-chip technology were compared with organoid and two-dimensional models, in which the model showed greater cell viability with greater expression of E-cadherin and zonula occludens-1, although non-biological supports were used to construct three-dimensional models, such as polystyrene membranes[69].

Decellularisation and recellularisation methods can present difficulties based on the microarchitecture of the ECM during re-endothelialisation, and differences in ECM components such as glycosaminoglycans, elastin and fibronectin can affect the development of the endothelium. It is therefore essential to develop and apply suitable methods using physical, chemical and enzymatic techniques. Finally, although the use of bioreactors has helped in this regard, there are still immunological and inadequate flow challenges. Modifications to the surface used heparin molecules can be improved according to regulatory requirements. In addition, other studies using mesenchymal stem cells and exosomes have been promising, but require further investigation[67].

Other relevant studies about intestinal fibrosis, a consequence of the chronic inflammation of IBD, point to new methodologies for decellularisation in the human duodenum, obtaining three-dimensional scaffolds of intestinal ECM. These techniques preserve essential tissue properties and maintain three-dimensional architecture that surpass traditional two-dimensional culture models and animal models. Recellularisation using primary intestinal myofibroblasts showed superior efficacy while preserving differentiation characteristics. In this way, these scaffolds can present an improvement in relation to IBD research and develop new therapies and targeting to optimise decellularisation and recellularisation techniques with a view to expanding clinical applicability[70].

However, decellularised ECM has excellent functionality as a biomaterial, although its application in the construction of organoids is limited. Based on this, studies have advanced in the search to combine decellularised ECM with other biomaterials to form hydrogels that optimise cell behaviour. It is therefore essential to understand the composition of decellularised ECM and to customise it for different organoids. Finally, it is necessary to adapt these models for clinical use and investigate them in different diseases to improve diagnosis and treatment[71].

CONCLUSION

In summary, IBD requires innovative approaches to improve the treatment of this disease. Regenerative medicine has emerged as a promising proposal for restoring intestinal function through the repair and regeneration of damaged

Table 1 A summary of some of the agents used in the decellularisation process, including the type of agent, concentrations applied, treatment times, characteristics and effects on the tissue, and the preservation of the extracellular matrix

Agent	Type	Concentration	Time	Characteristics and effects	Preservation of the ECM
Sodium dodecyl sulfate	Ionic detergent	0.05%-2%	12-24 hours	Highly effective at removing cells, solubilises cell membranes; can degrade ECM (especially glycosaminoglycans)	May degrade ECM, including collagen and glycosaminoglycans
Triton X-100	Non-ionic detergent	0.1%-1% (v/v)	Variable (*e.g.*, 24 hours)	Less damaging to the ECM, effective in removing cell debris in thick tissues	Good preservation of the ECM structure
Ammonium hydroxide	Alkaline	0.1% (v/v)	Overnight	Used in combination with Triton X-100, aids decellularisation	Preserves villous structures and ECM
Deionized water	Osmotic agent	N/A	Variable	Causes hyposmotic shock in cells, contributing to cell removal	Helps remove cellular content
Sodium deoxycholate	Ionic detergent	Variable (*e.g.*, 0.05%)	Variable	Removes cells effectively, but can compromise the MEC	May degrade glycosaminoglycans, impacting the ECM
Methanol/chloroform	Degreaser	1:1 (v/v)	Variable	Used to remove lipids before decellularisation	Helps remove cellular content
Trypsin/EDTA	Digestive enzymes	0.05%/0.05%	Variable	Used for cellular digestion, in combination with detergents	Helps remove cellular content
KI (potassium iodine)	Additional agent	1.0 M	Variable	Used in combination with Triton X-100 for decellularisation	Helps remove cellular content
Peracetic acid	Sterilising	Variable	Variable	Used to sterilise tissue after decellularisation	Preserves the structure of the mesentery and vascular channels
DNAse	Enzyme	Variable	Variable	Removes residual DNA after decellularisation	No direct effect on ECM, focuses on DNA removal

ECM: Extracellular matrix; N/A: Not applicable.

tissues. To this end, the development of decellularization techniques to obtain scaffolds that mimic the native environment of the intestine is essential. Research regarding the ECM alterations caused by IBD is therefore crucial, as are studies to advance the development of more effective and safer regenerative therapies for IBD.

FOOTNOTES

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MINIREVIEWS

Clinicopathological and molecular insights into odontogenic tumors associated with syndromes: A comprehensive review

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Abstract

The association between genetic syndromes and odontogenic tumors encompasses several entities, reflecting the intricate interplay between genetic factors and the development of these lesions. The present study aimed to comprehensively investigate the associations between genetic syndromes and odontogenic tumors. We delineated the diverse spectrum of syndromic connections, including key syndromes such as Gardner syndrome, Gorlin syndrome, Schimmelpenning syndrome, and others. Our findings underscore the clinical significance of recognizing odontogenic tumors associated with genetic syndromes as diagnostic indicators for early intervention. We advocate for multidisciplinary collaboration among clinicians, geneticists, and researchers to deepen our understanding of the underlying mechanisms driving these syndromic associations. In light of this, our study contributes to the growing body of knowledge in dentistry and medical genetics, offering insights that may inform clinical practice and enhance patient care for individuals affected by genetic syndromes and odontogenic tumors.

Key Words: Genetic syndrome; Odontogenic tumors; Head and neck tumors; Misdiagnosis; Genetic mutations

Core Tip: It is important for the health professional to know that some odontogenic tumors have a close relationship with some genetic syndromes. Knowledge of this relationship can help a correct diagnosis and comprehensive treatment of the patient. Thus, the aim of the present review was to comprehensively investigate the associations between genetic syndromes and odontogenic tumors.

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INTRODUCTION

Odontogenic tumors (OT) represent a heterogeneous group of lesions, ranging from non-neoplastic tissue malformations (*i.e.*, hamartomas) to tumors with varying degrees of malignancy and clinical implications. Although considered uncommon, they account for approximately 1% of all oral diagnoses[1,2]. This prevalence can vary according to geographic region, with higher incidence rates reported in countries located in Asia and Africa[3,4]. The origin of OTs is attributed to the proliferation of remnants of the soft and hard tissues that give rise to teeth; however, the exact pathogenesis of these tumors remains unknown[5].

Some cases of OTs associated with syndromes have been observed[6]. Although syndromes often do not directly relate to neoplastic development, their occurrence may indicate a relationship between genetic factors and the pathogenesis of OTs, thereby contributing to a more comprehensive understanding of these entities in the realm of oral pathology. Accordingly, the aim of the present review was to provide insights into the association or concurrent lesions of OT and genetic syndromes based on clinicopathological and molecular aspects.

GENETIC SYNDROMES AND ODONTOGENIC TUMORS

Hereditary aspects are associated with some head and neck tumors[7]. OTs can manifest in association with various syndromes (Table 1), predisposing individuals to the development of multiple tumors concurrently[8-10].

Gardner Syndrome (Familial Adenomatous Polyposis)

Gardner syndrome, a distinct subtype of familial adenomatous polyposis, is characterized by mutations in the *Adenomatous polyposis coli* (*APC*) gene[11]. The genetic link between Gardner syndrome and the *APC* gene, located on chromosome 5, specifically within band 5q21, was identified in research studies[12-14]. The *APC* gene functions as a tumor suppressor, producing a protein that regulates cell growth and ensures proper cell cycle timing[15]. In Gardner syndrome, mutations in the *APC* gene result in uncontrolled cell growth. Additionally, Gardner syndrome involves other genetic anomalies, including loss of DNA methylation, mutations in the *RAS* gene on chromosome 12, deletion of the *DCC* gene on chromosome 18, and mutations in the TP53 gene on chromosome 17.

Due to the genetic heterogeneity of Gardner syndrome, there can be diverse phenotypic expressions; however, the three primary features are multiple gastrointestinal polyps, osteomas, and soft tissue tumors[16]. This syndrome holds more historical than clinical significance today, as these extraintestinal growths are more closely associated with specific mutation locations in the *APC* gene rather than with familial patterns.

In terms of oral manifestations, individuals may present with osteomas, supernumerary teeth, impacted teeth, odontomas, and osteomyelitis[7,17]. Concerning OTs, several cases of compound odontomas located in the anterior maxilla or complex odontomas in the posterior mandible or anterior maxilla have been described in the literature[18-21]. Additionally, in 2018, Salti and coauthors[22] published a case involving a large odontogenic myxoma in a patient with Gardner syndrome, who also presented with multiple osteomas and a compound odontoma. Figure 1 illustrates a case of Gardner syndrome in a patient with multiple osteomas and an odontoma.

Interestingly, Preuss *et al*[23] conducted a systematic review addressing the prevalence of oral lesions associated with Gardner syndrome. Their investigation revealed that the syndrome could present with lesions localized in the proximity of the jawbones. Notably, unicystic ameloblastoma emerged as the most frequently reported lesion, documented in three cases.

Gorlin syndrome

Gorlin syndrome is a hereditary cancer syndrome inherited in an autosomal dominant manner[7]. This condition is caused by mutations in the patched gene, which encodes a transmembrane receptor responsible for recognizing sonic hedgehog signaling proteins[24,25]. The syndrome is characterized by the presence of numerous basal cell carcinomas, along with accompanying skeletal, ophthalmological, and neurological abnormalities[26]. A small proportion of patients may present with additional features such as hypertelorism, macrocephaly, and cleft lip and/or palate during childhood,

Table 1 Summarize findings of syndromic conditions and odontogenic tumors

Odontogenic tumor	Syndrome	Genetic alteration	General clinical condition
Odontoma	Gardner syndrome	Mutations in the *APC* gene	Multiple colorectal polyps and various types of tumors, both benign and malignant
	Otodental syndrome	Not yet established (chromosome 11q13 deletion syndrome)	Globodontia, sensorineural high-frequency hearing loss and ocular coloboma
Adenomatoid odontogenic tumor	Schimmelpenning syndrome	Postzygotic mutations in *RAS* genes	One or several nevus sebaceous with abnormalities of ocular, cardiac, skeletal, and nervous systems
	Attenuated familial adenomatosis polyposis	Mutations in the *APC* gene, with less common occurrences linked to mutations in the MUTYH gene	Hundreds or thousands of adenomatous polyps in the large bowel
Ameloblastoma	Gardner syndrome	Mutations in the *APC* gene	Multiple colorectal polyps and various types of tumors, both benign and malignant
	Gorlin syndrome	Mutations in the patched (PTCH) gene	Numerous basal cell carcinomas and accompanying skeletal, ophthalmological, and neurological abnormalities
	Schimmelpenning syndrome	Postzygotic mutations in *RAS* genes	One or several nevus sebaceous with abnormalities of ocular, cardiac, skeletal, and nervous systems
	Simpson-Golabi-Behmel syndrome	Mutations in a semi-dominant X-linked gene encoding Glypican 3	Pre- and postnatal overgrowth, distinctive facial anomalies, and abnormalities affecting internal organs, the skeleton, and occasionally, varying degrees of intellectual disability
	Williams syndrome	Deletion of genes on chromosome 7q11.23	Developmental delay, intellectual disability, a specific cognitive profile, unique personality characteristics, cardiovascular disease, connective tissue abnormalities, growth deficiency, endocrine abnormalities, and distinctive facies
Odontogenic myxoma	Gardner syndrome	Mutations in the *APC* gene	Multiple colorectal polyps and various types of tumors, both benign and malignant

APC: *Adenomatous polyposis coli*.

along with desmoplastic medulloblastoma. Furthermore, ovarian neoplasms, cardiac fibromas, mesenteric keratocysts, rhabdomyosarcomas, and meningiomas may also be observed in some cases[7].

The diagnostic criteria for Gorlin syndrome have evolved over time. Initially proposed by Evans *et al*[24] in 1993, the criteria were modified by Kimonis *et al*[27] in 1997 and later revised by Bree *et al*[28] in 2011. A diagnosis can be established based on one of the following: (1) One major criterion and genetic confirmation; (2) Two major criteria, or (3) One major criterion and two minor criteria. Specific criteria typically include the presence of multiple basal cell carcinomas, jaw keratocysts, and various skeletal, ophthalmological, and neurological abnormalities. It is important to highlight that a thorough medical and family history, along with a physical examination - including an assessment for dysmorphic features, skeletal abnormalities, and skin abnormalities - is essential in suspected cases of the syndrome[29].

In the gnathic bones, Gorlin syndrome is strongly associated with odontogenic keratocysts (OKCs)[30]. However, cases reporting individuals with Gorlin syndrome and ameloblastomas have been published in the literature, underscoring the diverse spectrum of syndromic associations, including those with OTs, and the potential for multifocal manifestations in affected individuals[31-33]. A systematic review by Atarbashi-Moghadam *et al*[6] summarizing syndromic conditions and ameloblastomas identified six cases of ameloblastoma associated with Gorlin syndrome. Interestingly, these cases demonstrated a female predilection and a tendency for maxillary involvement.

Careful clinical evaluation and genetic testing are essential to confirm the diagnosis and guide management strategies. Early intervention is crucial in preventing complications and improving patient outcomes. Comprehensive patient care often requires a multidisciplinary approach, involving dermatologists, geneticists, dentists, and other specialists to monitor and treat the diverse manifestations of this syndrome[34,35].

Schimmelpenning syndrome (Epidermal nevus syndrome)

Schimmelpenning syndrome, also known as epidermal nevus syndrome, is a rare congenital disorder characterized by the presence of epidermal nevi-patches of abnormal skin that typically appear as raised, warty, or thickened areas. These are often associated with cerebral, ocular, or skeletal defects[36,37]. Postzygotic mutations in *RAS* genes have been linked to the development and progression of this disorder[38].

In individuals with Schimmelpenning syndrome, abnormalities in the development of the jaws may occur, primarily presenting as multiple adenomatoid odontogenic tumors (AOT)[10]. A systematic review conducted by Neumann *et al* [39] identified synchronous OTs in two cases diagnosed with Schimmelpenning syndrome[40,41]. These cases presented with multiple odontomas and an AOT[40], with one case also exhibiting synchronous squamous OT[41].

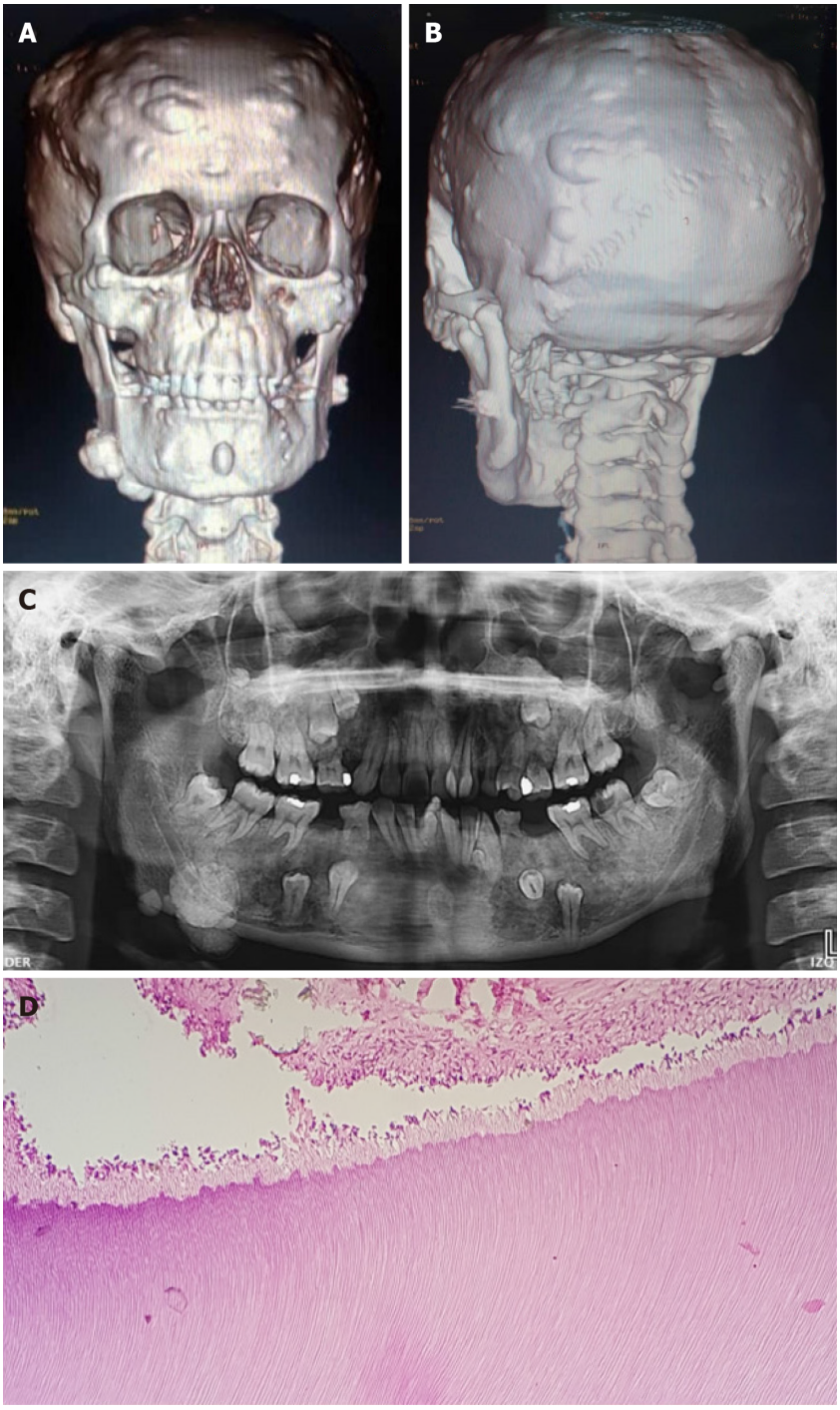

Figure 1 Gardner syndrome in a 22-years-female patient. A and B: Three-dimensional reconstruction images show multiple variable sized osteomas in craniofacial bones; C: Panoramic radiograph shows radiopacity in the right posterior region of the mandible diagnostic as odontoma; D: Haematoxylin and eosin staining section of the odontoma characterized by the presence of dentinal tubules, odontoblasts, and dental pulp.

Otodental syndrome

Otodental syndrome, also known as otodental dysplasia, features a distinctive dental condition called globodontia, often accompanied by sensorineural high-frequency hearing loss and ocular coloboma[42]. Although the specific gene associated with this syndrome remains incompletely elucidated, studies have indicated a deletion on chromosome 11q13 [43].

Interestingly, the dental characteristics alone can be definitive for diagnosis. The most consistent anatomical feature is the abnormal morphology of certain teeth[44]. This condition is marked by distinctive tooth fusion features, including abnormal bulbous enlargement of the crown, which appears spherical with poorly defined grooves[45]. Additionally, molars often exhibit taurodontism, characterized by an inverted crown-to-body ratio. There are also frequent alterations in the roots and pulp canals, making endodontic treatment unpredictable[45,46]. Other dental abnormalities associated with otodental syndrome include congenitally missing teeth and enamel hypoplasia[46]. Although few cases have been described, individuals with this syndrome may also exhibit a propensity for multiple odontomas[44,45,47].

Differential diagnoses for otodental syndrome include autosomal recessive sensorineural hearing impairment with dizziness and hypodontia, bilateral sensorineural hearing loss with multiple anterior dens invaginatus, and double dens invaginatus with developmental delay, progressive sensorineural hearing loss, and multituberculated mandibular incisors. For accurate diagnosis and management of the syndrome, a thorough medical, dental, and family history is crucial. An interdisciplinary approach is recommended.

Simpson-Golabi-Behmel syndrome

Simpson-Golabi-Behmel syndrome (SGBS) is a rare condition characterized by overgrowth and multiple congenital anomalies[48,49]. It results from mutations in a semi-dominant X-linked gene responsible for encoding Glypican 3 (GPC3). Information regarding the clinical and oral presentations of SGBS is limited in the literature. To the best of our knowledge, only one case of this syndrome associated with an OT has been reported[49]. The case involved a 16-year-old male who presented with multiple jaw lesions, including an OKC, ameloblastoma, lateral periodontal cyst, dentigerous cyst, and mucous retention cyst, affecting both the mandible and maxilla.

Williams syndrome

Williams syndrome results from the deletion of genes on chromosome 7q11.23, specifically involving the elastin gene. Since the initial description of the syndrome, understanding of the phenotype's complexity and its evolving characteristics has significantly advanced[50]. This includes insights into the genetic underpinnings, mechanisms driving specific phenotypes, and the benefits of various interventions. However, many questions remain unresolved, which limits the capacity to optimize care and enhance patient outcomes.

The syndrome is characterized as a multisystemic disorder, with diagnosis typically guided by the presence of indicative signs and/or symptoms[50]. Notable signs include supravalvular aortic stenosis, facial dysmorphisms, dental anomalies, neurodevelopmental delays, learning disabilities, and an excessively sociable demeanor[51]. The occurrence of OTs is exceedingly rare, with only one case of ameloblastoma reported thus far[52]. In this case, the authors suggest that further molecular and clinical studies are necessary to establish a definitive association between the syndrome and tumor development.

Attenuated familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is a genetic disorder characterized by the development of at least 100 adenomas in the large bowel and various associated manifestations. It is inherited in an autosomal dominant manner and primarily arises from mutations in the *APC* gene, with less common occurrences linked to mutations in the *MUTYH* gene. A milder form, known as attenuated FAP, is characterized by the presence of fewer than 100 adenomas in the large bowel, distinguishing it from classical FAP. To date, only one case of an OT (specifically, an AOT) has been described in the literature in association with this syndrome[53].

CONCLUSION

This manuscript provides a comprehensive exploration of the intricate relationship between genetic syndromes and OTs. Our findings underscore the importance of recognizing the oral and maxillofacial manifestations associated with genetic syndromes, as these can serve as diagnostic clues for early detection and intervention. In this context, a thorough clinical examination is crucial. This should include a detailed patient history, consideration of underlying conditions, and relevant imaging tests. Additionally, understanding the associations between OTs and genetic syndromes helps clinicians identify patterns of presentation and anticipate potential complications or comorbidities. This knowledge can guide diagnostic workup, treatment planning, and long-term management strategies for affected individuals. Furthermore, it is important to note that while cases in the literature report OTs occurring in syndromic patients, the syndrome may not necessarily be associated with the etiopathogenesis of the neoplasm.

A significant aspect highlighted is the impact of missed diagnoses of genetic syndromes. Delayed or inaccurate identification can lead to suboptimal management and potentially worsen patient outcomes. For example, in Gardner syndrome, a delayed diagnosis may result in the progression of colorectal polyps to cancer and the development of jawbone lesions, which can lead to facial asymmetry, discomfort, and an increased risk of cancer. In the case of Gorlin syndrome, delays can lead to the progression of jawbone lesions and skin cancers, resulting in severe deformities, chronic pain, and more complex surgical needs.

We also emphasize the importance of incorporating genetic testing into clinical practice when a syndromic condition is suspected. Comprehensive genetic evaluations can not only confirm diagnoses but also provide valuable insights into the hereditary nature of these syndromes. Finally, managing OTs in syndromic patients requires a collaborative approach, involving researchers with expertise across the diverse conditions and genetic factors associated with each syndrome. This approach should consider both the specific characteristics of the tumor and the syndromic condition, aiming to provide optimal care, minimize complications, and enhance the overall quality of life for the patient.

Due to the nature of the study design employed, several limitations are evident. Firstly, the lack of a systematic search method meant that some relevant studies could not be included. Secondly, some case reports lacked the comprehensive details necessary to fully understand the syndrome and its association with OTs. Finally, the findings may not be universally applicable across all healthcare settings or populations due to variations in clinical practices and patient demographics.

FOOTNOTES

Author contributions: Schuch LF and Bologna-Molina R designed and performed research, and wrote the paper; Silveira FM, Pereira-Prado V, Sicco E, and Pandiar D revised the article; Villarroel-Dorrego M contributed with the representative cases.

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MINIREVIEWS

Intravitreal therapy for the management of diabetic retinopathy: A concise review

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Abstract

Diabetic retinopathy (DR) is a serious microvascular complication of diabetes mellitus and may result in irreversible visual loss. Laser treatment has been the gold standard treatment for diabetic macular edema and proliferative diabetic retinopathy for many years. Of late, intravitreal therapy has emerged as a cornerstone in the management of DR. Among the diverse pharmacotherapeutic options, anti-vascular endothelial growth factor agents have demonstrated remarkable efficacy by attenuating neovascularization and reducing macular edema, thus preserving visual acuity in DR patients.

Key Words: Diabetic retinopathy; Intravitreal therapy; Anti-vascular endothelial growth factor; Macular edema; Proliferative diabetic-retinopathy

Core Tip: Intravitreal therapy has revolutionized the treatment for diabetic retinopathy (DR). The various treatment options include intravitreal anti-vascular endothelial growth factor injections, triamcinolone acetonide and steroid implants. Intravitreal therapy can be used for the management of Diabetic Macular Edema and Proliferative Diabetic Retinopathy. Additionally, corticosteroids have shown promising results by exerting anti-inflammatory effects and stabilizing blood-retinal barrier integrity. Recent advancements have introduced novel agents targeting various pathways implicated in DR pathogenesis, such as angiopoietin-2 and integrins, offering potential avenues for tailored therapeutic interventions. This review aims to comprehensively examine diverse facets of intravitreal therapy concerning the management of DR and its associated complications.

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INTRODUCTION

Diabetes mellitus (DM) has emerged as a major public health problem[1]. Diabetic retinopathy (DR) ranks as one of the most prevalent complications of DM and is the primary cause of visual impairment and blindness in working age groups (15–64 years)[1-5]. Visual impairment may occur due to diabetic macular edema (DME) or as a result of complications of proliferative DR (PDR) like vitreous hemorrhage, neovascular glaucoma and tractional retinal detachment[5-7].

In DR, prolonged high blood sugar levels are believed to harm endothelial cells and basement membrane proteins within the retinal blood vessels. This damage, along with increased vascular permeability and the production of proinflammatory cytokines, can cause retinal ischemia, which may trigger an excessive increase in vascular endothelial growth factor (VEGF) expression. VEGF along with proinflammatory cytokines increase vascular permeability and promote the breakdown of the retinal-blood barrier, exacerbating the disease and leading to vision loss[7-9].

This understanding has led physicians to adopt intravitreal therapy to address DR-related complications and improve vision in patients. By administering the drug directly into the vitreous cavity, therapeutic doses can be obtained for a longer duration without systemic adverse effects. Intravitreal drugs that have been used in the management of DR can be broadly divided into two groups: Anti-VEGF agents and corticosteroids. VEGF inhibitors that have been evaluated in prospective, randomized phase 2 and phase 3 clinical trials, and demonstrated favourable results for patients with DME and DR include bevacizumab, ranibizumab, aflibercept, brolucizumab and faricimab. Intravitreal corticosteroids include Triamcinolone and dexamethasone implant (Ozurdex®; Allergan, Inc., Irvine, CA, United States).

This review seeks to offer a thorough overview of intravitreal therapies used in the management of DR and DME. Additionally, we have discussed newer emerging agents that are being developed for intravitreal use in patients with DR.

METHODOLOGY

A thorough search on PubMed, Embase, Reference Citation Analysis, Embase and Google Scholar was performed of keywords – Intravitreal therapy in Diabetic Retinopathy. Latest and highly cited articles written in English were included.

Intravitreal therapy in the management of DME

Intravitreal therapy has revolutionized the treatment for DR. The various treatment options include intravitreal anti-VEGF injections, triamcinolone acetonide and steroid implants. Intravitreal therapy can be used for the management of DME and PDR.

Intravitreal agents used in the management of DME can be grouped into: (1) Intravitreal anti-VEGF agents; (2) Intravitreal corticosteroids; and (3) Intravitreal anti-VEGF agents.

VEGF plays a central role in the pathogenesis of sight-threatening retinal changes in DR. VEGF drives ischemic changes at the level of the retina and stimulates the growth of new vessels. These vessels are fragile, and highly permeable and leakage from them can lead to exudation in the macular area. Also, new vessels can easily rupture leading to pre-retinal and vitreous hemorrhage. Anti-VEGF agents, therefore, remain the cornerstone for the treatment of DR and DME.

Anti-VEGF agents include: (1) Bevacizumab; (2) Ranibizumab; (3) Aflibercept; (4) Brolucizumab; and (5) Faricimab (Table 1).

Bevacizumab

Bevacizumab is a recombinant humanized monoclonal antibody targeting VEGF. It is structurally composed of human immunoglobulin G1 with regions derived from murine antibodies. Its molecular weight is 145 kDa. Initially developed for cancer therapy, bevacizumab was first approved by the Food and Drug Administration (FDA) in 2004 for metastatic colorectal cancer. Its off-label use in ophthalmology began with the treatment of neovascular age-related macular

Table 1 Characteristic features of different anti – vascular endothelial growth factor drugs

Drug	Active molecule	Molecular weight	Dosage	Mechanism of action	Year of FDA approval for use in DR/DME
Bevacizumab	Recombinant humanized monoclonal antibody targeting VEGF	145 kDa	1.25 mg/0.05 mL	Non-specific blockade of VEGF on endothelial surface	2004
Ranibizumab	Recombinant humanized, monoclonal antibody fragment	45 kDa	0.5 mg/0.05 mL	Binds VEGF-A, VEGF-B, and placental growth factor	2012
Aflibercept	Recombinant protein and attaches to the VEGF receptor 1 and 2	115 kDa	2 mg/0.05 mL	Blocks VEGF-A, VEGF-B, Placental growth factor-1 and 2	2014
Brolucizumab	Humanized single-chain variable fragment	26 kDa	6 mg/0.05 mL	Inhibits VEGF A and VEGF 165	2022
Faricimab	Bispecific antibody	149 kDa	6 mg/0.05 mL	Targets both VEGF-A and angiopoietin-2	2022

FDA: Food and Drug Administration; DR: Diabetic retinopathy; DME: Diabetic macular edema; VEGF: Vascular endothelial growth factor.

degeneration (AMD), showcasing significant efficacy in reducing macular edema and improving visual acuity. Despite the lack of FDA approval for ocular use, cost-effectiveness makes bevacizumab an important tool in the armamentarium of DME management.

Dosage and administration

The recommended intravitreal dosage of bevacizumab (off-label use) for DME is 1.25 mg (0.05 mL). Typically, the injections are administered monthly. The treatment frequency may be adjusted based on the patient's response and visual acuity improvement.

Clinical studies

The BOLT (Bevacizumab or Laser Therapy) study, a two-year, randomized controlled trial compared bevacizumab to laser therapy in patients with DME. Patients who received intravitreal bevacizumab had significant improvement in visual acuity and central macular thickness compared to laser treatment. Specifically, at 12 months, patients treated with bevacizumab had a mean gain of 8.6 letters in visual acuity compared to a loss of 0.5 letters in the laser group. At 24 months, the bevacizumab group maintained a mean gain of 8.6 letters, while the laser group had a mean gain of only 0.9 letters[9-11].

The studies conducted by the Diabetic Retinopathy Clinical Research Network (DRCR.net) have been instrumental in establishing the role of bevacizumab in DME. The Protocol T study, a head-to-head comparison of aflibercept, ranibizumab, and bevacizumab, revealed that aflibercept and ranibizumab offered superior visual outcomes at one year for patients with baseline visual acuity of 20/50 or worse, but bevacizumab was comparable in those with better baseline vision and more cost-effective[11,12]. Post hoc analyses of DRCR Protocol T further confirmed bevacizumab's efficacy in various subgroups of DME patients[12].

Cochrane review has consistently supported the use of bevacizumab in DME. A comprehensive review of multiple clinical trials concluded that intravitreal bevacizumab is effective in improving visual acuity and reducing central macular thickness in DME patients. The review emphasized that while aflibercept and ranibizumab might offer slightly better outcomes in specific scenarios, bevacizumab remains a valuable option, especially in settings where cost is a significant concern[12].

Currently, bevacizumab's off-label use in DME is widespread and endorsed by numerous clinical guidelines, recognizing its balance of efficacy, safety, and cost. Its indication extends to patients with central-involved DME, offering an accessible alternative to more expensive anti-VEGF therapies. Despite the absence of FDA approval for ocular indications, bevacizumab continues to be an important treatment modality, supported by robust clinical evidence and extensive real-world application.

RANIBIZUMAB

Ranibizumab is a recombinant, humanized, monoclonal antibody fragment with a molecular weight of 48 kDa. The fragment is derived from a bevacizumab antibody and needs recombinant processing to produce the desired Fab-Y0317 molecule. The small size of the molecule increases the ocular penetration. The FDA approved Ranibizumab for DME in 2012.

Dosage and administration

The commonly used dosages of ranibizumab (0.1 mg/0.01 mL) are 0.5 mg (0.05 mL) and 0.3 mg (0.03 mL). The standard dosing regimen comprises an initial series of three four-weekly (q4) injections. Thereafter, treatment is often individu-

alized based on the patient's response, with the potential for adjustments in the injection frequency.

Treatment protocols

Phase III trials, such as RISE and RIDE[12-16] used a fixed dosing regimen, either monthly or bimonthly treatment after a loading phase. However, this regimen is difficult to follow in real-world clinical practice. To optimize the treatment effects and cost-effectiveness, different treatment protocols have been developed in recent years. Of these, the two most commonly used protocols are: (1) Pro re nata (PRN); and (2) Treat-and-extend (T&E) regimen; Pro re nata (PRN).

In the pro re nata (PRN) approach, also known as "as needed," the frequency of injections is minimized while adhering to a fixed follow-up schedule. This allows for close monitoring of the patient's response to treatment[17-19].

Treat-and-extend (T&E) regimen

The treat-and-extend (T&E) regimen involves incrementally increasing the interval between follow-up visits after the patient has demonstrated a sufficient response to treatment. During each visit, an injection is administered to maintain therapeutic efficacy[18,19].

Clinical studies

Pivotal phase 3 trials for the use of intravitreal Ranibizumab in patients with DME, such as RISE and RIDE, demonstrated significant visual acuity improvement with Ranibizumab treatment compared to sham injections. In these studies, approximately 39% and 45% of patients, respectively, achieved a ≥ 15-letter gain in best-corrected visual acuity (BCVA) at 24 months[13-16].

Further validation of Ranibizumab's efficacy came from the DRCR Protocol I study, which showed that Ranibizumab, combined with prompt or deferred laser, resulted in superior vision outcomes over laser treatment alone. At 2 years, 50% of Ranibizumab-treated patients gained ≥ 10 letters in BCVA, compared to 28% in the laser group[20-23].

Post hoc analyses of these trials provided additional insights, revealing sustained benefits in visual acuity and anatomical improvements over extended periods. For instance, patients initially treated with Ranibizumab maintained their visual gains and macular thickness reductions through extended follow-up[21-24].

The Cochrane systematic review analyzed 15 randomized controlled trials involving over 4000 participants to evaluate the efficacy of ranibizumab in DME. The findings revealed that ranibizumab significantly improves BCVA with an average gain of +6.6 letters at one year, compared to a minimal change of -0.5 letters in control groups receiving sham or laser treatment. Additionally, ranibizumab markedly reduced central retinal thickness (CRT), demonstrating its effectiveness in resolving macular edema. The review also affirmed ranibizumab's favorable safety profile, with a low incidence of adverse events[13-23].

Ranibizumab, therefore, remains a cornerstone in the therapeutic arsenal against DME, supported by extensive clinical trial data, post hoc analyses, and real-world evidence. Its ability to significantly improve and sustain visual outcomes while maintaining a favorable safety profile underscores its vital role in preserving vision in diabetic patients. Because of this, Ranibizumab remains the first-line agent and the standard of care in the management of DME (Figures 1 and 2).

AFLIBERCEPT

Aflibercept is a recombinant protein and attaches to the VEGF receptor 1 and 2. Its molecular weight is 115 kDa. The molecule blocks VEGF-A, VEGF-B, and Placental growth factor-1 and 2. Aflibercept was first developed as a treatment for wet AMD and received FDA approval for this indication in 2011. Its application for DME was approved by the FDA in 2014. Ever since, aflibercept has become a crucial component in the management of DR, providing an efficacious option for patients with DME.

Dosage and administration

The standard dosing regimen for aflibercept in DME is an initial series of five monthly injections (2 mg/0.05 mL), followed by injections every two months. In clinical practice, many physicians adopt a treat-and-extend approach, adjusting the interval between doses based on individual patient responses.

Clinical studies

Numerous clinical trials have established the efficacy of aflibercept in DME, providing a robust foundation of evidence for its use. The VIVID and VISTA trials were pivotal in this context, offering detailed data on their therapeutic impact[21-24]. In the VIVID trial, aflibercept administered at 2 mg every four weeks or every eight weeks resulted in a mean improvement in BCVA of 10.5 and 10.7 letters, respectively, at 52 weeks, compared to a 1.2-letter improvement in the laser group. Similarly, the VISTA trial reported mean BCVA gains of 12.5 and 10.7 letters for the same dosing regimens, *vs* a 0.2-letter improvement with laser treatment at 52 weeks. Furthermore, aflibercept-treated patients exhibited a substantial decrease in central retinal thickness, with reductions of 185.9 μm and 183.1 μm in the VIVID study and 191.1 μm and 189.3 μm in the VISTA study, corresponding to the two dosing schedules[20-24].

The DRCR Protocol V examined the outcomes of initial aflibercept treatment against laser photocoagulation and observation in patients with good baseline visual acuity. Immediate aflibercept treatment did not significantly prevent vision loss compared to the other strategies. This suggests the importance of personalized treatment, taking into account initial visual acuity and disease severity to optimize outcomes for each patient[20-24].

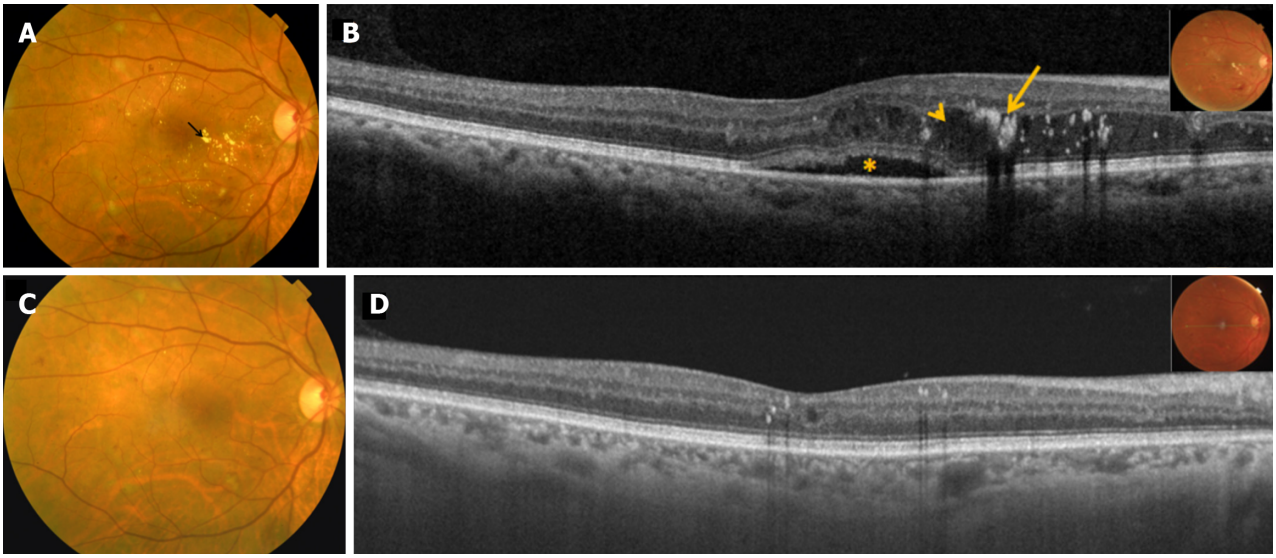

Figure 1 Ranibizumab remains the first-line agent and the standard of care in the management of diabetic macular edema. A: Fundus photograph of the right eye of a patient with moderate non-proliferative diabetic retinopathy shows retinal thickening and hard exudates (arrow) suggestive of clinically significant macular edema (CSME); B: Corresponding swept source optical coherence tomography scan shows intraretinal (arrow-head) and subretinal fluid (asterisk) along with hard exudates (arrow); C and D: Treatment with Intravitreal Ranibizumab (Accentrix®) resulted in resolution of CSME and improvement in visual acuity from 6/18 to 6/9.

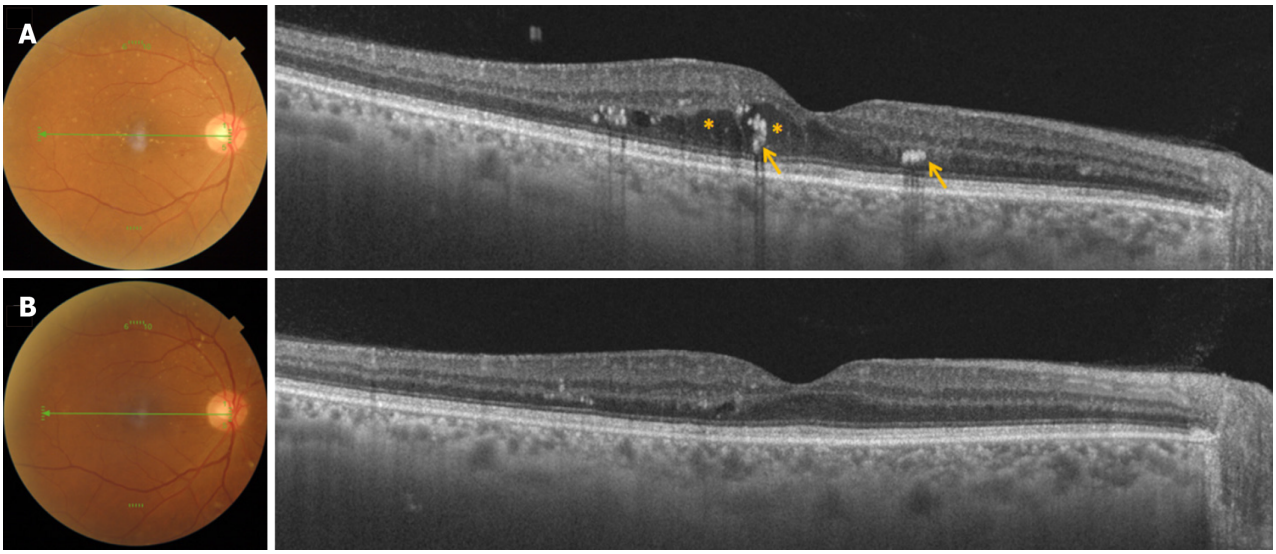

Figure 2 A 55-year-old Asian Indian female presented with decreased vision in her right eye for the past two months. She was diagnosed with type II diabetes mellitus 5 years back. A: She had moderate non-proliferative diabetic retinopathy with clinically significant macular edema (CSME) at presentation. Swept source optical coherence tomography scan showed the presence of intra-retinal cystic spaces (asterisk) and hard exudates (arrows); B: Treatment with intravitreal Aflibercept (Eylea®) resulted in the resolution of CSME.

Similarly, in another study by DRCR (Protocol T), patients with poorer visual acuity, when treated with aflibercept showed significantly greater improvements in BCVA compared to those receiving bevacizumab or ranibizumab[11-25].

Long-term studies have reinforced the sustained benefits of aflibercept. Five-year follow-up of patients in Protocol T confirmed that the visual acuity gain achieved with aflibercept could be maintained over an extended period, with a manageable safety profile[21-25]. Additionally, the extended results of the VISTA and VIVID studies at 148 weeks demonstrated that aflibercept continued to provide significant visual and anatomical improvement[21-26].

Thus, aflibercept remains a first-line treatment for DME, particularly in patients with severe visual impairment at baselinegiven its efficacy and manageable safety profile (Figure 3).

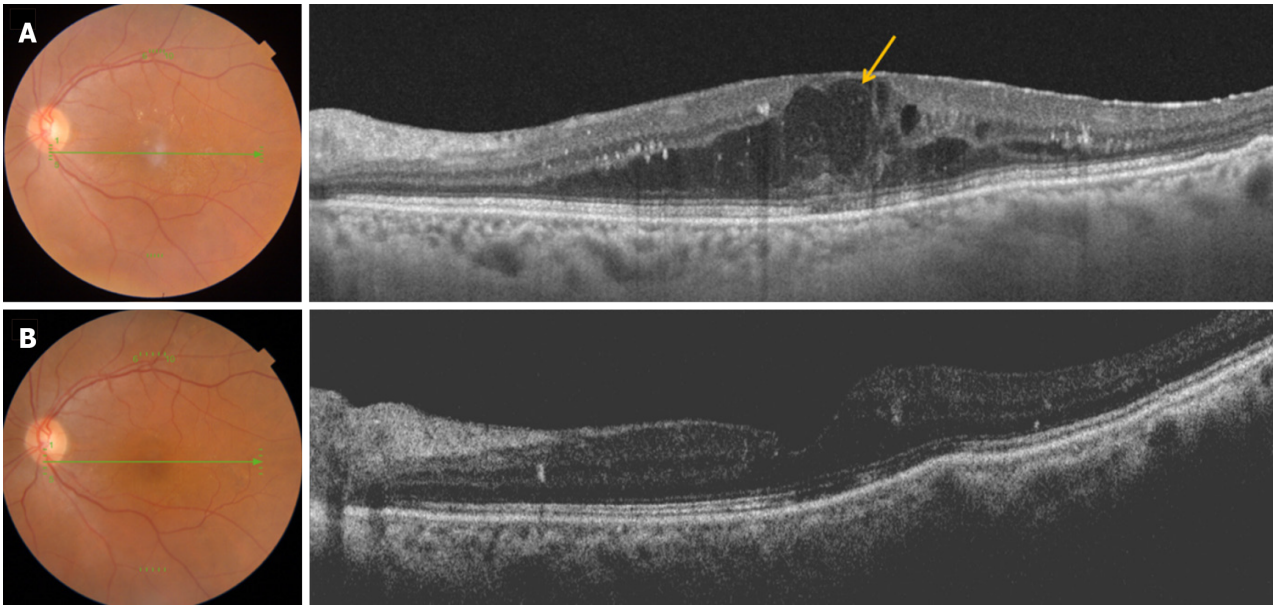

Figure 3 A 65-year-old Asian Indian male presented with decreased vision in his left eye. He was diagnosed with type II diabetes mellitus 2 years back. He also gave a history of myocardial infarction two months back. A: Fundus examination showed moderate non-proliferative diabetic retinopathy with clinically significant macular edema (CSME) in the left eye. Swept source optical coherence tomography scan showed presence of intra-retinal cystic spaces (arrow); B: Treatment with intravitreal dexamethasone implant (Ozurdex®) resulted in the resolution of CSME.

BROLUCIZUMAB

Brolucizumab is a humanized single-chain variable fragment (scFv) antibody that functions by binding with high specificity to VEGF(VEGF). It has a low molecular weight of 26 kDa. The structural design of brolucizumab enhances its specificity and potentially extends its therapeutic duration compared to conventional antibodies targeting VEGF. Brolucizumab was initially approved by the FDA for the treatment of neovascular AMD in October 2019. Its approval for DME followed in June 2022 based on the results of the pivotal KESTREL and KITE Phase 3 trials.

Dosing and administration

Brolucizumab is initially administered with a loading phase, involving injections (6 mg/0.05 mL) every six weeks for the first five doses. Subsequently, the maintenance phase adjusts to injections every 8 to 12 weeks based on individual response and disease activity.

Clinical studies

The KESTREL and KITE trials were randomized, double-masked studies that evaluated the efficacy and safety of brolucizumab compared to aflibercept in patients with DME[21-29]. 926 patients from 36 countries were enrolled. Both trials demonstrated the non-inferiority of brolucizumab to aflibercept in terms of improvement in BCVA. Patients treated with brolucizumab experienced a mean gain of 12.2 letters, compared to 11.0 letters in the aflibercept group. Also, patients who received brolucizumab showed superior anatomical outcomes, with a higher proportion of patients achieving a significant reduction in CRT and resolution of retinal fluid compared to aflibercept. More than half of brolucizumab 6 mg subjects could be maintained on q12w dosing after loading, thereby potentially reducing treatment burden for patients[21-29].

A study by Rübsam *et al*[25] comparing intravitreal brolucizumab and aflibercept in patients with DME revealed significant improvement in BCVA and reduction in CRT. The study evaluated 35 eyes from 24 patients treated with brolucizumab and 40 eyes from 31 patients treated with aflibercept. At week 36, treatment-naïve DME eyes treated with brolucizumab showed an improvement of +6.4 letters (P = 0.03), while those treated with aflibercept gained +9.5 letters (P = 0.001). Additionally, recalcitrant DME eyes exhibited BCVA improvement of +5 letters with brolucizumab (P = 0.006) and +5.5 letters with aflibercept (P = 0.02). Both treatments also led to significant CRT reduction. Notably, the mean treatment interval for brolucizumab was longer at 11.3 weeks, compared to 6.5 weeks for aflibercept in treatment-naïve eyes, and 9.3 weeks *vs* 5.3 weeks for pretreated eyes, suggesting that brolucizumab may offer the benefit of reduced injection frequency[22-29].

However, while brolucizumab shows promising efficacy, the incidence of intraocular inflammation (IOI) and retinal vasculitis in real-world settings remains a concern. Reports indicate that IOI rates range from 2% to 4%, with some studies citing higher rates in specific patient groups or dosages[22-29]. Even in KITE and KESTREL trials, rates of IOI were higher in the brolucizumab group compared to aflibercept, with incidence reported in 4.7% and 3.7% of patients receiving brolucizumab 3 mg and 6 mg, respectively[22-29].

Thus, brolucizumab offers superior anatomical and visual outcomes with the potential for extended dosing intervals. However, recently, serious side effects like retinal vasculitis and severe intraocular inflammation have been reported with the use of brolucizumab. As it is a relatively newer agent with limited availability of long-term safety data, continued monitoring through real-world studies is required to ascertain its long-term safety and efficacy in DME management.

FARICIMAB

Faricimab is a novel bispecific antibody targeting both VEGF-A and angiopoietin-2 (Ang-2) designed specifically for ocular use.

Faricimab was developed to overcome the limitations of current anti-VEGF therapies by concurrently targeting Ang-2, which plays a role in vascular maturation and stability.

Dosage and administration

Faricimab's recommended dosage regimen involves intravitreal injections (6 mg/0.05 mL) administered initially every 8 weeks during the loading phase, followed by an extended dosing interval based on disease activity assessment.

Clinical studies

YOSEMITE and RHINE trials by Wykoff *et al*[27] confirmed faricimab's efficacy and safety in DME. These double-masked, randomized phase 3 trials included over 1800 patients globally. A longer dosing regimen at 12 weeks and 16 weeks was evaluated. Both trials demonstrated that faricimab was non-inferior to aflibercept in improving BCVA, with patients gaining an average of 11.8 letters in the YOSEMITE trial and 10.7 letters in the RHINE trial. These gains were comparable to aflibercept, which showed gains of 10.3 and 10.2 letters, respectively. Additionally, faricimab led to a significant reduction in CRT, with a reduction of 187.1 μm in YOSEMITE and 195.8 μm in RHINE, compared to 170.3 μm and 169.1 μm for aflibercept. Notably, 52% of patients in YOSEMITE and 51% in RHINE achieved and maintained a 16-week dosing interval by the end of one year, indicating faricimab's potential for extended treatment intervals. The safety profile was comparable to aflibercept, though careful monitoring for intraocular inflammation and rare cases of retinal vasculitis is necessary[23-32].

Real-world studies and subgroup analyses have similarly supported the efficacy of faricimab across diverse patient demographics[23-33]. Thus, Faricimab represents a significant advancement in the management of DME, supported by robust clinical data demonstrating efficacy, durability, and manageable safety profile. Ongoing research continues to explore its long-term benefits and optimize treatment protocols, reaffirming its pivotal role in improving visual outcomes and quality of life for patients with DME. However, despite its promising efficacy, faricimab is associated with notable side effects, including IOI and rare instances of retinal vasculitis. Vigilance and prompt management of these adverse events are crucial for optimizing treatment outcomes[24-32].

Comparative analysis of the different anti-VEGF agents and corticosteroids in the management of DME

The DRCR Protocol T study conducted a randomized clinical trial comparing the efficacy of three anti-vascular endothelial growth factor (anti-VEGF) agents-aflibercept, bevacizumab, and ranibizumab—in treating DME associated with central retinal involvement and visual acuity loss. The trial revealed that all three agents improved visual acuity, with aflibercept showing superior results, especially in patients with worse baseline visual acuity. Specifically, aflibercept led to an average improvement of 18.9 letters, whereas ranibizumab and bevacizumab resulted in gains of 14.2 and 11.8 letters, respectively, after one year.

The study highlighted the potential for greater treatment benefits with aflibercept in patients starting with poorer vision. However, in cases of better initial visual acuity, the differences among the three agents were minimal, making bevacizumab a cost-effective option despite its slightly lower efficacy[10-25].

Cheng *et al*[29] performed a systemic review to compare the efficacy of glucocorticoids and various anti-VEGF, in the treatment of DME, and evaluated various clinical treatment regimens consisting of different therapeutic measures.

The study examined 39 randomized controlled trials and found that at a 3-month follow-up, the combination of intravitreal bevacizumab (IVB) and triamcinolone acetonide (TA) was the most effective in improving best-corrected visual acuity (BCVA) and reducing central macular thickness. However, at the 6-month follow-up, intravitreal dexamethasone (DEX) implants showed superior efficacy, particularly in patients with severe macular oedema and impaired vision. This again highlights the importance of tailoring treatment strategies based on the severity and duration of the condition.

INTRAVITREAL STEROIDS

Inflammation plays an important role in the pathogenesis of DME. Hyperglycemia results in elevated levels of inflammatory cytokines, such as interleukin-1β (IL-1β), nuclear factor-κB (NF-κB), Vascular endothelial growth factor (VEGF), tumor necrosis factor α (TNFα), transforming growth factor-beta, and Intercellular Adhesion Molecule-1 in retinal pericytes and Müller cells[26-34]. Chronic hyperglycemia leads to the loss of pericytes and the disruption of tight junctions in retinal endothelial cells, which promotes the release of VEGF and other inflammatory mediators, including IL-1, IL-6, IL-8, TNFα, matrix metalloproteinases, kallikrein-kinin, and monocyte chemoattractant protein-1[29-35]. These mediators, along with VEGF, further compromise tight junction integrity, increasing vascular permeability and

contributing to DME. Intravitreal corticosteroid injections can suppress these inflammatory pathways, thereby playing an important role in the treatment of DR and DME.

Types of intravitreal corticosteroids

Several corticosteroids are used in intravitreal injections, including: (1) Triamcinolone Acetonide: One of the earliest corticosteroids used for DME, known for its anti-inflammatory properties; (2) Dexamethasone Implant (Ozurdex): A biodegradable implant that provides sustained release of the drug over several months; and (3) Fluocinolone Acetonide Implant (Iluvien™): Another long-acting implant that releases corticosteroid for up to three years.

TRIAMCINOLONE ACETONIDE

TA is a potent corticosteroid with anti-inflammatory properties. Its application in ophthalmology, particularly for DME, gained attention in the early 2000s as an alternative to laser photocoagulation.

Dosage and administration

TA can be administered *via* intravitreal injection in different doses, typically ranging from 1 mg to 4 mg. The choice of dose may depend on the severity of DME, the patient's response to treatment, and the occurrence of side effects. A commonly used dose in clinical studies is 4 mg.

Clinical studies

The DRCR-Protocol B is a landmark study that compared the efficacy of intravitreal TA with focal/grid laser photocoagulation for DME. Laser photocoagulation was more effective than TA in improving visual acuity over two years. TA provided more rapid short-term improvement in visual acuity compared to laser treatment. However, the patients treated with TA showed a higher incidence of adverse events, including elevated IOP and cataract progression[30-38].

Similarly in a Cochrane review by Rittiphairoj *et al*[33], TA was found to be effective in improving visual acuity and reducing central macular thickness in patients with DME. However, its use was also associated with significant adverse events, particularly increased intraocular pressure and cataract formation, making it less favourable compared to anti-VEGF agents for the long-term management of DME.

Abdel-Maboud *et al*[34] performed a meta-analysis to compare the efficacy and safety of intravitreal TA *vs* intravitreal Bevacizumab alone or combined IVB+IVT in the treatment of DME. The authors found no significant difference in the long-term outcomes between the two treatments regarding visual acuity and central macular thickness. However, IVT was associated with higher rates of increased intraocular pressure (IOP).

To sum up, TA has been a valuable addition to the therapeutic arsenal for DME. Its anti-inflammatory and anti-permeability properties can provide significant short-term improvement in visual acuity. Its low cost makes TA a useful option in DME management in resource-starved populations. However, the risk of side effects such as elevated IOP and cataract formation limits its long-term use. Thus, the choice of TA should be individualized, taking into account the patient's specific condition, response to previous treatments, and potential risks.

DEXAMETHASONE IMPLANT (OZURDEX®; ALLERGAN, INC., IRVINE, CA, UNITED STATES)

Dexamethasone is a more potent anti-inflammatory agent compared to TA and is associated with fewer side effects. Ozurdex® (Allergan, Inc., Irvine, CA, United States) is a sustained-release dexamethasone implant and provides long-acting anti-inflammatory action. The frequency of intravitreal injections needed is markedly reduced resulting in better patient compliance and fewer hospital visits. The implant is made from sustained release biodegradable copolymer that gradually degrades into lactic acid and glycolic acid, which are further metabolized into carbon dioxide and water. Ozurdex was FDA-approved in September 2014 for the treatment of DME.

Dosage and administration

The drug delivery system is a single-use device which uses a 22 G needle to insert the implant intravitreally leaving a self-sealing suture-less wound. The implant contains 0.7mg of dexamethasone and provides long-lasting effects up to 6 months.

Clinical studies

The MEAD trials[33-43] were two large, randomized, multicenter phase 3 studies with identical protocols involving 1048 patients with center-involving DME. Based on the results of these very trials, the FDA approval of Ozurdex was granted for DME. The participants were divided into three groups: Ozurdex 0.7 mg, Ozurdex 0.35 mg, and a sham group. Upon three-year follow-ups, significant improvement in BCVA was observed with both doses of Ozurdex compared to sham, along with a notable reduction in central retinal thickness. Cataract formation and elevated IOP were significant side effects. 65% of phakic eyes receiving the DEX implant developed cataracts compared to 20% in the sham group. About 40% of patients in the Ozurdex group required IOP-lowering medications, *vs* only 10% in the sham group[33-44].

Following FDA approval, numerous studies have evaluated the efficacy and safety of the DEX implant. Special conditions such as vitrectomized eyes and DME refractory to anti-VEGF treatments have also been explored.

According to the EURETINA guidelines, dexamethasone implant can be considered a second-line treatment for patients who do not respond to anti-VEGF injections after 3 to 6 doses. It can also be considered as primary option for those with contraindications to anti-VEGF agents, such as a recent history of major cardiovascular events, pregnancy, or an unwillingness to adhere to monthly injections[34-44]. The PLACID trial was the first randomized multicenter study evaluating the use of Ozurdex in DME[38-44]. It compared two groups: One receiving Ozurdex plus laser at one month, and the other receiving laser monotherapy. Results showed a significantly higher proportion of patients achieving a 10-letter or more improvement in visual acuity in the Ozurdex plus laser group at nine months. The combination also proved superior in improving central retinal thickness in patients with diffuse macular edema. Elevated intraocular pressure was managed with medications in 16% of patients, and 20% of phakic eyes experienced cataract-related adverse events.

The MOZART study, a small retrospective analysis, assessed the 0.7 mg DEX implant's effectiveness and safety in DME patients with visual impairment. It demonstrated favorable anatomical and functional outcomes with a single injection and manageable side effects[38-45].

The CHROME study was a retrospective study and included patients with macular edema secondary to various retinal disease, including DME. For the DME cohort, while central retinal thickness reduction was achieved, significant visual acuity improvement was more noticeable in pseudophakic eyes[39-45].

The CHAMPLAIN study focused on the DEX implant's safety and efficacy in vitrectomized eyes, addressing the faster clearance of anti-VEGF agents in these eyes. This phase 2 clinical study found a 6-letter gain in BCVA at eight weeks and maintained a 3-letter gain at 26 weeks. Additionally, 30% of patients experienced a 10-letter gain in BCVA two months post-injection[40-44]. The recent ARTES study group conducted an extensive evaluation of dexamethasone in real-life settings for DME, considering previous treatment history, diabetes duration, and control levels. It concluded that treatment-naive eyes had better baseline and follow-up BCVA, while late DME and uncontrolled diabetes were associated with poorer outcomes. It also suggested shorter treatment intervals than the officially recommended six months[41-45].

DEX implant has been shown to be useful in in previously treated DME patients[42-47]. The DRCR.net Protocol U trial compared combination therapy of ranibizumab and a dexamethasone implant to ranibizumab alone in persistent DME, showing a significant reduction in central foveal thickness with the combination therapy[43-49].

A meta-analysis on single-dose dexamethasone implantation for persistent DME refractory to anti-VEGF therapy indicated significant anatomical and functional improvements[44-51]. The BEVORDEX trial, comparing bevacizumab and dexamethasone implant, found similar functional outcomes but greater CRT reduction with fewer injections in the dexamethasone group[45-51]. A consensus among Spanish experts recommended DEX implants as first-line therapy for specific DME patient groups, such as pseudophakic, poor-adherents, vitrectomized, candidates for cataract surgery, and those with a history of cardiovascular events. They also suggested using DEX implants after inadequate anti-VEGF response, typically after three injections[46-52].

Despite clinical trials suggesting the therapeutic effect of Ozurdex lasts at least six months, real-life practice indicates that more frequent dosing may be required for some patients. A study by Bucolo *et al*[49] on the long-term use of DEX implants in DME noted that about one-third of eyes needed retreatment before six months.

DEX implant has also been shown to be useful in patients with DME who undergo cataract surgery. Gupta *et al*[50] evaluated role of intraoperative intravitreal dexamethasone implant in patients with diabetic retinopathy with/without macula edema undergoing phacoemulsification in a two-arm, randomized, assessor-blinded trial. 151 patients with type-2 diabetes mellitus and cataract were divided into two groups: DEX group *vs* standard of care (SOC) group, *i.e.* phacoemulsification and intraocular lens implantation without injection DEX implant. The patients in DEX group had reduced central macular thickness and required lesser number of rescue interventions at the end of 12 weeks follow up compared to SOC group. The authors concluded that patients undergoing cataract surgery with DR with/without macular edema benefit from DEX implant with effects lasting for at least three months[48-53].

In summary, DEX implants have demonstrated favorable anatomical and functional outcomes in randomized controlled trials for DME. However, they exhibit a relatively higher incidence of ocular side effects, including an increased risk of cataract formation and elevated intraocular pressure, compared to the gold standard anti-VEGF agents. Consequently, DEX implants are typically considered a second-line treatment for DME (Figure 3). Nevertheless, they may be preferred as a first-line therapy in cases where anti-VEGF agents are contraindicated, such as during pregnancy or in patients with a recent history of major cardiovascular or cerebrovascular events. Additionally, DEX implants can be considered a primary treatment option for vitrectomized and pseudophakic eyes. They are also beneficial for patients with refractory or recalcitrant macular edema. The advantages of DEX implants over anti-VEGF agents include ease of administration, comparable cost, extended periods of remission, reduced frequency of injections, and consequently, fewer hospital visits.

FLUOCINOLONE ACETONIDE IMPLANT (ILUVIEN™)

The fluocinolone acetonide (FAc) implant, marketed as Iluvien™ was developed to address the need for sustained drug delivery to the retina, offering a steady release of the corticosteroid over an extended period, thereby providing sustained drug delivery to the retina. Iluvien™ received FDA approval in September 2014 for the treatment of DME in patients previously treated with corticosteroids without a significant rise in intraocular pressure.

Dosage and administration

The Iluvien™ implant contains 0.19 mg of fluocinolone acetonide. It is administered intravitreally, typically through a 25-gauge needle, and designed to continuously release the drug over three years. The implant releases fluocinolone acetonide at an initial rate of 0.25 μg/day, averaging 0.2 μg/day, and is effective for up to 36 months.

Clinical studies

Several studies have evaluated the efficacy and safety of Iluvien in DME.

The FAME (Fluocinolone Acetonide for Diabetic Macular Edema) phase 3 trials[49-55] were instrumental in validating the efficacy of the FAc implant, Iluvien, for the treatment of DME. These multicenter, randomized, double-masked trials included over 950 patients who were followed for 36 months. The primary endpoint was the proportion of patients achieving a ≥ 15-letter improvement in BCVA from baseline, a critical measure of clinical significance in visual function improvement. Patients receiving the FAc implant showed significant and sustained improvement in visual acuity compared to the control group, which received a sham injection. Specifically, at 24 months, 28.7% of patients in the low-dose FAc group (0.2 μg/day) achieved a ≥ 15-letter gain in BCVA, compared to 16.2% in the sham group. This benefit was maintained at the 36-month mark, with 28.7% in the FAc group *vs* 18.9% in the control group showing similar gains. In addition to visual acuity improvement, the FAME trials also reported a significant reduction in central retinal thickness, a key indicator of DME severity. Patients treated with the FAc implant exhibited a mean reduction in CRT from baseline, highlighting the implant's ability to mitigate retinal swelling effectively.

The safety profile of the FAc implant was also thoroughly assessed. The commonly identified side effects, such as increased intraocular pressure and cataract formation, were managed with standard ophthalmic interventions[49-55].

PALADIN, a phase 4 study, further confirmed the long-term safety and efficacy of the 0.19 mg FAc implant. It focused on patients with DME who had previously received corticosteroid treatment without significant IOP increase. Over a three-year follow-up, the study demonstrated sustained visual acuity improvement, with an average BCVA gain of 4.5 letters from baseline. The implant significantly reduced the treatment burden, with 76% of eyes not requiring additional therapy during the study period. Side effects were consistent with earlier trials, primarily increased IOP and cataract formation, manageable with standard ophthalmic care[51-56].

A systematic review of real-world studies by Kodjikian *et al*[54] involving 1880 eyes demonstrated that the FAc implant for DME resulted in a mean peak visual gain of +8.7 letters and a mean central retinal thickness reduction of 34.3%[52-57]. The review highlighted that patients with lower baseline BCVA and more recent DME experienced better outcomes. However, those with chronic DME often required more frequent rescue therapies. Additionally, ocular hypertension was reported in 20.1% of cases, but only 0.6% needed surgical intervention, and 43.2% of phakic patients underwent cataract extraction.

Thus, Iluvien has emerged as a useful option in managing chronic DME. It is currently positioned as a second or third-line treatment for DME, particularly beneficial for patients unresponsive to anti-VEGF therapies. Its long-term efficacy in reducing treatment frequency and improving visual outcomes has shown promising results, even though ocular hypertension and cataracts remain important adverse effects.

Intravitreal therapy in the management of PDR

PDR is a severe and advanced stage of diabetic retinopathy characterized by the neovascularization of the retina and optic disc. This pathological angiogenesis occurs in response to retinal ischemia, driven by upregulated VEGF. Newly formed vessels are fragile, leading to recurrent vitreous hemorrhage, tractional retinal detachment, and potential vision loss. Pan-retinal photocoagulation (PRP) is the standard of care for the management of PDR. However, PRP may result in reduced peripheral vision and worsening of DME. Recently, intravitreal anti-VEGF agents have been used in the management of PDR.

RANIBIZUMAB

Ranibizumab, an anti-VEGF agent, was approved by the FDA for the treatment of PDR in 2017. It works by inhibiting VEGF, a key molecule in angiogenesis, thereby preventing the formation of abnormal blood vessels in the retina and reducing vascular permeability.

Clinical studies

DRCR network has conducted extensive research on ranibizumab for PDR. The landmark Protocol S study compared ranibizumab with PRP, demonstrating that intravitreal ranibizumab was non-inferior to PRP in visual acuity outcomes at two years[53-56]. Mean visual acuity improvement was 2.8 letters in the ranibizumab group *vs* 0.2 letters in the PRP group ($P < 0.001$). A follow-up study evaluating five-year outcomes confirmed these findings, with 53% of the ranibizumab group maintaining stable or improved vision compared to 46% in the PRP group. Additionally, ranibizumab-treated eyes had significantly fewer cases of vision-impairing complications such as vitreous hemorrhage and tractional retinal detachment. Another analysis from the DRCR investigated patient-centred outcome, revealing higher satisfaction and quality of life scores among those treated with ranibizumab compared to PRP[53-57].

Lang *et al*[58] in the PRIDE study's second-year follow-up found that combination therapy of Ranibizumab and PRP could offer enhanced outcomes in some patients[54-61]. Ranibizumab was found to be more effective than PRP, particularly in reducing central retinal thickness and preventing progression to advanced DR stages. Additionally, combination

therapy involving ranibizumab and PRP has been shown to enhance overall efficacy, providing a comprehensive approach to managing PDR. Another study by Gross *et al*[56] highlighted the sequence effect in combined Ranibizumab and PRP therapy, emphasizing the timing and order of treatments for optimal efficacy.

Thus, Ranibizumab represents a significant advancement in the treatment of PDR, offering comparable, if not superior, outcomes to traditional PRP with added benefits in terms of patient quality of life and reduced procedural complications. Ongoing research and trials will continue to refine its application, potentially establishing it as the first-line therapy for PDR.

Perioperative use

Ranibizumab has also shown promise as a preoperative adjunct in vitrectomy for PDR with vitreous hemorrhage. Studies report that preoperative administration of Ranibizumab reduces intraoperative bleeding and postoperative complications, facilitating easier and safer surgical interventions.

A study by Li *et al*[59] demonstrated that patients pre-treated with Ranibizumab had significantly better surgical outcomes and faster recovery[58-63]. In this study, 50 patients with PDR and vitreous hemorrhage were divided into two groups: One group received a preoperative intravitreal injection of Ranibizumab, while the other group did not. The results indicated that the Ranibizumab pre-treatment group had a significantly lower incidence of intraoperative bleeding (12% *vs* 36%, $P < 0.05$) and postoperative complications such as recurrent vitreous hemorrhage and retinal detachment (8% *vs* 24%, $P < 0.05$). Additionally, patients in the Ranibizumab group had a shorter mean duration of surgery (45 minutes *vs* 60 minutes, $P < 0.05$) and a faster postoperative visual recovery, with a higher percentage achieving a visual acuity of 20/40 or better at three months post-surgery (68% *vs* 44%, $P < 0.05$).

These findings are consistent with other studies in the field. For instance, a meta-analysis conducted by Beaulieu *et al*[57] reviewed several randomized controlled trials and concluded that preoperative Ranibizumab significantly reduces intraoperative complications and improves surgical outcomes in patients undergoing vitrectomy for PDR. The pooled data showed a reduction in intraoperative bleeding by 50% and a 30% reduction in the incidence of postoperative complications.

Furthermore, a study by Li *et al*[59] supported these results, demonstrating that preoperative Ranibizumab administration not only facilitates vitrectomy but also enhances the overall anatomical and functional outcomes. Patients who received Ranibizumab had a higher rate of complete vitreous clearance and fewer required additional surgical interventions compared to the control group.

The mechanism behind these benefits is thought to involve Ranibizumab's ability to inhibit VEGF, thereby reducing neovascularization and vessel permeability. This leads to decreased intraoperative bleeding and a more stable intraocular environment during surgery, which contributes to better postoperative outcomes and faster recovery times.

Thus, preoperative use of Ranibizumab in vitrectomy for PDR with vitreous hemorrhage is associated with significant improvements in surgical safety and efficacy. These findings highlight the potential of Ranibizumab as a valuable adjunctive therapy in the management of complex diabetic retinal diseases.

AFLIBERCEPT

Aflibercept has emerged as a useful treatment option for PDR. FDA approved aflibercept for the treatment of PDR in 2019.

Clinical studies

Several studies have explored the efficacy of Aflibercept in PDR. The DRCR Protocol W is one of the most notable, examining the long-term visual outcomes of Aflibercept in preventing vision-threatening complications in DR. This four-year randomized trial demonstrated that patients receiving Aflibercept had significantly better visual outcomes compared to those undergoing standard treatment, with an average visual acuity improvement of 7.5 letters ($P < 0.001$) [64]. In another DRCR study[64], a randomized clinical trial, 205 adults with vitreous hemorrhage from proliferative DR were randomized to intravitreous aflibercept ($n = 100$) or vitrectomy with panretinal photocoagulation ($n = 105$). The primary outcome, mean visual acuity over 24 weeks, showed no significant difference between aflibercept (59.3) and vitrectomy group. At 4 weeks, aflibercept group had lower visual acuity compared to vitrectomy, but differences at 2 years were not significant. The study suggested that while initial treatment choice did not significantly affect visual outcomes over 24 weeks, larger studies may be needed to discern the potential long-term benefits of initial vitrectomy with panretinal photocoagulation.

Xie *et al*[62] conducted a comprehensive meta-analysis of Aflibercept for the long-term treatment of DME and PDR[64-70]. The analysis included multiple randomized controlled trials and found that Aflibercept significantly reduced central retinal thickness and improved visual acuity. Specifically, Aflibercept-treated eyes showed an average reduction in central retinal thickness of 137.5 microns and an improvement in visual acuity of 10.3 letters ($P < 0.01$). The CLARITY study[64-70], another significant trial, compared intravitreal Aflibercept with PRP for PDR. This non-inferiority trial concluded that Aflibercept was as effective as PRP in maintaining or improving BCVA at 52 weeks. Aflibercept-treated eyes had a mean BCVA improvement of 3.9 letters, while the PRP group showed a mean change of 3.3 letters ($P < 0.001$).

The ability of Aflibercept to reduce retinal neovascularization and stabilize or improve vision makes it a valuable tool in the management of PDR. However, some limitations and considerations must be addressed. The long-term sustainability of Aflibercept's benefits and the optimal frequency of injections remain areas requiring further research. Additionally, combination therapies involving Aflibercept and PRP or other treatment modalities are being explored to

enhance therapeutic outcomes. For instance, a study by Tao *et al*[64] found that combining Aflibercept with PRP resulted in better control of high-risk PDR compared to Aflibercept alone, suggesting that combination therapy might be more effective in certain patient populations[65].

In conclusion, both Ranibizumab and Aflibercept potentially offer comparable or superior outcomes to traditional PRP. Ranibizumab has demonstrated efficacy in visual acuity improvement and reduction of vision-impairing complications while Aflibercept has shown promising visual outcomes and central retinal thickness reduction. The perioperative use of Ranibizumab in vitrectomy further underscores its utility in enhancing surgical safety and outcomes. Ongoing research and clinical trials continue to refine the applications and optimize the treatment protocols of these anti-VEGF agents.

Role of biosimilars in the MDR

Biosimilars are biologic medical products that are highly similar to already approved reference biologics. Biosimilars are usually developed once the patent on the original biologic expires. They offer significant potential in reducing treatment costs and increasing accessibility. The introduction of biosimilars into clinical practice represents a transformative advancement in the management of DR and DME, providing more affordable therapeutic options without compromising efficacy or safety.

Ranibizumab biosimilars

Ranibizumab (Lucentis) biosimilars have been developed and approved in various regions. Razumab (Intas Pharmaceuticals Ltd.) was the first biosimilar of ranibizumab to be developed. Sufficient literature evidence is available on the clinical efficacy and safety of Razumab in real-world practice. The CESAR study[66] was a multicenter, prospective clinical trial that evaluated the efficacy and safety of Razumab in 324 patients with DME. The study demonstrated significant improvement in BCVA and reduction in Central macular thickness at 6 months follow-up. Importantly, the safety profile of Razumab was found to be comparable to the reference ranibizumab, with adverse events being mild to moderate and consistent with those reported for the innovator drug. In another multicenter retrospective study by Chakraborty *et al*[66] compared innovator ranibizumab with its biosimilar. The study included 250 patients, evenly split between those receiving the biosimilar and those receiving the reference product. Over a follow-up period of 12 months, both groups exhibited comparable improvement in BCVA and reduction in central macular thickness. The incidence of adverse events was similar across both cohorts, further supporting the equivalence of the biosimilar to its reference product in both efficacy and safety.

RanizuRel™ is another Ranibizumab biosimilar developed by Reliance Life Sciences. Sharma *et al*[67] assessed the safety of Ranizurel in clinical practice in Ranizurel Safety Evaluation in Real-World (RaSER) study. They concluded that Ranizurel has a favourable safety profile, with a low incidence of adverse effects reported among patients.

Aflibercept biosimilars

Aflibercept biosimilars are emerging as promising alternatives for treating DME. One notable biosimilar, Yesafili (aflibercept-jbvf), has recently been approved by the FDA in May 2024 as the first interchangeable biosimilar to Eylea (aflibercept) offering a cost-effective option for DME.

The advent of biosimilars for ranibizumab and aflibercept marks a significant milestone in the management of diabetic retinopathy and diabetic macular edema. These biosimilars offer efficacy and safety profiles comparable to their reference biologics, thus providing additional treatment options that can potentially reduce healthcare costs. Continuous research and post-marketing surveillance will be essential to further establish the long-term efficacy and safety of these biosimilars, ensuring their sustained integration into clinical practice.

NEWER DRUGS IN MANAGEMENT OF DME

In recent times, newer drugs are being developed to effectively control systemic disease as well as to act locally. Previously, trials like the United Kingdom Prospective Diabetes Study and Diabetes Control and Complications Trial have established the role of strict control of DM in reducing systemic complications of DM. Novel Drugs like Ruboxistaurin which modulate VEGF expression have been tested in clinical trials. The drug inhibits the beta isoform of protein Kinase C and downregulates VEGF expression. A reduction in the occurrence of sight-threatening complications after the use of Ruboxistaurin has been highlighted in the study conducted by PKC- DRS group. The group also highlighted the improvement in vision in some patients with DR. A decrease in the progression of DME and limited requirement of laser treatment was also noted in the study[69]. Other newer drugs being studied include octreotide, fiderestat and ranirestat. Increased levels of growth hormone and Insulin-like Growth factor can worsen DR. Octreotide is a synthetic somatostatin analogue which acts as a growth hormone-inhibitor. It has been used to prevent neovascularization and protect against the development of proliferative DR[70]. Fiderestat and ranirestat are aldose reductase inhibitors which lead to strict control of glycemia and have shown a reduction in retinal thickness secondary to their use in patients with DME[71]. Previously, long-term use of fiderestat has shown the suppression of the development of DR changes in animal models[72].

DRAWBACKS OF INTRAVITREAL THERAPY

The major drawback of intravitreal therapy is the temporary and short-lived effect of the agents used. The sight-threatening complications and ischemic changes may develop despite therapy. The risk for re-bleeding, progression and reappearance of pathological changes may persist after the time-bound effect of the drug wanes off. Also, once the severe ischemic changes have set in, the reversal of the effects is difficult even after therapy. Other side effects include cataract formation, glaucoma and endophthalmitis[72]. Diabetes mellitus is fast becoming a pandemic involving both the anterior as well as the posterior segment of the human eye. Artificial – intelligence will play a major role in a customized treatment for the ophthalmic – patients[73-75]. This futuristic modality will help in identifying the best suited anti – VEGF therapy for a particular patient of diabetic-retinopathy.

CONCLUSION

Upcoming novel therapies like stem cell therapy, the use of nanotechnology, and vesicular systems may revolutionize the therapy of DR in the near future. There have been serious concerns about the serious side effects of anti-VEGF therapy like cardiovascular risk factors and stroke. However, a recent meta-analysis did not highlight any significant risk after anti-VEGF therapy.

FOOTNOTES

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MINIREVIEWS

Melanocortin 4 receptor mutation in obesity

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Abstract

Obesity is increasingly prevalent worldwide, with genetic factors contributing to its development. The hypothalamic leptin-melanocortin pathway is central to the regulation of appetite and weight; leptin activates the proopiomelanocortin neurons, leading to the production of melanocortin peptides; these in turn act on melanocortin 4 receptors (MC4R) which suppress appetite and increase energy expenditure. MC4R mutations are responsible for syndromic and non-syndromic obesity. These mutations are classified based on their impact on the receptor's life cycle: *i.e.* null mutations, intracellular retention, binding defects, signaling defects, and variants of unknown function. Clinical manifestations of MC4R mutations include early-onset obesity, hyperphagia, and metabolic abnormalities such as hyperinsulinemia and dyslipidemia. Management strategies for obesity due to MC4R mutations have evolved with the development of targeted therapies such as Setmelanotide, an MC4R agonist which can reduce weight and manage symptoms without adverse cardiovascular effects. Future research directions must include expansion of population studies to better understand the epidemiology of MC4R mutations, exploration of the molecular mechanisms underlying MC4R signaling, and development of new therapeutic agents. Understanding the interaction between MC4R and other genetic and environmental factors will be key to advancing both the prevention and treatment of obesity.

Key Words: Leptin-melanocortin pathway; Downstream; G protein; Cyclic AMP; Mutation; Obesity syndromes; Screening; Setmelanotide

Core Tip: The leptin-melanocortin pathway regulates energy balance and body weight. Melanocortin-4 receptor (MC4R) plays a key role in this pathway by reducing hunger, inducing satiety and increasing energy expenditure. Mutations of MC4R result in obesity and hyperphagia in childhood. Setmelanotide is an MC4R agonist approved for use in obesity caused by leptin-melanocortin pathway dysfunction.

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INTRODUCTION

Body weight is maintained by a balance between energy intake and expenditure through the central hypothalamic leptin-melanocortin pathway. Among the hormones involved, leptin acts by activating the release by proopiomelanocortin (POMC) which is further cleaved into melanocortin ligands a and b; the b- melanocyte-stimulating hormone (MSH) binds and activates the melanocortin-4 receptor (MC4R) which results in reduced hunger, induction of satiety and increased energy expenditure. Variants of MC4R are associated with rare forms of recalcitrant obesity, usually manifesting in infancy or early childhood. A number of other mutations by MC4R and its proximate and downstream signals have been identified to cause syndromic obesity. Less serious disruptions by the pathway are responsible for intermediate degrees of obesity.

While syndromic or genetic causes of obesity are rare, the prevalence of obesity has been rising. A 2021 global report from the World Health Organization reported that obesity nearly tripled since 1975. As of 2016, more than 1.9 billion adults were overweight[1]. Adverse outcomes of obesity are dependent on race and ethnicity[2], which are projected to peak between 2026 and 2054, first in the United States, followed by European nations[3]. Similar findings were reported from the United Kingdom[4], South East Asia[5] and Africa[6].

A trend towards increasing obesity and overweight rates is observed even in childhood[7]. A 2017 Lancet report[8] on worldwide trends in body-mass index (BMI) showed that the rate of excess body weight was increasing in Asia. The increase in overweight outpaced the lowered prevalence of underweight[9]. The obesity pandemic in children was observed even in regions where obesity rates had plateaued before the coronavirus disease 2019 pandemic[10].

Gao *et al*[11] published a comprehensive global analysis on spatial and temporal trends in childhood overweight and obesity from 191 countries. Although genetics and environmental factors have a role in the pathogenesis, the rapid increase suggests a greater contribution by environmental factors. Since childhood obesity is the precursor of obesity in adulthood, prevention is essential[11]. The first step is to recognize the underlying lifestyle factors, which can then be addressed.

One of the proposed theories for the burgeoning rates of obesity in low and middle-income countries is the 'modernization theory'. The 'dependency/world systems theory view' proposes that external structural factors were mainly responsible for the rising obesity trends (*viz*, flooding countries with obesogenic, nutrient-poor foods). Fox *et al*[12] compared the dependency theory and modernization theory and concluded that the latter better accounted for increasing obesity. Modernization theory views that countries with progressing economies pass through phases of nutrition transition. from lower calorie, chiefly plant-based diet to a meat and processed food diet resulting in weight gain. The 2023 study by Gao *et al*[11] showed that worldwide, boys tended to be more overweight and obese than girls. Curating data from multiple cross-sectional studies from 1975 and projecting to 2020, the prevalence was reported to increase in boys from 4.1% in 1975 to 19.3% in 2016. In girls it was projected to increase from 4.6% to 17.5%. Before 2000, when assessed by income levels, the occurrence and rate of increase of obesity in both boys and girls was greater in higher income countries; however after 2020, highest rates and faster growth rates were seen in lower and middle income countries[11]. There was a global association between the rate of urbanization and childhood overweight and obesity[12].

These trends were attributed to global economic development, and cultural differences as well as intergeneration effects by malnutrition in early life. Countries with the most rapid growth shared rapid economic development, social and cultural changes leading to consumption of unhealthy ultra-processed foods. Reversal or stabilization of trends occurred due to interventions by governments[13].

CENTRAL REGULATION OF APPETITE AND WEIGHT

Weight and appetite are principally regulated by the ventromedial nucleus in the hypothalamus. Other brain areas include the arcuate nucleus, paraventricular nucleus and lateral hypothalamic area, where MC4R regulates energy metabolism by suppressing food intake and increasing energy expenditure. Identification of monogenic or non-syndromic obesity disorders revealed a complex interplay among different hormones and neurotransmitters, among which MC4R plays a central role. Appetite suppressing hormones (anorexigenic) and appetite stimulating hormones (orexigenic) communicate between the peripheral tissues and the hypothalamus[14].

Leptin, a hormone secreted by the adipose tissue binds to its receptor expressed on POMC neurons located in the arcuate nucleus of the hypothalamus. This leads to the formation of propeptide POMC, which is then cleaved to melanocortin ligands and MSH. MSH in turn activates MC4R, expressed in the paraventricular nucleus, leading to its effects on metabolism[15] (Figure 1).

MELANOCORTIN SYSTEM

POMC is an ancient gene, having been in existence for over 700 million years[16,17]. The melanocortin system has three components: Pro-peptide POMC, the melanocortin peptides and endogenous antagonists by these receptors [agouti and agouti-related protein (AgRP)][18].

Among the many genes related to obesity, MC4R is by far the most significant. It is localized in chromosome 18q22 and codes a 332 amino acid transmembrane protein[19]. The gene does not contain introns and has the highest homology with melanocortin 3 receptor (MC3R), another member of the MCR family[20]. Evolutionary analysis by MC4R showed that it underwent purifying selection, resulting in low levels of silent polymorphisms in humans[21].

Melanocortin receptors

Five melanocortin receptors (MCRs) are responsible for diverse actions[22] (Table 1) which are named 1-5 based on the sequence by their cloning. MC1R receptor, expressed in the skin and hair follicles regulates skin pigmentation. MC2R in the adrenal cortex regulates adrenal steroidogenesis. MCR3 and MCR4 are referred to as neural MCRs as they are principally expressed in the central nervous system. MC5R has a wide tissue expression, particularly the exocrine glands.

Research studies of MCR4 showed it was chiefly expressed in brain regions such as thalamus, hypothalamus and hippocampus. mRNA of MCR4 was identified in dentate gyrus, cortex and amygdala and in astrocytes. MC4R mRNA was first expressed on embryonic day 14, followed by other tissues by day 19[22]. MC3R and MC4R are chiefly expressed in the brain and MC5R in the peripheral tissues[18].

MC4R signaling

Upon binding by α-MSH to MC4R, adenylate cyclase is activated *via* G protein (guanine nucleotide-binding protein). Cyclic AMP (cAMP) is increased intracellularly, followed by activation of protein kinase A, exchange protein, extracellular regulated kinases 1 and 2 and cAMP response element binding protein. In addition, there is increased transcription by the proto-oncogene c-FOS with a simultaneous reduction of 5' AMP-activated protein kinase (AMPK) [23].

In addition to this pathway, different ligands induce different signals on binding, which are not mutually exclusive. In case of MC4R, food intake is controlled *via* biased signaling controls involving the Kir7.1, the inward rectifier potassium channel[24].

The understanding of G-protein-coupled receptor (GPCR) signaling, of which MC4R is a member, has expanded in recent years. Initially, GPCR was believed to act as a lock which was opened by the ligand, functioning as a key[25]. As the complexity of GPCR was revealed, other models were proposed, such as ternary-complex model, in which signaling was initiated by three principal components: Ligand, receptor and transducer such as GPCRs[26]. The ternary-complex model was further expanded where the receptor exists in two equilibrated states: The inactive state that cannot signal and the active state that can recruit transducers to render them functional. Finally the cubic-ternary complex model was proposed in which G-proteins and ligands were considered to belong to a common pool accessible to each receptor[27]. Metzger *et al*[28] recently suggested downstream MC4R signaling *via* β-restin recruitment and activation by MAPK. MC4R signaling was also shown to occur through MC4R/Gq/11 pathway[28].

MC4R in metabolism and energy regulation

MC4R, through its regulatory role in body weight homeostasis may influence the course of metabolic syndrome and multiple sclerosis *via* its anti-inflammatory and neuroprotective effects[29]. It also plays a role the regulation of glucose homeostasis, erectile function and cardiovascular tone[30].

The melanocortin peptides act through central MC4R to regulate appetite, body weight and energy expenditure[30]. Despite early evidence that intracerebrovascular administration of α-MSH and adrenocorticotropic hormone reduced food intake in rats, the critical importance of MC4R in regulation of energy homeostasis was not fully recognized until the mid 1990s[22]. Hypothalamic melanocortinergic neurons exert a tonic inhibitory action on feeding. Disruption of this pathway led to changes in food intake, suggesting that MC4R which is highly expressed in PVC is the primary mediator of melanocortin regulation[31], playing a pivotal role in the complex neural regulation of appetite[32]. The role of MC4R in energy homeostasis has been identified from mice knock out models which showed a gene dosage effect[33]. Energy balance is a result of reduced food intake (responsible for 60% of the effect) while 40% is due to changes in the expenditure of energy. Thermogenesis is regulated *via* activation of sympathetic nervous system-BAT-uncoupling protein 1 axis and hypothalamic-pituitary-thyroid axis[34].

To summarize, in states of starvation, leptin levels are low, POMC neuronal activity is reduced and AgRP neuronal activity is increased resulting in reduced MC4R signaling. In the fed state, POMC neurons are activated along with inhibition of AgRP neurons and increased MC4R signaling; all these resulting in diminished food intake and increased expenditure of energy[22].

Table 1 Melanocortin receptors

Name	Tissue expression	Principal actions
MC1R	Skin, hair follicles	Regulates pigmentation
MC2R	Adrenal cortex	Regulates adrenal steroidogenesis
MC3R	Central nervous system	Regulates energy homeostasis
MC4R	Central nervous system	Regulates energy homeostasis
MC5R	Exocrine glands; wide expression	Regulates exocrine gland secretion

MCR: Melanocortin receptors.

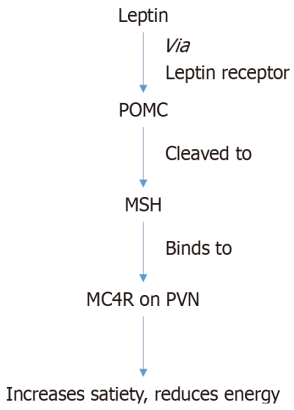

Figure 1 Overview of the leptin-melanocortin pathway. POMC: Proopiomelanocortin; MSH: Melanocyte-stimulating hormone; MC4R: Melanocortin 4 receptor; PVN: Paraventricular nucleus.

MC4R regulates energy homeostasis in other mammals and in lower vertebrates as well, including chickens and rainbow trout[22].

In a Chinese study, significant interactions were observed between variants near MC4R gene and obesity-related phenotypes (rs12970134), which was modified by physical activity[35-37].

MC4R MUTATIONS

Spontaneous and genetically induced variations of the melanocortin system showed the importance of this pathway in the regulation of body weight[38]. The first gene to be deleted in the mouse was the MC4R, which led to obesity. MC4R -/- phenotype was characterized by hyperphagia, adipocyte mass increase, increased growth and normal lean body mass [39]. The MC4R +/- model showed an intermediate phenotype in terms of body weight and food ingestion, supporting a gene dosage effect. Ste Marie *et al*[40] reported that inhibition of MC4R affected energy balance independent of food intake[40]. Therefore obesity in MC4R -/- mice was due to both increased food intake and diminished expenditure of energy[38]. Mutations of other genes in the melanocortin system such as MC3R and POMC also led to obesity.

Molecular classification by MC4R mutations

Identification of a variant MC4R in an obese individual does not necessarily imply that obesity is caused by the mutation [22]. Additional supporting information must be obtained such as familial co-segregation of the mutation and obesity; *in vitro* functional characterization by the mutant receptor confirms its causal role[41].

Classification of MC4R mutations is based on the receptor life cycle[22]: (1) Class I: Null mutations: impaired protein synthesis and/or enhanced protein degradation leads to low levels of protein (*e.g.* nonsense mutations); (2) Class II: Mutant receptors are produced in the cell but are misfolded and retained in the endoplasmic reticulum. This forms the largest group of mutations; (3) Class III: Binding defective mutants are expressed on the cellular surface but cannot bind with the ligands due to impaired binding capacity and/or affinity. Therefore signaling is impaired; (4) Class IV: Signaling-defective mutants properly reach the cell surface and bind the ligand, but transmit the signal with lower efficacy or not at all; and (5) Class V: Variants of unknown defect do not fit into any of the above.

More recently, Courbage *et al*[42] showed that MC4R variants can impact functions in three ways: (1) High: Nonsense, frameshift and splice variants, missense variants and rare variants with conclusive functional tests; (2) Moderate: Missense variants that are predicted as 'damaging' by at least four of the seven prediction tools; and (3) Low: Missense variants predicted as 'less likely damaging' by at least four of the seven prediction tools. The classification was used for

the analysis of 6467 subjects to assess the significance of heterozygous variants on the leptin-melanocortin system among the severely obese[42].

Feeding behavior in humans harboring MC4R mutants

To assess the prevalence and phenotypic effects of MC4R mutants, 20537 electronic medical records and genomics (eMERGE) participants with MC4R coding region sequencing data were studied; in addition 77454 independent persons with genome-wide genotyping data at this locus were also studied. The authors identified 125 coding variants (*n*: 1839 eMERGE participants), including 30 variants that were unreported earlier[43]. MC4R associated obesity was the most common form of monogenic obesity spectrum among 170 rare genetic variants associated with hyperphagia and early onset obesity[44,45].

CLINICAL PRESENTATION

The genotype-phenotype association of heterogeneous variants in leptin-melanocortin pathway was studied in 6467 subjects (6347 probands and 120 relatives)[42]. Specifically the MC4R gene was sequenced for 1165 subjects of the 1486 probands. To assess the combined effect of heterozygous variants on phenotype, subjects were chosen who were sequenced on the five genes, *viz LEP*, *LEPR*, *POMC*, *PCSK1* and *MC4R*. BMI of subjects with combined heterozygous variants was higher than in those with a single heterozygous variant (BMI65.2+/-13.2 kg/m^2 *vs* 49.0 +/-9.1 kg/m^2, $P < 0.01$)[42].

Body weight and growth

Aggregated data from 200 MC4R genetic mutations among nearly 1000 patients[46], (homozygous or compound heterozygous) showed early-onset severe obesity.

In heterozygous carriers, obesity was variable: Children carrying heterozygous mutation had similar BMI to wild-type children with obesity. In contrast, adults with heterozygous carriers had higher BMI compared to wild type patients with obesity. Gender differences were also observed; BMI was higher in middle aged women compared to middle aged men.

Where information on age of obesity was available (n = 104) mean age of onset of obesity was at 1.2 years old in homozygous carriers and 3.8 years old in those with heterozygous mutation. Children had an initial accelerated height, although the final height was lower than average.

Eating behavior

Hyperphagia was common in patients with MC4R mutations. When recorded (n = 175), hyperphagia was observed in 95% (100% in homozygous patients and 95.1% in heterozygous patients).

Other features

There was no specific change in the course of puberty and ultimate fertility in patients with MC4R mutations. Data on cognitive function was available in 30 cases; nine had mild disability: Speech delay, motor retardation, and mild mental retardation. Acanthosis nigricans was observed in 31%-41% of subjects.

Metabolic abnormalities

Hyperinsulinemia was observed more often in children (55%) than in adults (20%). Type 2 diabetes mellitus was reported in 16.9% of the cohort (n = 148). Dyslipidemia was present in 33% of mutation carriers (n = 20/60: 32% children and 34.8% adults). Advanced bone age and greater bone density were also reported.

In Qatar two subjects with MC4R mutation identified reduced expression of MC4R on the cell surface, intracellular retention by the MC4R protein, and failure to activate downstream signaling of the MC4R[47].

The obesity phenotype can be modified by the interaction of other genetic factors which may either be protective or deleterious, as well as by environmental factors[48].

SCREENING FOR MC4R MUTATIONS

Currently there are no established guidelines for newborn genetic screening. However with the availability of drugs to treat subjects with MC4R mutations (setmelanotide)[49,50], a case is made for childhood genetic screening in obesity. In childhood addition, genetic analysis by MC4R mutations could help predict responsiveness to drug treatment[51,52].

MANAGEMENT OF OBESITY DUE TO MC4R MUTATIONS

An understanding of the MC4R receptor, its expression and downstream signaling enabled the identification of agonists which mimic its physiological actions. Initially, analogues were hampered by MC4R activation of sympathetic nervous system, thereby affecting blood pressure and heart rate[53]; sexual arousal was another serious side effect. Development of a number of molecules was stalled early due to adverse effects including tachycardia, hypertension, sexual arousal and

skin pigmentation driven by MC1R activation[53]. These were largely due to the complex intracellular signaling by MC4R. It now seems likely that internalization of agonist induced MC4R could be the key element in the control of energy homeostasis.

Setmelanotide

Setmelanotide, an MC4R agonist has been approved for use in the treatment of obesity due to MC4R mutations. It showed no adverse effects on heart and blood pressure in humans, while having a substantial weight loss effect, with a favorable therapeutic index[53].

Animal studies published in 2016 showed that setmelanotide had the potential to be used as a replacement therapy for rare syndromic forms of obesity due to impaired POMC neuronal function[54]. Coulter *et al*[55] proposed that setmelanotide is a potential treatment for obesity caused by mutations of POMC system[55].

A number of case series showed that setmelanotide was effective in genetic and syndromic forms of obesity[49,50]. The Anti-Obesity Medications and Investigational Agents: An Obesity Medicine Association Clinical Practice Statement 2022 published guidelines for the use of setmelanotide along with other anti-obesity agents[56].

Setmelanotide is indicated for chronic weight management in adult and children aged 6 years or older when obesity is due to *POMC*, *PCSK1*, or *LEPR* deficiency. The diagnosis must be based on genetic testing which shows 'variants in *POMC*, *PCSK1*, or *LEPR* genes that are interpreted as pathogenic, likely pathogenic, or by variant of uncertain significance'. The drug is discontinued if, after 12–16 weeks of treatment, there is no weight loss by 5% from baseline or 5% baseline BMI for children with continued growth potential.

Setmelanotide is started at a dose of 2 mg subcutaneously once daily for 2 weeks with monitoring for gastrointestinal (GI) adverse reactions. If this dose cannot be tolerated, it can be reduced to 1 mg once daily. If the lower dose is tolerated, uptitration to 2 mg can be attempted for additional weight reduction, with a further increase to 3 mg a day; however the lowest tolerable dose must be used for maintenance treatment.

In children between the ages of six and 12, the drug is begun at a dose of 1 mg injected subcutaneously once daily for 2 weeks, while monitoring for adverse GI events. If the dose is not tolerated, it must be reduced to 0.5 mg once daily. If it is tolerated and for additional weight reduction, the dose can be increased to 1 mg once daily. It can be further increased to 2 mg once daily. In case the higher dose is not tolerated, it can be reduced to 1 mg once daily. The highest dose that can be given is limited to 3 mg once daily.

Common side effects, seen in about 25% of subjects consist of injection site reactions, skin hyperpigmentation, nausea, headache, GI effects, depression, upper respiratory tract infection, and spontaneous penile erection.

Potential for newer agents

In addition to MC4R agonists and antagonists, inverse agonists are potential areas for investigation; these include AgRP and its mimics for MC4R[27] (Table 2).

Table 2 Drugs for use in genetic forms of obesity

Drug	Analogue
Setmelanotide	Analogue of α-melanocyte melanocyte-stimulating hormone
Metreleptin	Human leptin analogue
Liraglutide	Glucagon-like peptide-1 analogue
Semaglutide	Long-acting glucagon-like peptide-1 analogue
Others	

CONCLUSION

Identification of monogenic obesity syndromes due to dysfunction of MC4R clarified the role of leptin-melanocortin pathway in regulating energy balance[57]. Impaired activity of MC4R led to rare monogenic forms of obesity whereas gene polymorphisms were related to weight gain and metabolic syndrome. Other MC4R gene polymorphisms were protective against obesity. MC4R could also have anti-inflammatory and neuroprotective effects[57]. Understanding the expression and downstream effects of MC4R resulted in the development of agonists such as setmelanotide which is approved for treatment of obesity due to POMC disorders[56]. In addition to MC4R agonists and antagonists, inverse agonists could be developed such as AgRP and its mimics for MC4R[27]. Early identification of genetic forms of obesity helps in tailoring management from a young age, which can prevent progressive metabolic abnormalities and improve long term prognosis[52]. Future research on MC4R and its role in obesity should focus on studying larger and more diverse population groups. This enhances knowledge of the MC4R signaling pathways, and aids in the development of personalized and effective therapies. Finally, interdisciplinary collaboration combining genetics, endocrinology, and pharmacology is necessary for translation into clinical applications and better patient outcomes.

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ORIGINAL ARTICLE

Retrospective Study

Value of autopsy in the modern age: Discrepancy between clinical and autopsy diagnoses

Cecilia Salzillo, Roberta Basile, Gerardo Cazzato, Giuseppe Ingravallo, Andrea Marzullo

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Abstract

BACKGROUND

Autopsy is a medical procedure that consists of the examination of the corpse to determine the cause of death and obtain information on pathological conditions or injuries. In recent years, there has been a reduction in hospital autopsies and an increase in forensic autopsies.

AIM

To evaluate the utility of autopsy in the modern age and the discrepancy between clinical and autopsy diagnoses.

METHODS

A retrospective observational study was conducted on the reports of all 645 hospital autopsies performed at Polyclinic of Bari from 2006 to 2021.

RESULTS

Group A, 2006-2009, 174 cases were studied: 58% male, 58% adults, 55% neonatology; pulmonary disease was the cause of death in 23% of cases; and there was a discrepancy between clinical and autopsy diagnosis in 55% of cases. Group B, 2010-2013, 119 cases: 52% male, 46% infants, 48% neonatology; pulmonary disease was the cause of death in 25% of cases; and there was a discrepancy between clinical and autopsy diagnosis in 56% of cases. Group C, 2014-2017, 168

cases: sex equality, 37% infants, 25% gynecology; pulmonary disease was the cause of death in 24% of cases; and there was a discrepancy between clinical and autopsy diagnosis in 58% of cases. Group D, 2018-2021, 184 cases: 56% male, 38% adult, 32% gynecology; pulmonary disease was the cause of death in 27% of cases; and there was a discrepancy between clinical and autopsy diagnosis in 58% of cases.

CONCLUSION
The study of hospital autopsies reveals a 56.75% discrepancy between clinical diagnosis and autopsy, highlighting the importance of autopsies, especially for fetal and neonatal diseases, which represent 59% of cases.

Key Words: Hospital autopsy; Modern age; Clinical diagnosis; Autopsy diagnosis; Public health

Core Tip: The hospital autopsy is useful in the modern age, especially for the diagnosis of fetal and neonatal pathologies. Genetic and non-genetic diagnoses are important to future parents for subsequent pregnancies and can thus be studied.

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INTRODUCTION

The term "autopsy" is derived from Greek and means "to see for oneself". There are two types of autopsy: hospital autopsy and forensic autopsy.

The first law authorizing human dissection was enacted in 1231 by Frederick II. In the 16th century, Andreas Vesalius initiated the modern study of anatomy with the publication of "De Humani Corporis Fabrica"[1]. Giovanni Morgagni correlated clinical symptoms with organic changes, introducing the anatomical-clinical concept, and published "De sedibus et causis morborum per anatomen indagatis"[2].

In the 19th century, Karl von Rokitansky performed over 30000 autopsies and wrote the "Handbook of Pathological Anatomy"[3], while Rudolf Virchow, the founder of modern pathology, introduced detailed autopsy techniques[4]. Maurice Letulle described the technique of mass organ removal[5], and Albrecht Ghon introduced the technique of *en bloc* removal.

Over the last decades, there has been a significant decrease in the number of hospital autopsies and an increase in judicial autopsies[6]. This phenomenon may be due to various factors, including changes in medical practices, economic issues and the evolution of diagnostic techniques. Hospital autopsies are used as a tool to improve the quality of care and diagnostic accuracy. However, their utilization has declined due to budget constraints and growing confidence in imaging technologies[7,8]. Forensic autopsies are performed to determine the cause of death for forensic purposes. Their frequency has increased due to a greater emphasis on medico-legal responsibility and the need for thorough investigations in cases of suspicious or violent death[9,10]. This underlines how our society has an interest in the legal aspect rather than in knowing the cause of death.

In this study, we aimed to analyze 645 hospital autopsy cases from 2006 to 2021 retrieved from the digital archive of the Pathology Unit, Department of Precision and Regenerative Medicine and Ionian Area, Polyclinic of Bari, to study the rate of concordance between clinical and autopsy diagnosis and evaluating whether the execution of hospital autopsies is helpful in the modern age.

MATERIALS AND METHODS

Study design

In our retrospective observational study, we analyzed the autopsy case history of the Pathology Unit, Department of Precision and Regenerative Medicine and Ionian Area, Polyclinic of Bari.

Patient data source

We used the digitalized archive of autopsy reports between 2006 and 2021 for a total of 645 cases, and all cases in the archive were included in the study.

Subgrouping for analysis

The 645 cases were divided into groups of 4 years to make the samples uniform and comparable: group A: 2006-2009 of 174 cases, group B: 2010-2013 of 119 cases, group C: 2014-2017 of 168 cases, and group D: 2018-2021 of 184 cases.

Each subgroups was divided by age: Fetus < 180 days or < 6 months gestational age, infant/newborn 1 year of age, child/adolescent 1-16 years of age, and adult > 16 years of age.

The total divisions into subgroups included the following: Sex, age, specialty, autopsy diagnosis, and correlation between clinical and autopsy diagnosis.

To analyze the pathological diagnosis, we grouped the cause of death into: Cardiovascular, infectious, miscellaneous, neoplastic, placental, pulmonary, and syndromes/malformations.

Finally, we analyzed the discrepancy between clinical and autopsy diagnoses.

RESULTS

Group A

Out of 174 cases in group A (Table 1), 58% were male, 41% female, and 1% undefined, of which 58% were adults, 1% children, 29% infants, and 12% fetuses. Out of these 174, the specialty were: neonatology 55%, gynecology 19%, internal medicine 10%, external 7%, and other departments 9%. The cause of death was classified as pulmonary disease in 23%, syndromes and/or malformations 10%, infections 8%, cardiovascular diseases 6%, placental disease 4%, neoplasms 4%, and miscellaneous 45%. In 55% of the analyzed cases, there was discrepancy between clinical and autopsy diagnoses (Figure 1A).

Group B

Group B (Table 2) comprised 119 cases; 51% male, 48% female, and 1% undefined, of which 31% were adults, 6% children, 46% infants, and 17% fetuses. The specialty of the origin of the deceased was 48% neonatology, 19% gynecology, 10% cardiac surgery, 12% general surgery, 4% neurology, and 7% others. The cause of death was categorized as 25% pulmonary diseases, 15% cardiovascular diseases, 11% syndromes and/or malformations, 9% infectious diseases, 3% neoplasms, 1% placental pathology, and 36% miscellaneous. In 56% of the cases, a discrepancy between clinical and autopsy diagnoses was observed (Figure 1B).

Group C

Group C (Table 3) comprised 168 cases, of which 50% were male and 50% female; 26% were adults, 1% were children, 37% infants, and 36% fetuses. The origin of the deceased was 30% gynecology, 25% neonatology, 17% cardiac surgery, 6% emergency room, 5% general surgery, 4% from other regional hospitals, and 13% others. The cause of death was classified as 24% pulmonary diseases, 15% cardiovascular diseases, 11% syndromes and/or malformations, 6% placental pathology, 3% infections, 2% neoplasms, and 39% miscellaneous. In 58% of the cases, there was a discrepancy between clinical and autopsy diagnoses (Figure 1C).

Group D

Group D (Table 4) comprised 184 cases; 56% male, 43% female, and 1% undefined; 38% were adults, 3% children, 26% infants, and 33% fetuses. Of these 184 cases, the specialty of origin was 32% gynecology, 21% other regional hospitals, 21% neonatology, 14% emergency department, and 12% others. In 27% of patients, the cause of death involved pulmonary diseases, 23% cardiovascular diseases, 6% syndromes and/or malformations, 5% placental disease, 4% infections, 2% neoplasms, and 33% miscellaneous. In 58% of the cases, there was a discrepancy between clinical and pathological diagnoses (Figure 1D).

Statistical analysis of changes over time

From our study it is evident that sex distribution has remained constant over time. In fact, the percentage of males showed only slight variations, going from 58% in group A to 56% in group D; whereas the percentage of females remained stable, fluctuating between 41% and 50%. This stability suggests that there were no significant changes in the sex distribution over the study period.

We observed an important change in age distribution. The percentage of newborns and fetuses increased over time, with a peak in Group C where newborns were 37% of cases and fetuses were 36% of cases; however, the percentage of adults significantly decreased, from 58% in Group A to 38% in Group D. This trend demonstrates the growing attention of autopsies to pediatric and neonatal cases in recent years.

Neonatology is one of the main specialties involved in autopsies, but the percentage fell from 55% in group A to 21% in group D. In contrast, gynecology had a significant increase, becoming the predominant specialty in group D with 32%. This change reflects possible changes in clinical practices and autopsy patient populations.

Lung and cardiovascular diseases consistently have high rates as causes of death across all groups. However, diagnoses classified as "miscellaneous" made up a significant proportion in each group, with a slight decrease from 45% in Group A to 33% in Group D. This broad and generic category of diagnoses could include a variety of conditions that do not fit into major disease categories, likely reflecting the complexity and diversity of autopsy cases examined.

The percentage of discrepancy between the clinical diagnosis and the autopsy diagnosis remained high and constant throughout the study period, with a variation between 55% and 58%. This discrepancy highlights the importance of

Table 1 Group A: Analyzing the 174 hospital autopsies, 2006-2009

Group A	Sex	Age	Specialty	Autopsy diagnosis	Discrepancy
174 cases, 2006-2009	58% male, 41%female, 1% undefined	58% adults, 29% infants, 12% fetuses, 1% children	55% neonatology, 19% gynecology, 10% internal medicine, 9% others, 7% external	23% pulmonary, 10% syndromes/malformations, 8% infections, 6% cardiovascular, 4% placental, 4% neoplasms, 45% miscellaneous	55%

Table 2 Group B: Analyzing the 119 hospital autopsies, 2010-2013

Group B	Sex	Age	Specialty	Autopsy diagnosis	Discrepancy
119 cases, 2010-2013	51% male, 48% female, 1% undefined	31% adults, 46% infants, 17% fetuses, 6% children	48% neonatology, 19% gynecology, 12% general surgery, 10% cardiac surgery, 7% others, 4% neurology	25% pulmonary, 15% cardiovascular, 11% syndromes/malformations, 9% infectious, 3% neoplasms, 1% placental, 36% miscellaneous	56%

Table 3 Group C: Analyzing the 168 hospital autopsies, 2014-2017

Group C	Sex	Age	Specialty	Autopsy diagnosis	Discrepancy
168 cases, 2014-2017	50% male, 50% female	37% infants, 36% fetuses, 26% adults, 1% children	30% gynecology, 25% neonatology, 17% cardiac surgery, 13% others, 6% emergency room, 5% general surgery, 4% external	24% pulmonary, 15% cardiovascular, 11% syndromes/malformations, 6% placental, 3% infections, 2% neoplasms, 39% miscellaneous	58%

Table 4 Group D: Analyzing the 184 hospital autopsies, 2018-2021

Group D	Sex	Age	Specialty	Autopsy diagnosis	Discrepancy
184 cases, 2018-2021	56% male, 43% female, 1% undefined	38% adults, 33% fetuses, 26% infants, 3% children	32% gynecology, 21% neonatology, 21% external, 14% emergency room, 12% others	27% pulmonary, 23%cardiovascular, 6% syndromes/malformations, 5% placental, 4% infections, 2% neoplasms, 33% miscellaneous	58%

autopsies as a crucial diagnostic tool, underscoring the limitations of clinical diagnoses and the utility of autopsy in revealing conditions not identified during life.

DISCUSSION

By analyzing the 645 hospital autopsies (Table 5), we observed a predominance of 53.75% males, 59% newborns and fetuses. The most frequent specialty of origin was 37.25% neonatology and 25% gynecology. The cause of death in our study was primarily pulmonary diseases followed by cardiovascular diseases, and the discrepancy between clinical and autopsy diagnoses was over 56.75%.

Our study highlights that hospital autopsies were mainly performed in fetuses and infants, and the requirement of autopsy in adults was progressively reduced.

The decrease in hospital autopsies in adult patients is probably due to the excessive trust in medical diagnostic technology, whereby it is believed that the autopsy does not provide any additional information that is not already known at the time of death[11].

Although some studies have underlined the importance of carrying out a hospital autopsy[6,11,12], medical doctors often do not recognize the importance and therefore do not explain the advantages to the deceased's relatives. Autopsy is a fundamental tool for understanding pathological processes, the effectiveness of treatments, the correct diagnostic approaches, and for preventing medical errors and supporting public health[13,14].

In a study conducted in Sweden by Friberg *et al*[15] on 2410 hospital autopsies of adult patients, there was an overall reduction in the request for autopsy examination with prevalence of cardiovascular disease as the cause of death, and with a discrepancy of more than 30% between clinical diagnosis and autopsy. The authors highlighted how the hospital autopsy provides information about the disease and cause of death that is likely unknown to the doctor and presumably to the relatives of the deceased and explained how this can have a negative impact on public health[15,16].

In a prospective cohort study, Latten *et al*[17] investigated the relationship between clinical cause of death, a history of cancer, and the rate of medical autopsies. The authors observed that the autopsy rate was positively correlated with the number of causes of death, suggesting a higher value of interest in autopsies in complex medical cases. According to the authors, healthcare and individuals would benefit from an increase in post-mortem investigations[17].

Table 5 Analyzing the 645 hospital autopsies, 2006-2021

Group	Sex	Age	Specialty	Autopsy diagnosis	Discrepancy
A + B + C + D = 645 cases, 2006-2021	53.75% male, 45.5% female, 0.75% undefined	38.25% adults, 34.5% infants, 24.5% fetuses, 2.75% children	37.25% neonatology, 25% gynecology, 10.25% others, 8% external, 6.75% cardiac surgery, 5% emergency room, 4.25% general surgery, 2.5% internal medicine, 1% neurology	24.75% pulmonary diseases, 14.75% cardiovascular diseases, 9.5% syndromes/malformations, 6% infections, 4% placental disease, 2.75% neoplasms, 38.25% miscellaneous	56.75%

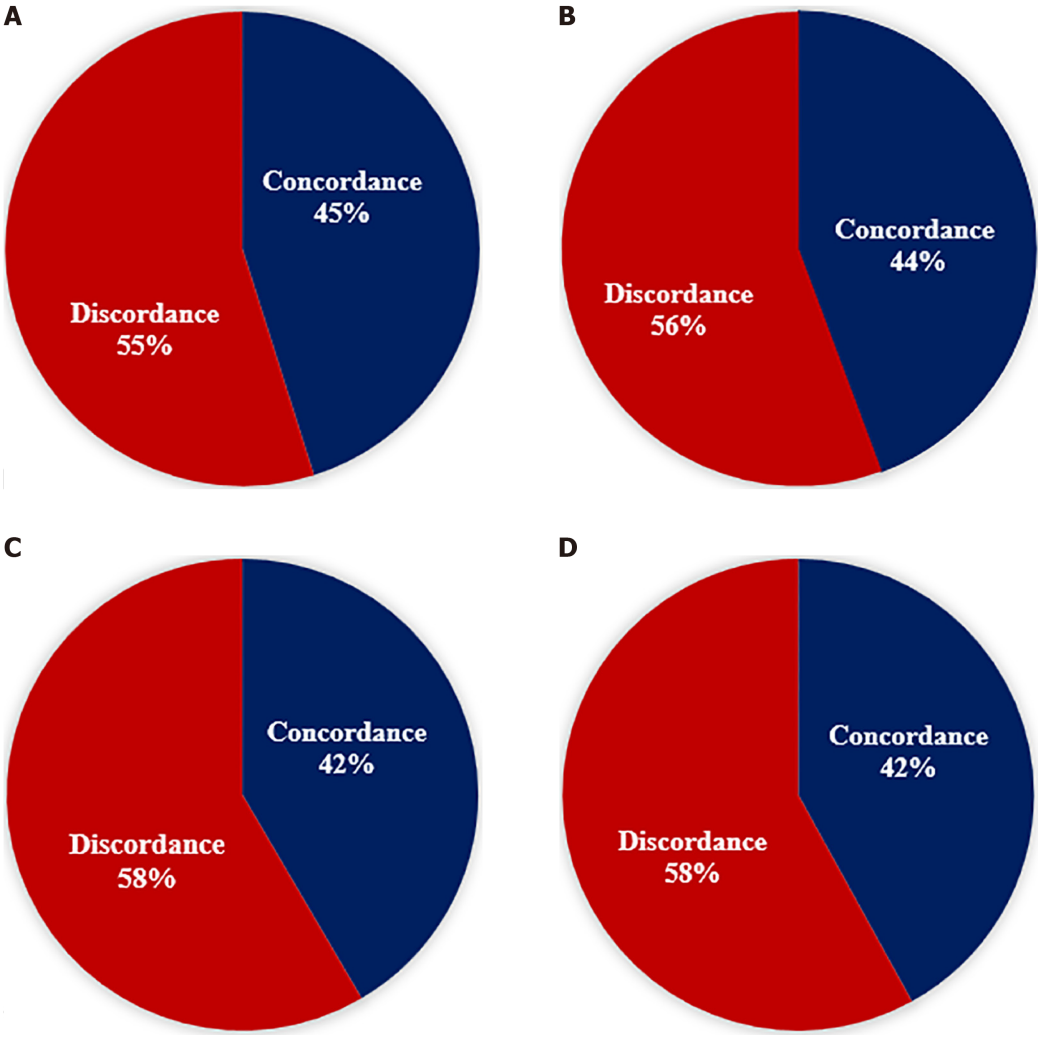

Figure 1 Discrepancy between clinical and autopsy diagnosis. A: Group A discrepancy of 55%; B: Group B discrepancy of 56%; C: Group C discrepancy of 58%; D: Group D discrepancy of 58%.

In their study, O'Rahelly *et al*[18] observed a 40% reduction in the autopsy rate and other studies in the literature[9,19,20].

One factor that can have a positive influence on reducing the performance of hospital autopsies is the communication by the doctor of the importance of the autopsy to provide clarification of the cause of death[21], especially in sudden death in fetuses, infants and young people, from cardiac or non-cardiac causes and from genetic or non-cardiac causes.

Studies in the literature[22-25] report that male infants and children tend to have higher mortality rates than females, which may be explained by the greater vulnerability of males to perinatal complications, congenital diseases and genetic syndromes. In particular, male newborns are more likely to be born prematurely with low birth weight and to develop respiratory distress syndrome, as they have slower lung maturation than females, making them more susceptible to respiratory problems and consequently significantly increases the risk of neonatal mortality. Furthermore, another crucial aspect is the genetic vulnerability of males due to the presence of only one X chromosome compared to XX females, which makes them more susceptible by increasing mortality. In addition, it is important to underline that stillbirth represents a dramatic experience, not only for parents, but also for professionals, especially if it occurs in the last weeks of gestation.

Interestingly, in our case history, the major cause of death was pulmonary pathologies. This result may be due to the fact that lung pathologies are often subtle in their clinical manifestation and are among the pathologies that predominantly manifest themselves in long-term hospitalizations in patients hospitalized for another pathology, but also that the cause of death is often not clear and therefore clinicians are more sensitive to requesting a hospital autopsy. Therefore, it is clear that the hospital autopsy is still useful in the modern age to evaluate the clinical diagnostic accuracy. It is particularly important for fetuses and newborns to identify the various causes of death, both genetic and otherwise, and to be able to help future parents plan for subsequent pregnancies[26]. From a public health perspective, the autopsy can become a preventive tool for family members and the community and play a role in grieving[27].

Strategies to increase autopsy rates

Our study and others demonstrate that hospital autopsies have significantly decreased in recent years despite being a fundamental tool for understanding causes of death and for improving medical practices. Consequently, this reduction has resulted in a loss of valuable clinical information that could contribute to medical training, scientific research and improving the quality of care. Effective strategies to increase autopsy rates should involve raising awareness of healthcare personnel, patients and hospital policies.

A crucial first step is to invest in the continuous education and training of healthcare personnel, who must be constantly updated on the importance of autopsies and the procedures for requesting them through periodic refresher courses. The next step is empathetic communication by health professionals with families who are unaware of the benefits of autopsies, both for the medical community and for society. It is important to educate families and provide them with clear and easily understandable information through information leaflets and other resources that explain the autopsy process, its benefits and address ethical and religious issues.

Additionally, hospital policies are instrumental in providing guidelines and recommendations to establish mandatory autopsies in specific circumstances, such as in cases of sudden deaths.

CONCLUSION

In conclusion, the study of hospital autopsies at the Polyclinic of Bari shows that the discrepancy between clinical and autopsy diagnosis is 56.75%. Moreover, the hospital autopsy is still useful in the modern age, especially for the diagnosis of fetal and neonatal pathology who together account for 59% of autopsies, so that genetic and non-genetic diagnoses can be studied to help future parents for subsequent pregnancies. Focusing on the problems of stillbirth means ensuring adequate support for mothers and relatives, who are all too often left alone to face this traumatic event. Furthermore, analysis of hospital autopsy data over time reveals significant changes in patient demographics, medical specialties involved and causes of death. Despite these changes, the discrepancy between clinical and autopsy diagnoses remains an ongoing problem, underscoring the crucial importance of autopsies to improve diagnostic accuracy and the quality of medical care.

FOOTNOTES

Author contributions: Salzillo C and Basile R contributed equally; Salzillo C and Basile R contributed to the conceptualization and methodology; Salzillo C and Cazzato G performed the investigation; Basile R and Cazzato G were involved in data curation; Salzillo C, Basile R, and Cazzato G contributed to writing—original draft preparation; Marzullo A and Ingravallo G contributed to writing—review and editing; Marzullo A and Ingravallo G supervised the work; All authors have read and agreed to the published version of the manuscript.

Institutional review board statement: The data used in the document were obtained from autopsy reports in the database of the Pathology Unit, Department of Precision and Regenerative Medicine and Ionian Area, Bari Polyclinic, Italy. Access to the hospital archive for consultation of histopathological reports was approved and allowed by the director of the Pathology Unit. Furthermore, as this is a retrospective study of deceased patients, institutional review board approval is not required.

Informed consent statement: Data used in the paper were obtained from autoptic reports and do not need to be authorized for publication because they are considered anonymous and do not contain sensitive data. Moreover, according to Italian Law, the relatives of the deceased patients give their authorization to the use of the data for scientific purposes with the consent to the autopsy.

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ORIGINAL ARTICLE

Retrospective Study

HIPPO artificial intelligence: Correlating automated radiographic femoroacetabular measurements with patient-reported outcomes in developmental hip dysplasia

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Abstract

BACKGROUND

Hip dysplasia (HD) is characterized by insufficient acetabular coverage of the femoral head, leading to a predisposition for osteoarthritis. While radiographic measurements such as the lateral center edge angle (LCEA) and Tönnis angle are essential in evaluating HD severity, patient-reported outcome measures (PROMs) offer insights into the subjective health impact on patients.

AIM

To investigate the correlations between machine-learning automated and manual radiographic measurements of HD and PROMs with the hypothesis that artificial intelligence (AI)-generated HD measurements indicating less severe dysplasia correlate with better PROMs.

METHODS

Retrospective study evaluating 256 hips from 130 HD patients from a hip preservation clinic database. Manual and AI-derived radiographic measurements were collected and PROMs such as the Harris hip score (HHS), international hip outcome tool (iHOT-12), short form (SF) 12 (SF-12), and Visual Analogue Scale of the European Quality of Life Group survey were correlated using Spearman's rank-order correlation.

RESULTS
The median patient age was 28.6 years (range 15.7-62.3 years) with 82.3% of patients being women and 17.7% being men. The median interpretation time for manual readers and AI ranged between 4-12 minutes per patient and 31 seconds, respectively. Manual measurements exhibited weak correlations with HHS, including LCEA (r = 0.18) and Tönnis angle (r = -0.24). AI-derived metrics showed similar weak correlations, with the most significant being Caput-Collum-Diaphyseal (CCD) with iHOT-12 at r = -0.25 (P = 0.042) and CCD with SF-12 at r = 0.25 (P = 0.048). Other measured correlations were not significant (P > 0.05).

CONCLUSION
This study suggests AI can aid in HD assessment, but weak PROM correlations highlight their continued importance in predicting subjective health and outcomes, complementing AI-derived measurements in HD management.

Key Words: Hip dysplasia; Patient reported outcome measures; Deep-learning; Artificial intelligence; Radiographs; Lateral center edge angle

Core Tip: In this study, we compared an artificial intelligence (AI) tool measuring anteroposterior hip radiographs against manual readers for assessing hip dysplasia (HD) associations with patient-reported outcome measures (PROMs). The AI tool, HIPPO, efficiently generated radiographic measurements but showed poor correlations with PROMs, highlighting its current limitations in predicting clinical outcomes solely from radiological data. This indicates that while AI can aid radiographic assessments, PROMs remain crucial for capturing subjective patient experiences. The findings underscore the importance of integrating PROMs as an additional element in the clinical decision-making processes for HD, while also incorporating efficient radiographic assessment by AI tools.

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INTRODUCTION

Acetabular or hip dysplasia (HD) is a developmental condition that is characterized by a shallow or upsloping acetabulum that can be accompanied by femoral head incongruency[1]. HD often presents in the pediatric and adult population with symptoms of hip pain and/or instability. When left untreated, it can lead to hip osteoarthritis (OA) due to stress overload, shear forces, and improper mechanics progressively affecting joint cartilage[2]. Several conservative and surgical treatment options currently exist; among them, the most used modalities include physical therapy and lifestyle modifications, periacetabular osteotomy, hip arthroscopy, and total hip arthroplasty. The treatment modality chosen depends upon the time of discovery, symptom severity, and status of the hip labrum and cartilage, and functional disability[3-5].

Hip radiographs are the current gold standard for the initial screening and assessment of HD[6].There are a multitude of validated diagnostic radiographic measurements employed to assist the diagnosis of HD. Among them, lateral center edge angle (LCEA) is most commonly used, as measured on a standing anteroposterior (AP) pelvis radiograph[7]. Additionally, the Tönnis angle and extrusion index are also commonly used in clinical practice[8]. Following radiographic assessment, advanced imaging such as magnetic resonance imaging or computed tomography can be used for pre-operative planning and further assessment of the health of the labrum or hyaline cartilage[9].

While a diagnosis of HD is established by a combination of clinical presentation, examination findings and radiographic measurements, patient-reported outcome measures (PROMs) are equally important to illustrate the perception of patients' subjective hip health status[10]. These are gleaned from different surveys administered at the time of clinical presentation, such as the Harris hip score (HHS), international hip outcome tool (iHOT-12), Visual Analogue Scale (VAS) for Pain, VAS of the European Quality of Life Group (EQ-VAS) (health status), and short form (SF) 12 (SF-12) (quality of life), among others. Each patient reported outcome survey provides a different evaluation of the patient's condition. For instance, the HHS is a reliable indicator for patient function, while iHOT-12 provides a good indication for quality-of-life changes[11-13].

PROMs have become increasingly important in evaluating indications for treatment and prognosis for HD patients[14-16]. Despite their common use in the clinical evaluation of patients with HD and pain, the International Hip-related Pain Research Network meeting in 2018 ruled that more studies are needed to further evaluate the usefulness of PROMS[17].

Thus, it is important to examine the relationships between validated radiographic HD measurements and PROMs[11]. One prior study evaluated the by Takegami *et al*[18] evaluated the relationship between manual individual radiographic parameters with the patient-reported outcome measurements in Japanese patients. However, it is time consuming to routinely measure the above-described parameters, let alone control for the associated inherent reader variance and need to remember how to obtain such parameters. If these measurements could be automatically produced by machine learning using artificial intelligence (AI), the clinical note and/or radiographic interpretation report could be auto-populated. In addition, the correlations between radiographic parameters and PROMs can be studied in a more standardized manner and for longitudinal data collection. To that end, AP radiographic measurements can be auto-evaluated by HIPPO software, which is a validated AI hip measurement tool validated in a European study and Conformite Europeenne certified [ImageBiopsy Lab Inc. (Vienna, Austria)][19]. Yet, it is not known how these standardized deep-learning software generated measurements obtained in the United States population correlate with their PROMs data. Additionally, it is not known if a validated AI tool can assist in predicting PROMs data and providing comprehensive evaluation for HD patients.

Our hypothesis was that AI-generated HD measurements indicating less severe dysplasia correlate with better PROMs. Thus, the aim was to assess the correlation between AI-derived hip measurement and initial PROMs in a consecutive series of patients. This is the first study to evaluate manual and AI measures of radiographs in patients with HD and associate radiographic findings with preoperative PROMs data.

The SF-12 questionnaire is a short form of the SF-36, where a patient provides a subjective assessment of their own health status and its influence on their respective lifestyle; it reports on psychological features of the condition[20]. Another tool to assess patient outcomes is the iHOT-12 adapted from the 33-question survey that defines changes in quality of life due to hip pathology[21]. An additional meaningful measure is the EQ-VAS-a visual analog scale from 0 to 100-through which the general overall health status of the patient can be observed[22]. In terms of radiographic assessment, multiple parameters provide an indication of the hip's mechanical profile. For instance, the Caput-Collum-Diaphyseal (CCD) angle between the femoral neck and shaft axes contributes to the evaluation of femoral alignment[23]. Additionally, the Sharp's angle, LCEA, Tönnis angle, and the extrusion index represent important radiographic parameters that help assess acetabular coverage, which is important in assessing the severity of dysplasia[23,24]. These PROMs, in concert with the described standardized radiographic measurements, enable the clinician to have a comprehensive understanding of the severity and impact of HD on patients.

MATERIALS AND METHODS

Institutional Review Board approval was received for retrospective use of a longitudinally gathered patient registry data and surveys. Anonymous survey data involving PROMs was collected in our institutional hip preservation practice. All Health Insurance Portability and Accountability Act regulations were followed.

Patients

Using our anonymized electronic database of patients who visited the institutional hip preservation clinic, we identified 325 hips from 276 patients with a complete radiographic series from December 2016 to December 2021. Each patient had a reference final HD diagnosis based on consensus radiographic opinions of an independent fellowship trained musculoskeletal radiologist and hip preservation surgeon using the 4-view radiographic series (AP pelvis, 45° Dunn, Frog-leg lateral, and false profile views) and clinical findings. Only patients with a concordant final diagnosis of HD were included in this study, resulting in 256 hips from 130 patients. Six of the 136 patients did not return an output from HIPPO (Figure 1). The hips with prior surgical interventions or avascular necrosis were excluded. Patient demographic data including age, gender, and body mass index (BMI) were extracted from the electronic health records. Additionally, dates of the patient's first office visit and survey, along with the dates and details of any surgeries were collected. The surveys were obtained at the time of the initial clinic visit when the radiograph was obtained to avoid delay between imaging and initial PROM survey.

PROMs

The patients were surveyed at the time of their initial office visit, which included HHS, iHOT-12, SF-12, and EQ-VAS as shown in Table 1. Survey data was obtained using an online REDCap form and was retrieved into an excel document for each of the included deidentified study patients. Each survey result was manually calculated and normalized to 100% by two medical students under the training and supervision of the senior orthopedic hip specialist.

Manual measurements

Tönnis grade of hip OA was evaluated in all cases by the senior orthopedic surgeon. Manual HD measurements were obtained as a control for the AI measurements. Measurements were taken for each patient by three readers under the supervision and training of a senior musculoskeletal (MSK) radiologist. The three readers underwent extensive training under the MSK radiologist and were assessed for accuracy on a series of training images before obtaining the measurements for the study. The study measurements were then averaged and correlated with PROMs (Table 1)[16,20-22]. Time required to assess these measurements was recorded using a stopwatch from the time images were loaded on IntelliSpace Picture Archiving and Communication System (Philips, Best, Netherlands) to completion of the reads using a built-in measurement tool. Measurement data from the AI algorithm and manual measurements with their detailed inter-reader and inter-modality correlations between manual measurements and AI was published and showed good to

Table 1 Patient-reported outcome measures surveys

Survey	Description
HHS	The HHS is a joint-specific 10-question survey evaluating hip function. The survey parameters include- ability to climb stairs, take public transport, and put on shoes and socks. The test has been shown to have strong construct validity, and thus would be appropriate as a comprehensive assessment of the affected joint's impact on the patient[16]
SF-12	The SF-12 survey, which was adapted from the SF-36 survey, assesses the patient's view of their own health and how it relates to their lifestyle. It includes questions, such as asking the patient if they achieved as much as they have liked and whether they have felt calm and peaceful. Thus, the SF-12 can provide insight into the psychological aspect of the patient's condition[20]
IHOT-12	The iHOT-12 is a 12-question survey adapted from the 33-question survey. The survey evaluates quality of life changes[21]
EQ-VAS	EQ-VAS is a scale from 0 (worst health) to 100 (best health) that allows the patient to indicate their overall perspective of their health state[22]

IHOT: International hip outcome tool; EQ-VAS: Visual Analogue Scale of the European Quality of Life Group; HHS: Harris hip score; SF: Short form.

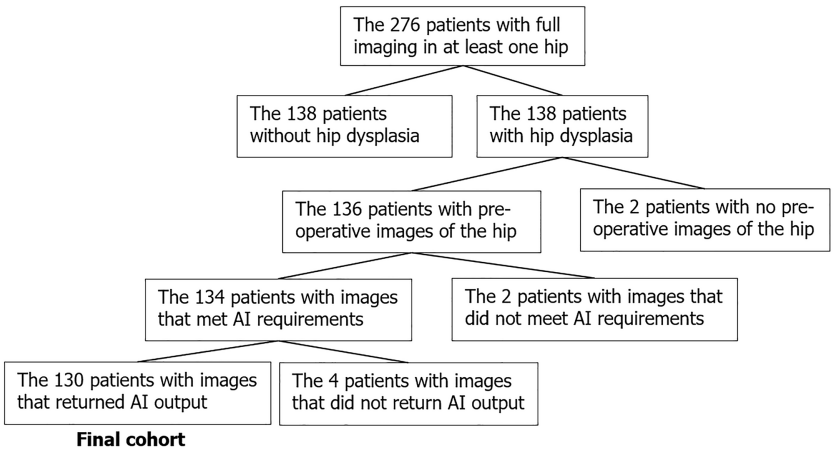

Figure 1 It Shows the final cohort for hip dysplasia patients with patient-reported outcome measures data and compatible imaging.

excellent inter-method reliability for common HD landmarks including LCEA and Tönnis angle[19].

AI measurement tool–HIPPO

'HIPPO' is an AI deep-learning software [ImageBiopsy Lab Inc. (Vienna, Austria)] that automatically locates anatomical landmarks on AP full leg standing radiographs. Using these landmarks, the tool measures various radiographic parameters. These parameters are LCEA, Tönnis Angle, Sharp Angle, CCD angle and pelvic obliquity (Table 2 and Figure 2)[12,23,24]. The software accepts images in Digital Imaging and Communications in Medicine (DICOM) format and returns a DICOM compatible AI report. When the software returns an error report or does not return a report at all, a software failure is indicated. A software failure could be due to errors in the software itself or anatomical subtleties in the radiograph that heavily affected how the software interprets the images. All images in the study were securely transferred to the picture archiving and communication system server at our institution, and from there were pushed to a local installation of the AI software. Measurements were then downloaded onto an excel document after being processed through the software (Windows 11, Microsoft, Redmond, WA). In our study, the median HIPPO reading time per patient was 41 seconds.

Statistical analysis

Descriptive statistics were calculated for patient demographics. All hip measurements were on per-hip level while PROMs except HHS were on per-patient level. Therefore, one hip from each patient was selected when comparing hip measurements to iHOT-12, SF-12, and EQ-VAS. The hip with the worst mean LCEA score from the 3 readers was selected. Correlations between hip measurements and HHS were calculated on the same selected hips. Spearman's rank correlation coefficients were reported with corresponding 95%CI. Hypothesis tests for non-zero correlation were conducted at a 0.05 significance level. *P*-values were adjusted for false discovery rate *via* the Benjamini and Hochberg method for each PROM. Correlation coefficients were interpreted as negligible: 0-0.1, weak: 0.1-0.39, moderate 0.4-0.69, strong: 0.7-0.89 and very strong: 0.9-1[25]. With 80% power to detect a correlation of at least 0.26 at 0.05 significance level, the study needed 130 patients before adjustments for multiple comparisons.

Table 2 HIPPO radiographic hip measurements and landmarks

Measurement	Description
CCD	The CCD angle was measured as the angle between the femur neck and shaft axis[23]
Pelvic obliquity	The pelvic obliquity was measured with a tangential line from the apex of the femoral heads and a line parallel to the horizontal plane as in Figure 1
Sharps angle	Sharp's angle was measured with a line connecting the inferior ischial tuberosities and a line connecting the lower medial edge of the acetabular teardrop and the lateral edge of acetabular sourcil[23]
LCEA	The LCEA was measured as a perpendicular line through the center of the femoral head perpendicular to the acetabular tuberosities and the angle between a line from the center of the femoral head to the lateral acetabular sourcil[24]
Extrusion index	The extrusion index was measured by the difference of medial and lateral femoral head and the lateral edge sourcil with three vertical lines at edge aspect. The femoral head coverage was represented by the percentage of femoral head covered: Lateral femoral head to lateral edge sourcil distance minus the total horizontal head diameter[23]
Tönnis angle	The Tönnis angle was measured as the angle between a line connecting the inferior and lateral aspects of the acetabular sourcil and a line connecting the inferior portion of the ischial tuberosities[12]

CCD: Caput-Collum-Diaphyseal; LCEA: Lateral center edge angle.

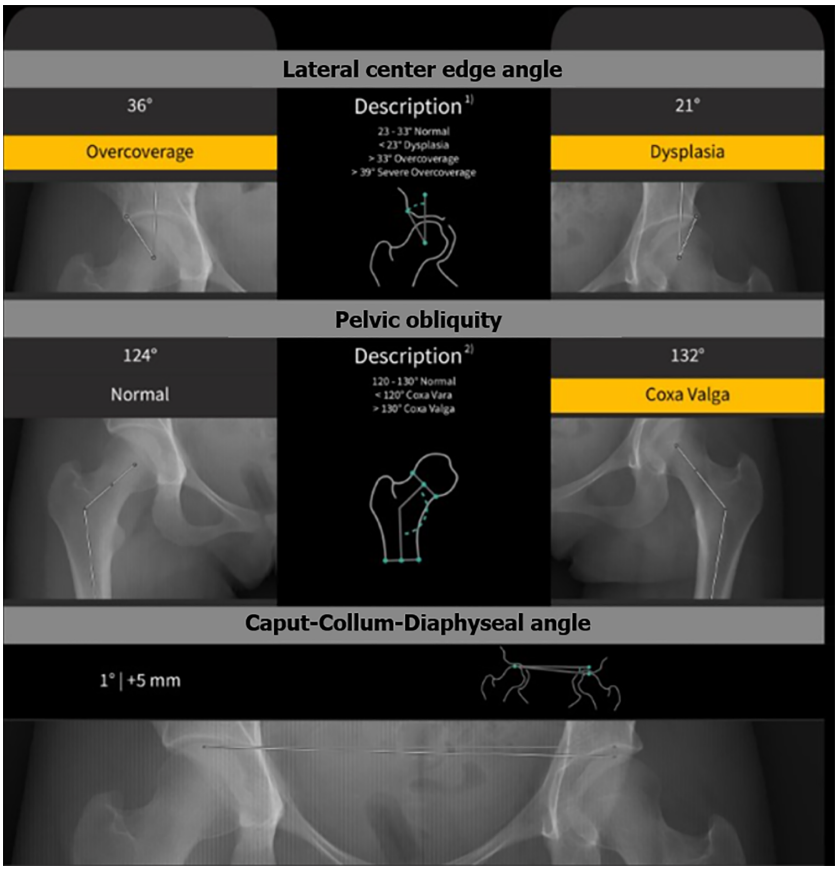

Figure 2 HIPPO Digital Imaging and Communications in Medicine output showing lateral center edge angle, Caput-Collum-Diaphyseal angle, and pelvic obliquity as measured by HIPPO on anteroposterior radiograph.

RESULTS

Patients

Descriptive statistics were calculated for appropriate demographic factors. The median patient age was 28.6 years with a maximum of 62.3 years and a minimum of 15.7 years. The 82.3% of patients were women and 17.7% were men. The BMI ranged from 17 kg/m^2 to 38 kg/m^2, with 24 kg/m^2 as the median. An orthopedic surgeon classified the hips according to the Tönnis grade. The median Tönnis grade was 0 with the majority (204 hips, 79.7%) having Tönnis grade 0, 51 hips (19.9%) with Tönnis grade 1, and 1 hip (0.4%) with Tönnis grade 2.

Manual measurements

Measurement data from the AI algorithm and manual measurements showed good to excellent inter-method reliability for common HD landmarks including LCEA and Tönnis angle. The median read time for manual readers ranged between 4 and 12 minutes per patient[19].

Manual hip measurements vs PROMs

The largest estimated correlation coefficients were between LCEA and HHS [0.18 (0.00, 0.35)], Tönnis Angle and HHS [-0.24 (-0.40, -0.06)], CCD and SF-12 [0.19, (0.01, 0.36)], and CCD and iHOT-12-12 [-0.19, (-0.36, 0.00)]; however, these weak correlations were not significant at a 0.05 level after adjustment for multiple comparisons (Table 3). No other significant correlation was observed between the remaining manual measurements and PROMs. A scatter plot is shown in Figure 3A.

AI hip measurements vs PROMs

CCD were significantly correlated with iHOT-12 and SF12, but the correlation strength was weak [CCD *vs* iHOT-12: -0.25 (-0.42, -0.07), P_{adj} = 0.042; CCD *vs* SF12: 0.25 (0.07, 0.42), P_{adj} = 0.048]. Other notable correlations of similar magnitude were estimated for Obliquity and EQ-VAS [-0.22, (-0.39, -0.4)], as well as Tönnis angle and HHS [-0.20, (-0.36, -0.02)]; however, these estimates were not significant at a 0.05 level after adjustment for multiple comparisons (Table 4 and Figure 3B).

PROMs

HD patients before intervention had an average survey scores of 69% EQ-VAS suggesting moderate pain[26] and 63% SF-12, which is slightly above the depression threshold[27]. They also had 61% iHOT-12, which is nominally above the acceptable symptom threshold (pass) of 59% indicating the patients had a greatly affected quality of life[28], and 62% HHS, which is poor function as defined by the standard less than < 70%[29].

DISCUSSION

This study aimed to evaluate the correlation between AI-generated radiographic measurements and PROMs in individuals with HD. Our findings suggest that while there is a presence of weak correlations between certain AI-derived radiographic measurements and PROMs, these relationships did not achieve statistical significance after adjustments for multiple comparisons. This indicates that the current capacity of AI, specifically the HIPPO deep-learning software, to predict clinical outcomes based on radiological data is limited, although not entirely negligible.

HIPPO is a novel tool for acquiring rapid hip measurements, successfully processing most cases with notable efficiency as reported previously[30]. Where manual readers required a median time of 6 minutes and 48 seconds per hip, and trained radiologists require on average 83 seconds per AP hip radiograph, the AI completed the same task in an average of 41 seconds, highlighting a significant reduction in time and cost per radiograph[19,30]. In this study, HIPPO AI found a significant association between the CCD angle and iHOT-12/SF-12 PROMs compared to manual readers. An elevated CCD angle (Coxa Valga) has been associated with HD, although it is a less commonly used measurement diagnostically [6,31]. While the exact reason for this significant association is not known, the authors hypothesize that the difficulty of measuring CCD among manual readers compared to a standardized AI tool introduced sufficient variation to prevent an observed association[32]. These findings further highlight the importance of standardization in assessment and interpretation of radiographic measurements. The results of this study differ from those of Takegami *et al*[18], where the LCEA angle in 108 Japanese HD patients was independently associated with the Japanese Orthopaedic Association's hip disease questionnaire. However, the end point PROMs examined in our study were different and applied to a heterogeneous United States population, limiting direct comparison. Despite the potential for AI to streamline clinical workflow, our study highlights the difficulty and current unfeasibility of correlating radiographic findings with patient-centric outcomes such as PROMs. Although HIPPO is efficient at measuring, it may require more training to recognize patterns that better match patients experience. This highlights an area where AI can develop to become more clinically meaningful.

An additional consideration is our patient cohort. Overall, the study's patient cohort was symptomatic, presenting with moderate pain, slightly above the depression threshold, and poor functional scores as per EQ-VAS, SF-12, iHOT-12, and HHS, respectively[26-29]. The homogeneity of this group may have diluted the potential to discern a stronger correlation between radiographic measurements and PROMs. Including asymptomatic individuals in future studies may provide a broader spectrum of disease and potentially unveil more defined associations.

It is important to note the subjective nature of PROMs and their potential to be affected by factors beyond the HD diagnosis. For instance, while HHS mainly measures hip function, SF-12 encompasses wider quality of life and mental health parameters, which can be affected by multiple socio-economic and demographic factors[33]. Similarly, individual variability in physical fitness and factors such as hamstring strength play a role in hip stability and perceived symptoms and functionality, contributing to an observed variability in PROMs that may make it difficult to correlate any radiographic measurement, no matter the tool used[34,35].

The weak correlations observed challenge our initial hypothesis that improvements in HD radiographic measures would linearly correlate with better PROMs. The authors do not believe that these weak correlations are due to inaccuracies in the AI measurement tool, which was previously validated by Archer *et al*[19] revealing moderate to strong associations with trained manual readers. Additionally, the vast majority of observed correlations were nonsignificant and contained similar results to the manual readers, with exception of CCA angle and certain PROMs on AI reads, thus

Table 3 Spearman correlation between manual hip measurements and various patient-reported outcome measures surveys

Patient-reported outcome measures	Hip measures	Estimate	Lower 95%CI	Upper 95%CI	Raw *P* value	Adjusted *P* value
Visual Analogue Scale of the European Quality of Life Group	CCD	0.07	-0.11	0.25	0.450	0.802
	Extrusion index	0.02	-0.16	0.20	0.823	0.823
	LCEA	-0.04	-0.22	0.15	0.688	0.823
	Obliquity	-0.17	-0.34	0.01	0.063	0.378
	Sharp	0.06	-0.13	0.24	0.535	0.802
	Tönnis	-0.08	-0.25	0.11	0.419	0.802
Harris hip score	CCD	0.02	-0.16	0.20	0.791	0.791
	Extrusion index	-0.14	-0.31	0.04	0.122	0.183
	LCEA	0.18	0.00	0.35	0.049	0.147
	Obliquity	-0.16	-0.33	0.02	0.081	0.162
	Sharp	-0.06	-0.24	0.12	0.493	0.592
	Tönnis	-0.24	-0.40	-0.06	0.009	0.054
International hip outcome tool	CCD	-0.19	-0.36	0.00	0.045	0.270
	Extrusion index	-0.03	-0.21	0.16	0.764	0.999
	LCEA	0.00	-0.18	0.18	0.999	0.999
	Obliquity	0.13	-0.06	0.30	0.183	0.549
	Sharp	0.00	-0.18	0.18	0.998	0.999
	Tönnis	0.07	-0.12	0.25	0.469	0.938
Short form 12	CCD	0.19	0.01	0.36	0.042	0.252
	Extrusion index	0.03	-0.16	0.21	0.778	0.870
	LCEA	-0.03	-0.22	0.15	0.720	0.870
	Obliquity	-0.13	-0.30	0.06	0.186	0.558
	Sharp	0.06	-0.13	0.24	0.530	0.870
	Tönnis	-0.02	-0.20	0.17	0.870	0.870

CCD: Caput-Collum-Diaphyseal; LCEA: Lateral center edge angle.

suggesting a similar radiographic accuracy between groups as previously described. These results call into question the clinical utility of radiographic measurements alone in predicting patient-reported outcomes and highlights the complexity of HD as a disease entity. While AI can rapidly provide quantitative data valuable for initial screenings and monitoring disease progression, it should complement-not replace-PROMs, which encapsulate the patient's subjective experience and the functional impact of the disease. PROMs remain essential for capturing the holistic impact on quality of life, guiding more personalized treatment approaches. Therefore, clinicians are encouraged to use various means of information-gathering including the use of PROMs. They capture a spectrum of patient experiences and outcomes that are not obvious through radiographic data, reinforcing their role in comprehensive care for patients with HD.

Our study has several limitations. The gender distribution in our study was predominantly female, reflecting the higher incidence of HD in women[36]. This distribution may influence the correlations observed and thus may not be generalizable to a male population. Additionally, most participants were middle-aged adults, so our results might not reflect the bone density and joint health variations found in older patients, and thus may affect the generalizability of this study[37]. Finally, the manual measurements, while performed by medical students under the supervision of an MSK radiologist, are not immune to human error. Anatomical variability might have led to inaccuracies; however, extensive training aimed to mitigate such errors, and their impact on the study's validity is considered minimal. Future studies should also incorporate prospective clinical validation studies to assess AI tools against traditional radiographic measurements, post-implementation in patient care settings. Additionally, randomized controlled trials comparing patient outcomes using AI-derived data with those using manual radiographic assessments are critical to establish the

Table 4 Spearman correlation between artificial intelligence hip measurements and various patient-reported outcome measures surveys

Patient-reported outcome measures	Hip measures	Estimate	Lower 95%CI	Upper 95%CI	Raw *P* value	Adjusted *P* value
Visual Analogue Scale of the European Quality of Life Group	CCD	0.09	-0.09	0.27	0.330	0.509
	Extrusion index	0.07	-0.11	0.25	0.424	0.509
	LCEA	-0.09	-0.27	0.10	0.354	0.509
	Obliquity	-0.22	-0.39	-0.04	0.015	0.090
	Sharp	0.06	-0.13	0.23	0.557	0.557
	Tönnis	-0.11	-0.29	0.07	0.235	0.509
Harris hip score	CCD	0.11	-0.07	0.28	0.238	0.286
	Extrusion index	-0.15	-0.32	0.03	0.112	0.255
	LCEA	0.13	-0.05	0.30	0.170	0.255
	Obliquity	-0.13	-0.30	0.05	0.154	0.255
	Sharp	-0.03	-0.21	0.15	0.723	0.723
	Tönnis	-0.20	-0.36	-0.02	0.033	0.198
International hip outcome tool	CCD	-0.25	-0.42	-0.07	0.007	0.042[a]
	Extrusion index	0.03	-0.15	0.22	0.718	0.718
	LCEA	-0.05	-0.23	0.14	0.608	0.718
	Obliquity	0.17	-0.02	0.34	0.079	0.237
	Sharp	0.04	-0.15	0.22	0.703	0.718
	Tönnis	0.05	-0.13	0.24	0.565	0.718
Short form 12	CCD	0.25	0.07	0.42	0.008	0.048[c]
	Extrusion index	0.00	-0.18	0.19	0.972	0.972
	LCEA	-0.07	-0.25	0.12	0.476	0.714
	Obliquity	-0.16	-0.34	0.02	0.088	0.264
	Sharp	0.08	-0.10	0.26	0.385	0.714
	Tönnis	0.02	-0.16	0.21	0.817	0.972

[a]$P < 0.05$.
[c]$P < 0.001$.
CCD: Caput-Collum-Diaphyseal; LCEA: Lateral center edge angle.

effectiveness of AI in clinical decision-making for HD.

CONCLUSION

In conclusion, this study validated fast measurements using AI-software. Some correlations between AI-derived radiographic measurements and PROMs were seen in HD patients but these findings are mostly insignificant and weak, with most of the associations mirroring that of manual readers. Thus, at present, AI interpretations of radiographic data should be used with caution when predicting patient-reported outcomes. The potential of AI in clinical decision-making for HD patients remains promising in providing quick and accurate radiographic hip measurements. AI software has massive potential in streamlining physician workflow and in performing measurements that can have influence on the clinical decision-making process for patients with HD. It is through these continued efforts that we may fully realize the role of AI in the management of HD, while PROMs will continue to play a crucial role in assessing the broader implications of treatment on patient quality of life.

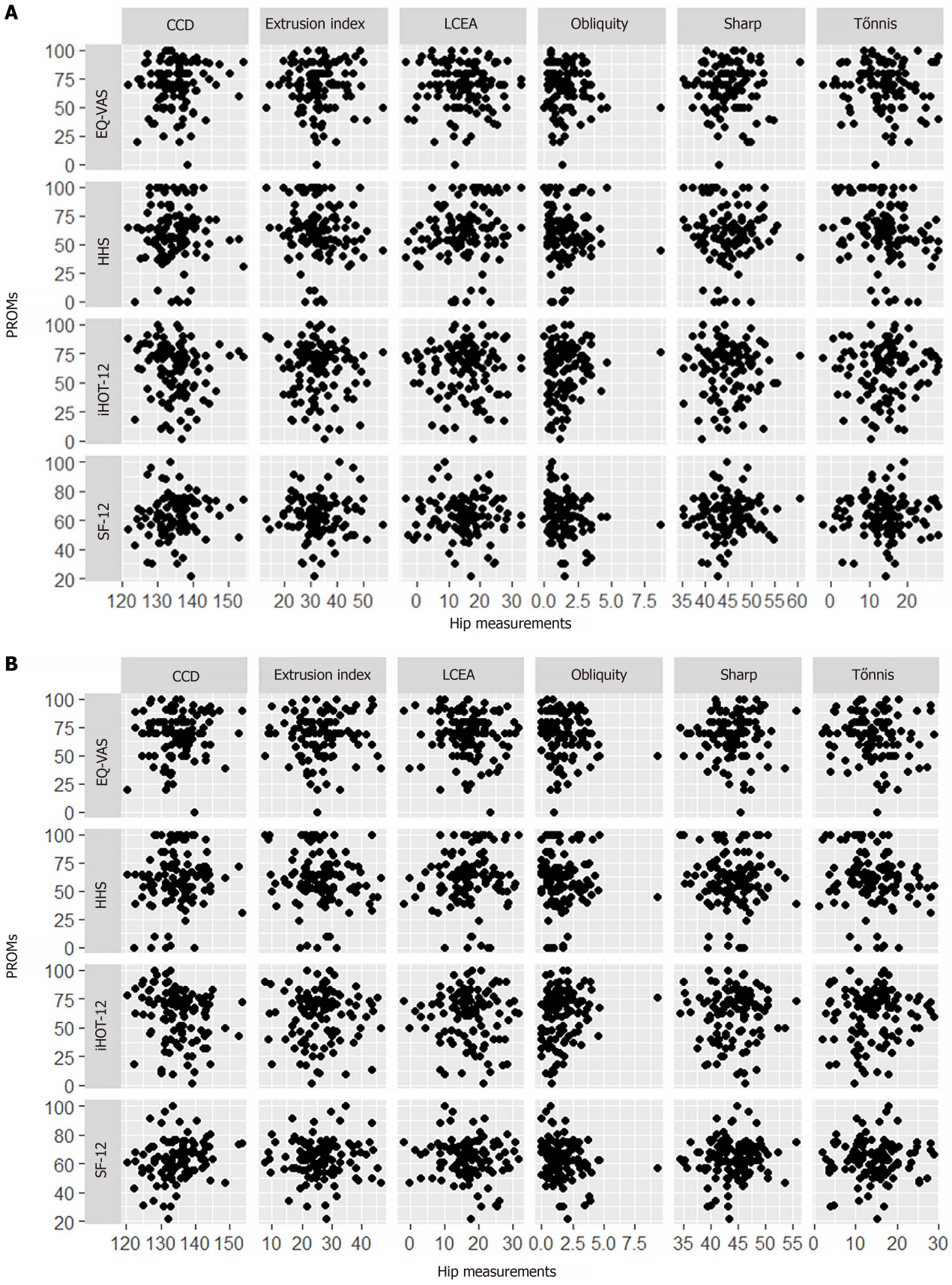

Figure 3 Scatterplot. A: Manual reader measurements and patient-reported outcome measures correlations; B: Artificial intelligence measurements and patient-reported outcome measures correlations. SF-12: Short form 12; IHOT-12: International hip outcome tool; HHS: Harris hip score; EQ-VAS: Visual Analogue Scale of the European Quality of Life Group; CCD: Caput-Collum-Diaphyseal; LCEA: Lateral center edge angle.

FOOTNOTES

Author contributions: Alshaikhsalama A and Archer H were involved in the conception, design, data collection, writing and editing of the manuscript; Xi Y supervised and edited the manuscript and performed the statistical analysis; Ljuhar H, Wells J, and Chhabra A involved in the conception, design, and supervision of the manuscript; all of the authors read and approved the final version of the manuscript to be published.

Institutional review board statement: The study was reviewed and approved by the University of Texas Southwestern Institutional Review Board (approval No. Stu-2022-1014).

Informed consent statement: The University of Texas Southwestern Institutional Review Board determined informed consent was not required for this study since the data is fully anonymized.

Conflict-of-interest statement: Wells JE had received fees for serving as a consultant for Ethicon; Ljuhar R was an employee of Image Biopsy Labs that developed HIPPO AI software; Chhabra A had received fees for serving as a consultant for ICON Medical and TREACE Medical Concepts Inc and for serving as a Siemens Medical advisor for Image Biopsy Inc.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at ahmed.alshaikhsalama@utsouthwestern.edu. Consent was not obtained but the presented data are anonymized and risk of identification is low.

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ORIGINAL ARTICLE

Clinical Trials Study

Dapagliflozin as an oral antihyperglycemic agent in the management of diabetes mellitus in patients with liver cirrhosis

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Abstract

BACKGROUND

The use of dapagliflozin in patients with cirrhosis has been relatively restricted due to concerns regarding its overall safety and pharmacological profile in this population.

AIM

To determine the safety and effectiveness of dapagliflozin in the co-management of diabetes mellitus and cirrhosis with or without ascites.

METHODS

The patients studied were divided into two groups: 100 patients in the control group received insulin, while 200 patients received dapagliflozin. These patients were classified as Child A, B, or C based on the Child–Pugh classification. Child A or B and Child C were administered doses of 10 mg and 5 mg of dapagliflozin, respectively.

RESULTS

The rate of increased diuretics dose was markedly elevated in the group that received insulin compared to the group that received dapagliflozin. In addition, dapagliflozin treatment substantially reduced weight, body mass index, and fasting blood glucose compared to the insulin group during follow-up. However,

there were no significant differences in hemoglobin A1c, liver function, or laboratory investigations between both groups during the follow-up period. The incidence of hypoglycemia, hepatic encephalopathy, variceal bleeding, and urinary tract infection was significantly higher in the insulin group compared to the dapagliflozin group. In contrast, the dapagliflozin group experienced significantly higher rates of frequent urination and dizziness. In addition, the insulin group exhibited a marked worsening of ascites compared to the dapagliflozin group.

CONCLUSION

Dapagliflozin demonstrated safety and efficacy in the treatment of diabetic patients who have cirrhosis with or without ascites. This resulted in an improvement of ascites, as well as a decrease in diuretic dose and Child–Pugh score.

Key Words: Dapagliflozin; Cirrhosis; Diabetes mellitus; Hemoglobin; Liver diseases

Core Tip: To assess the effectiveness and safety of dapagliflozin in the co-management diabetes mellitus and cirrhosis with or without ascites, patients were categorized into a control group of 100 patients administered insulin and 200 patients administered dapagliflozin. On follow-up, there was a significantly higher incidence of hypoglycemia, hepatic encephalopathy, variceal bleeding, and urinary tract infection in the insulin group than in the dapagliflozin group. Frequent micturition and vertigo were significantly higher in the dapagliflozin group. Dapagliflozin demonstrated safety and efficacy in the treatment of diabetic patients with cirrhosis, leading to improvement of ascites, decrease in diuretic dose, and Child–Pugh score.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder closely associated with the onset and progression of chronic liver diseases (CLD). It is characterized by changes in insulin sensitivity and impaired blood glucose regulation[1]. The number of patients affected by DM is expected to increase, with a prevalence of 22%-40% in cirrhotic cases[2]. In addition to serving as the primary site for insulin clearance, the liver plays a substantial role in maintaining blood glucose homeostasis.

Liver cirrhosis (LC) is diabetogenic, and diabetes is one of the risk factors for LC. DM leads to an increase in complications and mortality among patients with cirrhosis. Treatment of DM can be challenging in cases of liver failure[3]. Selecting antidiabetic drugs for patients with both CLD and DM is complex due to the potential impact of liver damage on the metabolism of diabetes medications.

The long-term effects of antidiabetic medications have received significant interest in patients with CLD[4]. These patients experience different severe comorbidities such as lactic acidosis, hypoglycemia, malnutrition, impaired renal function, and hypoalbuminemia. The pharmacokinetics of most antidiabetic drugs, except for insulin, necessitate dose titration. There are concerns regarding the management of diabetes in CLD cases, particularly those that involve the use of novel antidiabetic agents. To our knowledge, there are no explicit guidelines pertaining to this issue[5].

The mechanism of action of sodium-glucose cotransporter-2 (SGLT2) inhibitors as antidiabetic agents involves the inhibition of glucose reabsorption in the proximal renal tubule, which leads to a decrease in serum levels and a loss of glucose in urine. SGLT2 is a significant enzyme in the kidney that is responsible for the reabsorption of glucose. Inhibiting this enzyme lowers the threshold for glucose excretion in urine. Increased glucose loss results in calorie loss, a decrease in serum glucose levels, and mild osmotic diuresis. Additionally, SGLT2 inhibitors induce a slight decrease in blood pressure (BP) and moderate weight loss, contributing to their beneficial effects. Empagliflozin, dapagliflozin, and canagliflozin, which are SGLT2 inhibitors, reduce cardiovascular complications and mortality in individuals with type 2 diabetes (T2D) and cardiovascular issues. In cases of chronic kidney disease and T2D, they also reduce the risk of hospitalization for heart failure and end-stage renal disease[6]. In high-risk individuals with T2D, long-term therapy is advised to mitigate cardiovascular events, end-stage renal failure, renal disease progression, and hospitalization and mortality due to heart failure. Administering dapagliflozin early in acute heart failure hospitalization is both safe and contributes to one aspect of optimization[7], and dapagliflozin does not disrupt liver function tests. The observed decrease in aspartate aminotransferase (AST) levels and lack of impact on other liver function parameters indicate that dapagliflozin may be used as an adjunctive treatment to metformin in diabetic liver diseases[8].

Dapagliflozin is available in 10 mg and 5 mg tablets under the brand name Forxiga. The recommended dosage is 5-10 mg, once daily. SGLT2 inhibitors commonly have adverse effects, including genital mycotic infections, increased thirst, and urinary tract infections (UTIs). Less frequently reported side effects include ketoacidosis, hypersensitivity reactions, hypoglycemia, hypovolemia, dehydration, and elevated levels of creatinine and serum cholesterol. Dapagliflozin is associated with an increased risk of necrotizing fasciitis of the perineum in T2D patients[9].

MATERIALS AND METHODS

This study was performed at the Gastroenterology and Hepatology Unit, National Liver Institute Hospital, Menoufia University, Egypt, from November 2020 to November 2022. Following the institutional review board's approval (IRB number: 00248/2021), all patients who participated provided written informed consent. This prospective study was conducted on patients with cirrhosis and DM (with or without ascites) to determine the safety and efficacy of dapagliflozin in the co-management of DM and cirrhosis.

The patients were divided into 100 patients as controls who received insulin as a treatment for diabetes, those classified as Child A, B, or C based on the Child–Pugh classification[10], and 200 patients administered dapagliflozin as a treatment for diabetes. Participants classified as Child A or B received a daily 10 mg dose of dapagliflozin, while Child C patients received a daily 5 mg dose of dapagliflozin. All patients were adults over the age of 18 who had been diagnosed with cirrhosis and diabetes based on the criteria set by the American Diabetes Association, including random plasma glucose ≥ 200 mg/dL, oral glucose tolerance test ≥ 200 mg/dL, 2-h plasma glucose (during 75 g), fasting plasma glucose ≥ 126 mg/dL, and a hemoglobin A1c (HbA1c) ≥ 6.5%[11].

We excluded patients under the age of 18, diabetic patients receiving combination therapy, and patients with hepatic dysfunction along with renal impairment. Age, sex, duration of diabetes, changes in insulin doses, changes in diuretic doses, and the presence of comorbidities such as hypertension, coronary heart disease, and dyslipidemia were collected during baseline and at 12-week assessments of all patients. All groups were matched in terms of age and sex. The duration of insulin use was established, and we evaluated weight loss, alterations in body mass index (BMI), and changes in BP. Furthermore, modifications in HbA1c, fasting glucose level, lipid profile, estimated glomerular filtration rate (eGFR) value, serum potassium, uric acid level, gamma-glutamyl transferase, alanine aminotransferase (ALT with normal range 10-44 IU/L), AST with normal range 10-34 IU/L, serum sodium, and modifications in abdominal ultrasound findings were evaluated. The documented side effects of the treatment included UTI, proteinuria, frequent micturition, vertigo, hepatic encephalopathy, variceal bleeding, hepatocellular carcinoma, pyelonephritis, hypoglycemia, genital infection, syncope, hypotension, dehydration, abdominal discomfort, back pain, dizziness, rash, and phlebitis.

Statistical analysis

Data was collected and entered into the computer utilizing the 22nd version of SPSS software (IBM Corp., Armonk, NY, United States). Descriptive statistics were expressed as mean ± standard deviation (SD) and range (for quantitative data). The χ^2 test was utilized to assess the association between qualitative variables. In addition, the student *t*-test was utilized to compare the SD and mean of two data sets with normal distribution. In contrast, the Whitney test was used for data with non-normal distribution. Additionally, Pearson's correlation was utilized to examine the correlation between two normally distributed variables, while Spearman's correlation was utilized in non-normally distributed variables. The Fisher exact test was utilized for 2 × 2 qualitative variables when > 25% of the cells have an expected count of < 5. A *P*-value < 0.05 was considered statistically significant.

RESULTS

There were no significant differences between patients receiving dapagliflozin treatment and controls in terms of age, sex, duration of illness, smoking, contact with canal water, and associated comorbidities ($P > 0.05$). Table 1 summarizes clinical data among the study groups. The dapagliflozin group comprised 56 patients classified as Child A, 81 as Child B, and 63 as Child C, with percentages of 28%, 40.5%, and 31.5%, respectively. The insulin group included 24 patients in Child A, 32 in Child B, and 44 in Child C, with percentages of 24%, 32%, and 44%, respectively. Of the patients in the dapagliflozin group, 151 had positive hepatitis C virus (HCV). In contrast, the group receiving insulin consisted of 78 HCV patients. Direct-acting antivirals (DAAs) were administered to 146 patients in the dapagliflozin group who tested positive for HCV, while interferon therapy was administered to five patients. DAAs were administered to 76 patients in the insulin group, and two patients underwent interferon therapy. The diuretic dosage did not change in any of the 150 patients in the dapagliflozin group. However, 50 patients in this group did increase their diuretic dose. The insulin group did not experience any changes in their diuretic dose. A total of 18 patients maintained their diuretic dose, while 12 patients experienced a reduction in their dose. Additionally, diuretic medication was ceased in eight patients, while the dosage increased in 41 patients. The result was highly statistically significant ($P < 0.0001$) (Figure 1).

The results displayed in Table 2 show a significant decrease in weight, BMI, fasting blood glucose, HbA1c, and serum K following the administration of dapagliflozin ($P < 0.05$). Prior to dapagliflozin, the AST level was 49, which decreased to 44 after treatment. Similarly, the ALT level remained at 30 both before and after dapagliflozin treatment. Therefore, no substantial difference was observed in liver enzymes.

Table 1 Comparison of demographic data among the study groups

Parameter	Dapagliflozin group, *n* = 200	Insulin group, *n* = 100	Test of sig.	
			t/χ^2	*P* value
Age in years				
mean ± SD	56.84 ± 7.48	56.96 ± 9.29	0.121	0.904
Range	42–76	33–80		
Sex				
Male	96 (48)	44 (44)	0.258	0.611
Female	104 (52)	56 (56)		
Smoking				
Yes	59 (29.5)	38 (38)	2.202	0.137
No	141 (70.5)	62 (62)		
Contact with canal water				
Yes	119 (59.5)	59 (59)	0.007	0.933
No	81 (40.5)	41 (41)		
Comorbidities[1]				
Cardiac ischemia	16 (8)	11 (11)	0.733	0.392
HTN	32 (16)	21 (21)	1.146	0.284
Dyslipidemia	16 (8)	11 (11)	0.733	0.392
None	160 (80)	73 (73)	1.883	0.170
Child classification				
A	56 (28)	24 (24)	4.599	0.100
B	81 (40.5)	32 (32)		
C	63 (31.5)	44 (44)		
Causes of cirrhosis				
Unknown	33 (16.5)	14 (14)	0.321	0.956
HBV	2 (1)	1 (1)		
HCV	151 (75.5)	78 (78)		
Bilharziasis	14 (7)	7 (7)		
Treatment of HCV Infection				
No	49 (24.5)	22 (22)	0.329	0.849
Interferon	5 (2.5)	2 (2)		
DAAs	146 (73)	76 (76)		
Diuretic dose change				
No change	150 (75)	18 (18)	55.162	< 0.0001[1]
Decreased dose	0 (0)	12 (12)		
Stopped	0 (0)	8 (8)		
Increased	50 (25)	41 (41)		

[1]Some patients have more than 1 comorbidity.
Data are *n* (%) unless otherwise indicated. DAAs: Direct-acting antivirals; HCV: Hepatitis C virus; HTN: Hypertension; SD: Standard deviation.

Table 2 Comparison of body mass index, blood pressure and laboratory investigations before and after dapagliflozin treatment

Parameter	Dapagliflozin before, *n* = 200		Dapagliflozin after, *n* = 200		Test of sig.	
	Mean	SD	Mean	SD	*t*	*P* value
Body weight in kg	90.58	17.14	83.18	13.22	4.836	0.0001
BMI in kg/m^2	31.74	5.24	29.20	4.41	5.251	0.0001
SBP in mmHg	110.18	12.01	109.48	11.62	0.592	0.554
DBP in mmHg	72.30	8.92	71.58	8.66	0.825	0.410
HbA1c as %	9.64	1.74	6.88	1.12	18.906	0.0001
Fasting blood sugar in mg/dL	225.83	92.09	142.98	55.16	10.916	0.0001
Triglycerides in mg/dL	180.46	82.07	167.40	66.19	1.752	0.081
Cholesterol in mg/dL	205.36	58.85	199.02	56.94	1.096	0.274
Creatinine in mg/dL	0.87	0.35	0.86	0.44	0.176	0.860
MELD	11.07	5.09	10.88	5.17	0.37	0.711
MELD-Na	14.40	6.82	14.31	7.06	0.13	0.897
eGFR value in mL/min/1.73 m^2	125.93	59.37	116.12	49.50	1.788	0.075
Uric acid in mg/dL	5.07	1.05	4.89	0.85	1.908	0.057
AST in IU/L	49.06	68.16	44.36	61.00	0.727	0.467
ALT in IU/L	30.74	23.73	30.69	24.57	0.021	0.983
GGT in IU/L	78.64	126.52	77.84	185.29	0.05	0.960
Serum Na in mmol/L	133.48	4.77	133.05	5.64	0.832	0.406
Serum K in mg/dL	4.22	0.50	4.10	0.37	2.554	0.011

ALT: Alanine transaminase; AST: Aspartate aminotransferase; BMI: Body mass index; DBP: Diastolic blood pressure; eGFR: Estimated glomerular filtration rate; GGT: gamma-glutamyl transferase; HbA1c: Hemoglobin A1c; K: Potassium; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease-sodium; NA: Sodium; SBP: Systolic blood pressure.

Table 3 shows a marked decline in weight, BMI, fasting blood glucose, HbA1c, and eGFR on follow-up after 3 months in controls treated with insulin ($P < 0.05$). Furthermore, there was a statistically significant decline in weight, BMI, and fasting blood glucose on follow-up of the dapagliflozin treatment compared to the insulin group (controls) ($P < 0.05$). Conversely, no substantial differences were observed in HbA1c, liver function, laboratory investigations in the insulin (control) group follow-up, and dapagliflozin-treated patients, as shown in Table 4.

As depicted in Table 5, insulin levels in the control group had markedly elevated hypoglycemia, variceal bleeding, hepatic encephalopathy, and UTIs compared to the dapagliflozin-treated group. Moreover, the dapagliflozin-treated group exhibited higher levels of frequent urination and vertigo.

Follow-up abdominal ultrasonography revealed that 94 patients in the dapagliflozin group had improved ascites, while 106 patients remained stationary. In the insulin group, 30 patients had improved ascites, 34 were stationary, and 36 had worse ascites. Ascites deteriorated significantly more in the insulin (control group) group than in the dapagliflozin-treated group. While the classification of the Child did not change before or after treatment with dapagliflozin, the Child scores decreased from 7.53 to 7.09, with $P = 0.0001$. There was a significant decrease in Child scores following dapagliflozin treatment compared to before dapagliflozin treatment ($P < 0.05$), with non-significant differences ($P > 0.05$).

The Child score increased from 8.68 to 8.74, while the Child classification did not change before and after insulin treatment. In addition, there were no significant differences in Child scores before or after insulin treatment ($P > 0.05$).

DISCUSSION

All patients were matched in sex, age, duration of illness and smoking, contact with canal water, Child classification, causes of cirrhosis, treatment of HCV infection, hypertension, dyslipidemia, and cardiac ischemia. Consequently, no other factors affected the scope of our results. A total of 300 patients were enrolled, 140 of whom were males (46.66%), with a median age of 57 years.

The study demonstrated that the overall dapagliflozin safety profile was comparable (for females and males). Similarly, O'Donoghue *et al*[12] reported no signs of modification regarding dapagliflozin impact in terms of sex. Overall,

Table 3 Comparison of clinical and laboratory investigations at baseline and 3 months later in control group

Parameter	Baseline, *n* = 100		3 months later, *n* = 100		Test of sig.	
					t	*P* value
	Mean	SD	Mean	SD		
Body weight in kg	95.86	19.13	84.60	14.21	4.725	0.0001
BMI in kg/m^2	33.45	6.08	29.54	5.29	4.86	0.0001
SBP in mmHg	112.00	15.04	111.20	12.97	0.403	0.688
DBP in mmHg	70.80	10.22	70.80	9.18	0.0001	0.999
HbA1c as %	10.22	2.03	7.47	1.11	11.9	0.0001
Fasting blood sugar in mg/dL	299.26	77.57	174.74	63.29	12.438	0.0001
Triglycerides in mg/dL	240.04	78.18	221.62	51.48	1.968	0.050
Cholesterol in mg/dL	230.20	77.26	223.64	79.44	0.592	0.555
Creatinine in mg/dL	0.98	0.44	0.99	0.57	-0.144	0.886
MELD	12.46	5.11	12.32	5.60	0.185	0.854
MELD-Na	17.46	6.72	16.70	7.08	0.778	0.437
eGFR value in mL/min/1.73 m^2	119.29	68.19	101.53	46.25	2.141	0.034
Uric acid in mg/dL	4.96	0.80	4.96	0.80	0.0001	0.999
AST in IU/L	74.56	90.09	67.58	81.21	0.575	0.566
ALT in IU/L	40.40	30.79	41.72	31.73	-0.299	0.766
GGT in IU/L	131.98	199.44	131.31	269.69	0.02	0.984
Serum Na in mmol/L	131.42	5.81	131.36	6.78	0.067	0.946
Serum K in mg/dL	4.33	0.62	4.19	0.45	1.857	0.065

ALT: Alanine transaminase; AST: Aspartate aminotransferase; BMI: Body mass index; DBP: Diastolic blood pressure; eGFR: Estimated glomerular filtration rate; GGT: gamma-glutamyl transferase; HbA1c: Hemoglobin A1c; K: Potassium; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease-sodium; NA: Sodium; SBP: Systolic blood pressure.

the dapagliflozin safety profile was comparable for females and males[12]. In this study, we found that the dapagliflozin group had a mean BMI of 31.74 kg/m^2 prior to treatment and a mean BMI of 29.20 kg/m^2 following dapagliflozin treatment (P = 0.0001). According to our study, the administration of dapagliflozin reduced systolic blood pressure (SBP) from 110.2 to 109.5 mmHg, while diastolic blood pressure (DBP) remained unchanged (P = 0.554).

Hassoun *et al*[13] investigated the impact of sex, age, and BMI on secondary outcomes. Their results revealed that sex substantially impacted systolic BP change, as evidenced by patient pulse (P = 0.048) and time (P = 0.047). In contrast, patients aged > 50 experienced considerably less eGFR change than patients < 50 (P = 0.012). Furthermore, the baseline BMI did not show any substantial associations with alterations in secondary outcomes[13]. Hao *et al*[14] illustrated that the reduction in BP associated with dapagliflozin use is attributable to intravascular volume depletion due to its natriuretic and diuretic activities. Nevertheless, studies demonstrated that direct vasodilators have impacted the modulation of the sympathetic nervous system renin–angiotensin–aldosterone system, the efferent arteriole, and increased urinary excretion of uric acid. Notably, the decline in BP is not correlated with the estimated glomerular filtration rate[14]. An examination of 13 clinical trials involving a total of 2360 patients diagnosed with T2D who received a daily dose of dapagliflozin at 10 mg and 2295 patients with T2D who received a placebo revealed that the most significant decrease in BP was observed in patients with pre-existing hypertension at the beginning of the trials. For baseline hypertensive patients (SBP ≥ 140 mmHg), the average decline in DBP and SBP from the starting point to week 24 was -1.2 mmHg and -3.6 mmHg, respectively, after accounting for the placebo effect.

In non-hypertensive patients, the decline in DBP and SBP was -1.2 mmHg and -2.6 mmHg, respectively[15]. Montalvo-Gordon *et al*[16] found that SGLT2 inhibitors can reduce the renin–angiotensin–aldosterone system overactivation by inhibiting sodium and glucose reabsorption in the proximal convoluted tubule. This contributes to restoring the sympathetic nervous system and tubuloglomerular feedback and promotes natriuresis. These effects address the primary mechanisms involved in the development of portal hypertension in cirrhotic patients. Unlike angiotensin-converting enzyme inhibitors, spironolactone, SGLT2 inhibitors, and angiotensin receptor blockers are less effective in lowering overall BP and may be better tolerated by individuals with clinically significant portal hypertension[16]. Heerspink *et al*[17] demonstrated that SGLT2 inhibitors effectively reduce high blood sugar levels and BP by relatively hindering SGLT2 receptors in the kidney's proximal convoluted tubules. This inhibition prevents the reabsorption of filtered glucose and

Table 4 Comparison of clinical and laboratory investigations changes on follow-up in both groups

Parameter	Dapagliflozin, *n* = 200		Control group, *n* = 100		Test of sig.	
					t	*P* value
	Mean	SD	Mean	SD		
Body weight in kg	11.26	12.12	7.40	21.56	-1.982	0.048
BMI in kg/m^2	3.92	4.35	2.54	6.91	-2.103	0.036
SBP in mmHg	0.70	16.77	0.80	15.68	-0.051	0.959
DBP in mmHg	0.73	11.81	0.00	12.55	0.481	0.631
HbA1c as %	2.77	2.03	2.75	1.67	0.072	0.943
Fasting blood sugar in mg/dL	124.52	66.17	82.86	104.24	-4.206	0.000
Triglycerides in mg/dL	13.06	95.16	18.42	54.61	-0.619	0.537
Cholesterol in mg/dL	6.35	74.66	6.56	41.71	-0.032	0.975
Creatinine in mg/dL	0.01	0.55	-0.01	0.32	0.345	0.730
MELD	0.19	5.76	0.14	3.20	0.097	0.923
MELD-Na	0.09	8.48	0.76	3.09	-0.993	0.321
eGFR value in mL/min/1.73 m^2	10.96	63.42	19.79	57.57	-1.21	0.228
Uric acid in mg/dL	0.18	1.02	0.00	1.14	1.349	0.179
AST in IU/L	4.71	89.72	6.98	37.89	-0.308	0.758
ALT in IU/L	0.05	32.30	-1.32	23.08	0.422	0.673
GGT in IU/L	0.79	218.46	0.67	167.59	0.005	0.996
Serum Na in mmol/L	0.44	7.50	0.06	4.03	0.563	0.574
Serum K in mg/dL	0.11	0.63	0.14	0.61	-0.379	0.705

ALT: Alanine transaminase; AST: Aspartate aminotransferase; BMI: Body mass index; DBP: Diastolic blood pressure; eGFR: Estimated glomerular filtration rate; GGT: gamma-glutamyl transferase; HbA1c: Hemoglobin A1c; K: Potassium; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease-sodium; NA: Sodium; SBP: Systolic blood pressure.

sodium, leading to the excretion of glucose and sodium in the urine. SGLT2 inhibitors reduce preload and afterload, induce volume contraction, and decrease BP (by 1 to 2 mmHg) through the physiological effects of osmotic diuresis and natriuresis, resulting in cardiorenal protection[17]. This study demonstrated a marked elevation in diuretic dosage among individuals treated with insulin compared to those who received dapagliflozin. This can be attributed to the diuretic properties of dapagliflozin treatment. Our results indicated that 75% of patients treated with dapagliflozin did not require any change in their diuretic dose. However, 25% of patients who received dapagliflozin did require an increase in their diuretic dose. Furthermore, a statistically significant finding revealed that 41% of patients undergoing insulin therapy required an increase in their diuretic dosage ($P < 0.0001$). Charaya *et al*[18] demonstrated that the dapagliflozin group had a lower likelihood of increasing the dosage of loop diuretics (14% *vs.* 30%; $P = 0.048$), lower loop diuretic average doses (78.46 ± 38.95 mg/day *vs.* 102.82 ± 31.26 mg/day; $P = 0.001$) and more substantial weight loss [4100 (2950; 5750) g *vs.* 3000 (1380; 4650) g; $P = 0.02$]. The administration of dapagliflozin was associated with a more significant reduction in body weight and reduced requirement for increased diuretic treatment without severe renal function deterioration[19].

Furthermore, we observed a significant decrease in serum K, HbA1c, fasting blood glucose, weight, and BMI levels following dapagliflozin treatment. Dapagliflozin decreases body weight by excretion of glucose in urine and loss of fluids. A significant proportion of the weight loss reported in a composition study, approximately two-thirds, can be ascribed to the reduction in fat mass[19]. In our research, hypoglycemia risk was substantially reduced with dapagliflozin compared to with insulin. None of the patients treated with dapagliflozin experienced hypoglycemia compared to 5% of patients treated with insulin ($P = 0.0001$). Consistent with our results, Liu *et al*[20] demonstrated that SGLT-2 inhibitors have a minimal risk of hypoglycemia and can cause slight weight loss while reducing BP[20]. The results of our study indicate that dapagliflozin significantly reduced HbA1c levels (from 9.64 to 6.88, 3 months post-treatment), with a P value = 0.0001. Moreover, randomized, double-blind, multicenter, phase 3 trials have demonstrated that dapagliflozin (as monotherapy and combination therapy) effectively enhanced glycemic control and lowered BP and body weight in numerous T2D patients. This includes cases with elevated baseline HbA1c ≥ 9%[21], as well as old cases (aged ≥ 65 years) [22].

Table 5 Comparison of complications rate in the studied groups

Parameter	Dapagliflozin, *n* = 200	Control group, *n* = 100	Test of sig.	
			χ^2	*P* value
UTI	0 (0)	19 (19)	40.569	0.0001
Proteinuria	0 (0)	0 (0)		
Frequent micturition	20 (10)	0 (0)	10.714	0.001
Vertigo	20 (10)	0 (0)	10.714	0.001
Hepatic encephalopathy	0 (0)	6 (6)	6.091	0.014
Variceal bleeding	0 (0)	13 (13)	13.437	0.002
HCC	0 (0)	0 (0)		
Pyelonephritis	0 (0)	0 (0)		
Hypoglycemia	0 (0)	5 (5)	10.169	0.0001
Genital infection	8 (4)	4 (4)	0.098	0.755
Syncope	0 (0)	0 (0)		
Hypotension	12 (6)	5 (5)	0.125	0.724
Dehydration	12 (6)	5 (5)	0.125	0.724
Abdominal discomfort	0 (0)	0 (0)		
Back pain	0 (0)	0 (0)		
Dizziness	0 (0)	0 (0)		
Rash	0 (0)	0 (0)		
Phlebitis	0 (0)	0 (0)		

Data are *n* (%). HCC: Hepatocellular carcinoma; UTI: Urinary tract infection.

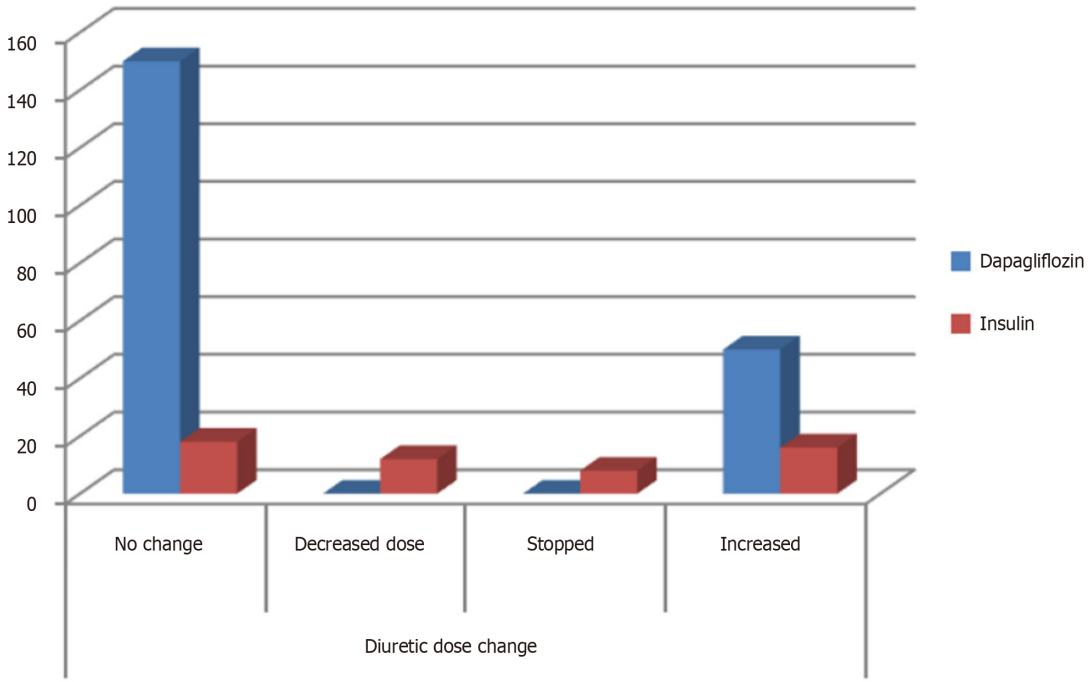

Figure 1 Diuretic dose change in the studied groups.

Regarding the impact of insulin treatment, the present study detected a substantial decline in eGFR, fasting blood glucose, weight, HbA1c, and BMI on follow-up after 3 months in controls treated with insulin. In line with our findings, Mottalib *et al*[23] proved that insulin therapy yields significant clinical advantages, including decreased HbA1c levels and alleviated long-term microvascular complications[23]. In our study, dapagliflozin reduced glomerular filtration rate (GFR) from 125.93 to 116.12, but was statistically non-significant (P = 0.075). Meeme and Kasozi demonstrated that glycemic control lowers the GFR in diabetic patients admitted for short-term treatment. A reduction in GFR reflects a reduction of hyperfiltration, a process that starts diabetic nephropathy[24]. Compared to the insulin (control) group, the present study demonstrated a statistically significant reduction in weight, BMI, and fasting blood glucose during the follow-up of dapagliflozin treatment. However, there were no statistically significant differences in HbA1c levels, liver function, or laboratory parameters between the insulin (control) group follow-up and dapagliflozin-treated patients.

In addition, dapagliflozin treatment resulted in a notable enhancement in glycemic control, accompanied by weight reduction, insulin sparing, and reduced occurrence of hypoglycemia compared to insulin therapy[25]. Ahmed *et al*[26] demonstrated that treatment with dapagliflozin resulted in enhancements in various biomarkers, including serum creatinine, blood glucose, serum malondialdehyde, urinary protein, serum urea, urinary glucose level, serum glutathione level, and serum insulin[26]. The present study detected a marked elevation in hepatic encephalopathy, hypoglycemia, UTI in insulin, and variceal bleeding (control group) compared to the treated group. Although frequent urination (experienced by 10% of cases in the dapagliflozin-treated group) and dizziness (experienced by 10% of cases in the dapagliflozin-treated group) were more prevalent in the dapagliflozin-treated group, these side effects were pervasive among females. According to Halimi *et al*[27], the most reported adverse events (AEs) associated with SGLT-2 inhibitors are urinary tract and female genital mycotic infections, increased urination, constipation, and nausea[27].

Similarly, Yen *et al*[28] examined the progression of cirrhotic complications in individuals with compensated cirrhosis who were either using insulin or not. The researchers found that insulin users had a greater likelihood of experiencing variceal bleeding, hepatic encephalopathy, ascites, and hepatic failure compared to those who did not use insulin[28]. Insulin activates adrenergic hormones and triggers the release of endothelin-1[29]. These substances cause the narrowing of blood vessels in isolated arterioles, leading to a rise in both portal pressure and systemic vascular resistance. Cirrhosis can exacerbate insulin resistance and disrupt the molecular insulin mechanisms in hepatocytes. Furthermore, consequent hyperinsulinemia and exogenous insulin may trigger signaling molecules and influence hepatocyte apoptosis[30]. Consequently, they have the potential to exacerbate the progression of LC and result in hepatic failure. Additionally, a prior study revealed that insulin therapy resulted in an elevated susceptibility to hypoglycemia[31].

The present study revealed statistically significantly worse ascites outcomes in the insulin group (controls) than in the dapagliflozin-treated group. In agreement with our results, prior research revealed that SGLT2 inhibitors improved peripheral edema and refractory ascites[5,16]. Furthermore, Kalambokis *et al*[32] documented a case of refractory ascites caused by alcoholic LC that was successfully managed using SGLT2 inhibitors. Before administering an SGLT2 inhibitor, this patient did not respond to the standard diuretics furosemide and spironolactone, and required frequent cell-free and concentrated ascites reinfusion therapy (CART). Nevertheless, the ascites considerably declined upon introducing an SGLT2 inhibitor to manage the patient's hyperglycemia, thereby reducing CART[32]. The present study revealed a marked decline in Child scores following dapagliflozin treatment, but Child classification did not markedly differ. In our research, dapagliflozin treatment decreased Child scores from 7.53 to 7.09, which was statistically significant (P = 0.0001). However, there was no alteration in the categorization of children, as the proportions of patients classified as Child A, B, or C remained consistent (28%, 40.5%, 31.5%, respectively) both before and after dapagliflozin treatment. Miyamoto *et al*[33] discovered that enhancing nutritional status may be attributed to a reduction in ascites, leading to decreased abdominal distention and increased food consumption. Moreover, the Child–Pugh score exhibited improvement, indicating enhanced liver function and nutritional status. Nevertheless, there was no statistically significant disparity in the Child's score before and after insulin treatment[33].

In contrast, Yen *et al*[28] demonstrated that individuals who use insulin have a greater likelihood of experiencing hypoglycemia, liver-related complications, cardiovascular events, and mortality when compared to non-users[28]. Dapagliflozin enhances glycemic control and alleviates weight with no increase in major hypoglycemic episodes in cirrhotic cases with DM. In our study, the incidence of UTI was lower in dapagliflozin than in insulin-treated patients. Additionally, 19% of patients in the group receiving insulin treatment experienced UTIs, whereas none receiving dapagliflozin treatment had a statistically significant P value (P = 0.0001). In contrast to our study, Zheng *et al*[34] demonstrated that dapagliflozin presented a greater susceptibility to UTIs when compared to both placebo and other active treatments. When using high doses of dapagliflozin for an extended period or when using it as an additional treatment, it is essential to carefully consider the risk of UTI in T2D patients[34]. Our study found that the incidence of genital infection was higher among patients who received dapagliflozin treatment, affecting approximately 4% of those treated with dapagliflozin. In our study, a comprehensive analysis of 13 studies utilizing dapagliflozin revealed no notable rise in the occurrence of renal dysfunction. Genito-UTI is the predominant adverse effect experienced by approximately 5% of patients who receive treatment with dapagliflozin. Most of these infections are uncomplicated genital infections that can be avoided by practicing good hygiene and using antifungal treatment[35]. Additionally, 6% of patients treated with dapagliflozin experienced hypotension and dehydration in our study. Jabbour *et al*[36] found that the incidence of volume depletion (hypotension/hypovolemia/dehydration) was 1.1% and 0.7% with dapagliflozin and placebo, respectively. During the 1st 2 weeks of treatment, 18.5% (5/27) of AEs associated with volume depletion occurred in the dapagliflozin group, while 17.6% (3/17) occurred in the placebo group. When categorized by age, the occurrence of volume depletion was comparable between patients under the age of 65 and those aged ≥ 65 years in the placebo group. However, in the dapagliflozin group, patients ≥ 65 years had a higher likelihood of experiencing volume depletion.

In both treatment groups, the incidence of volume depletion in patients using loop diuretics was 2.5 times greater compared to patients not using them. Furthermore, the occurrence of volume depletion was less common in patients with a baseline eGFR of ≥ 60 mL/min/1.73 m^2 compared to those with a baseline eGFR of 30 to < 60 mL/min/1.73 m^2, regardless of the treatment group. Hypotension was the most frequent AE associated with volume depletion in both treatment groups. However, most of these events were deemed unrelated to the study drug, were of mild-to-moderate severity, and did not necessitate the interruption or discontinuation of the drug. Syncope was the second most reported adverse event associated with volume depletion, with episodes thereof occurring at various times during the 24-week treatment period[36]. Yen *et al*[4] demonstrated that SGLT2 inhibitors enhance the excretion of glucose in the urine, reduce blood glucose levels, and alleviate insulin resistance in individuals with T2D. Patients with T2D and mild or moderate liver disease (LD) did not experience any significant alterations in their pharmacokinetic parameters. Nevertheless, it is advisable to exercise caution and administer lower quantities of these substances to patients with cirrhosis, to mitigate the potential dangers of hypotension and dehydration. Co-administering SGLT2 inhibitors with β blockers in patients with LC is likely safe[4]. This study's limitations were the short follow-up period and lack of financial support. The study was done during the endemic coronavirus disease in 2019, which affected the enrollment of more patients.

CONCLUSION

Dapagliflozin demonstrated both safety and efficacy in treating diabetic patients with cirrhosis, regardless of the presence of ascites. The treatment resulted in improved ascites, reduced diuretic dosage, and a lower Child–Pugh score. In our study, the incidence of UTI was lower in the dapagliflozin-treated group, while the incidence of genital infection was higher among patients who received dapagliflozin treatment. Not one of our studied patients complained of naso-pharyngitis.

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FOOTNOTES

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META-ANALYSIS

Statins decrease the risk of hepatocellular carcinoma in metabolic dysfunction-associated steatotic liver disease: A systematic review and meta-analysis

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Abstract

BACKGROUND

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a leading cause of chronic liver disease with a significant risk of developing hepatocellular carcinoma (HCC). Recent clinical evidence indicates the potential benefits of statins in cancer chemoprevention and therapeutics. However, it is still unclear if these drugs can lower the specific risk of HCC among patients with MASLD.

AIM
To investigate the impact of statin use on the risk of HCC development in patients with MASLD.

METHODS
A systematic review and meta-analysis of all the studies was performed that measured the effect of statin use on HCC occurrence in patients with MASLD. The difference in HCC risk between statin users and non-users was calculated among MASLD patients. We also evaluated the risk difference between lipophilic versus hydrophilic statins and the effect of cumulative dose on HCC risk reduction.

RESULTS
A total of four studies consisting of 291684 patients were included. MASLD patients on statin therapy had a 60% lower pooled risk of developing HCC compared to the non-statin group [relative risk (RR) = 0.40, 95%CI: 0.31-0.53, I^2 = 16.5%]. Patients taking lipophilic statins had a reduced risk of HCC (RR = 0.42, 95%CI: 0.28-0.64), whereas those on hydrophilic statins had not shown the risk reduction (RR = 0.57, 95%CI: 0.27-1.20). The higher (> 600) cumulative defined daily doses (cDDD) had a 70% reduced risk of HCC (RR = 0.30, 95%CI: 0.21-0.43). There was a 29% (RR = 0.71, 95%CI: 0.55-0.91) and 43% (RR = 0.57, 95%CI: 0.40-0.82) decreased risk in patients receiving 300-599 cDDD and 30-299 cDDD, respectively.

CONCLUSION
Statin use lowers the risk of HCC in patients with MASLD. The higher cDDD and lipophilicity of statins correlate with the HCC risk reduction.

Key Words: Metabolic dysfunction-associated steatotic liver disease; Hepatocellular carcinoma; Statins; Lipophilic statin; Hydrophilic statin; Meta-analysis

Core Tip: Current clinical evidence regarding the effect of statins on lowering the risk of hepatocellular carcinoma (HCC) among patients with metabolic dysfunction-associated steatotic liver disease (MASLD) is inconclusive. We performed a systematic review and meta-analysis of all the studies that evaluated the impact of statins on HCC occurrence in MASLD patients. The pooled data from four studies involving 291684 patients was included in the final analysis. Our findings show that statin use reduces the risk of HCC among patients with MASLD. The use of higher cumulative defined daily doses and lipophilic statins results in a significant reduction in HCC risk.

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INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease worldwide[1-3]. A recent meta-analysis revealed the global prevalence of MASLD has increased from 25.3% (1990–2006) to 38.2% (2016–2019)[4]. Studies have shown that patients with MASLD have an increased incidence of hepatocellular carcinoma (HCC)[5-9]. A systematic review showed that the HCC incidence in MASLD patients with and without cirrhosis at 10 years was up to 15% and 2.7%, respectively[10]. It is critical to understand that MASLD not only increases HCC risk but also liver cancer-related mortality[11]. A nationwide study from the United States found that HCC patients with alcoholic liver disease and MASLD as causes had significantly higher mortality rates than those with viral etiologies[12]. Furthermore, MASLD-associated HCC (MASLD-HCC) has a lower survival rate compared to hepatitis C virus-related HCC[13-15]. MASLD has also been linked to a lower reception of HCC surveillance, decreasing the detection of cancer in its early stages[16]. A Swedish multigenerational cohort study also revealed that first-degree relatives of MASLD patients had significantly increased hazards of HCC, major adverse hepatic outcomes, and liver-associated mortality[17]. The growing evidence of this troubling association has led to the curation of prevention strategies aimed at reducing HCC occurrence among MASLD patients[18-21]. While several modifiable risk factors are identified for MASLD-HCC, interest has also increased in the potential cancer chemopreventive role of certain widely used drugs[22].

Statins are a common class of drugs used for primary and secondary prevention of cardiovascular diseases[23,24]. These drugs also inhibit the growth of tumor cells through varied pharmacological activities and regulation of the methyl-valerate pathway[25-28]. Consequently, statins are among the most studied chemopreventive agents, potentially reducing the risk of cancers of the breast, prostate, pancreas, and liver[29-33]. Despite initial safety concerns, subsequent

research has proven the safety and efficacy of these medications in patients with liver disease, especially MASLD[34-37]. With regard to their chemopreventive effects against HCC, previous studies have predominantly evaluated patient populations other than MASLD[38-42]. Therefore, the role of statin treatment in MASLD-HCC risk reduction has remained largely underinvestigated. A recent meta-analysis has reported that statin use decreases the HCC incidence among patients with MASLD[43]. However, the data on the clinical benefit of statins stratified by solubility status and doses is still incongruous. To our knowledge, this is the first meta-analysis to assess the variation in HCC risk in patients with MASLD between statin users and non-users. We also aim to calculate the risk difference between hydrophilic and lipophilic statins, as well as the effect of cumulative dose on HCC risk reduction.

MATERIALS AND METHODS

Data search and screening

The meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis statement[44]. We performed a detailed search of electronic medical databases, including MEDLINE/PubMed, Web of Science, Embase, and Scopus, from January 1990 to June 2023. The following keywords were used in different combinations: (1) Hydroxymethylglutaryl-CoA reductase inhibitors OR HMG-CoA reductase inhibitors OR statins OR atorvastatin OR cerivastatin OR fluvastatin OR simvastatin OR lovastatin OR pitavastatin OR pravastatin OR rosuvastatin; (2) NAFLD OR nonalcoholic fatty liver disease OR NASH OR nonalcoholic steatohepatitis; and (3) HCC OR hepatocellular carcinoma OR liver cancer. Only articles in the English language were included. A manual bibliographic search of the included articles was also performed to find any missing studies. The search strategy in the current study is outlined (Figure 1).

Study selection and data extraction

Three researchers (Tarar ZI, Inayat F, and Gandhi M) independently searched for eligibility and screened abstracts, titles, and full manuscripts without the use of automation tools. Any disagreement was resolved through discussion with the senior author (Ali AH). Three reviewers (Tarar ZI, Inayat F, and Gandhi M) extracted data on an Excel sheet. The data on study design, year of publication, country of study, first authors, patient demographics, type and duration of statin use, study quality, and outcome measures were extracted. A fourth reviewer (Farooq U) reviewed the extracted data, and the final datasheet was drafted after a discussion between all four authors.

Eligibility criteria

The Population, Intervention, Control, and Outcome (PICO) framework was used to formulate the inclusion criteria as described in the Cochrane Collaboration Handbook[45,46]. The PICO characteristics for eligibility were: (1) Population: Patients older than 18 years with a history of MASLD; (2) Intervention: Use of statin therapy; (3) Comparison group: Statin non-users; and (4) Outcome: Risk of HCC development.

We excluded studies in which the underlying etiology of cirrhosis was a chronic liver disease other than MASLD, such as hereditary, alcohol-related, viral, and autoimmune causes.

Outcomes

The primary outcome of interest was the risk stratification for HCC among MASLD patients between statin users and non-users. We performed a subgroup analysis to examine the effect of lipophilic versus hydrophilic statins on HCC risk. We further analyzed the dose-dependent effect of statin on the risk of HCC development.

Statistical analysis

The random-effects model was used to calculate the pooled hazard ratios (HR) along with a 95%CI. Cochrane χ^2 and I^2 were applied to assess the heterogeneity and variance. Forest plots were used to present the results of the meta-analysis. The funnel plot and Egger's test for asymmetry were used to determine the publication bias. We utilized Comprehensive Meta-Analysis software version 3.0 (Biostat Inc., Englewood, NJ, United States) to conduct the analysis.

Quality assessment

We relied on the Methodological Index for Nonrandomized Studies (MINORS) criteria for assessing the quality of the included studies[47]. We scored comparative studies on 12 items of the MINORS criteria, and each item was scored from 0 to 2: (1) 0 if not reported; (2) 1 when reported but inadequate; and (3) 2 when reported and adequate. Therefore, a maximum ideal score of 24 could be obtained for comparative studies and 16 for non-comparative studies.

RESULTS

Literature search and study selection

A total of 1782 citations were found in the initial literature search; 1245 articles were removed as duplicates. We screened the remaining 537 reports and shortlisted 26 articles deemed relevant to our study question. These 26 articles were retrieved, and a comprehensive review was undertaken. Four studies were included in the final analysis[48-51]. The total

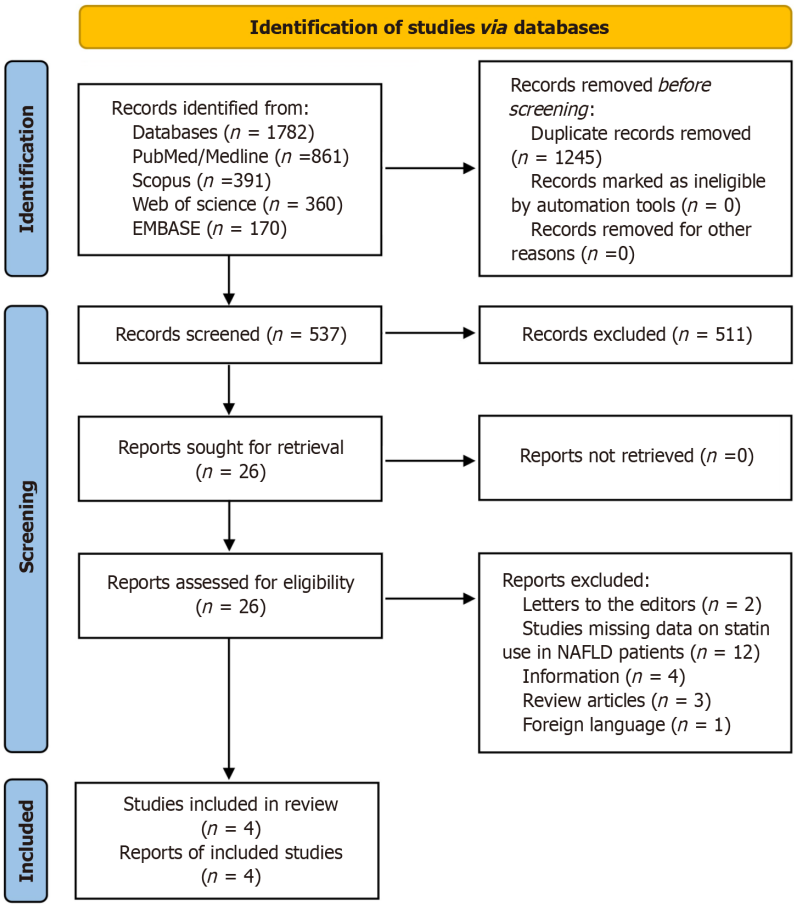

Figure 1 Preferred Reporting Items for Systematic Review and Meta-Analysis flow diagram of the search strategy.

number of patients was 291684. Of these, 80246 were statin users, and 211438 were not under therapy with statins. The mean age of the study population was 57.0 ± 12.2 years. The gender distribution of included patients showed that 46.9% (136804) were male and 53.1% (154880) were female. Three studies were retrospective cohorts, and one was a case-control (Table 1)[48-51]. The three studies provided the outcome data in HR, whereas one study reported results as odds ratios (OR). We converted the OR results of this study to relative risk (RR) prior to its inclusion in the final analysis.

Outcomes

Patients with MASLD who were on statin therapy had a 60% less pooled risk of developing HCC compared to the non-statin group (RR = 0.40, 95%CI: 0.31-0.53, I^2 = 16.5%) (Figure 2A)[48-51]. We performed a subgroup analysis of lipophilic versus hydrophilic statins reported in two studies. Lipophilic statins were associated with a lower risk of HCC (RR = 0.42, 95%CI: 0.28-0.64). No statistically significant difference was noted among hydrophilic statin users (RR = 0.57, 95%CI: 0.27-1.20) (Figure 2B)[50,51].

Dose-dependent risk reduction

We analyzed the data based on the dose of statins and concluded that > 600 cumulative defined daily doses (cDDD) decrease the risk of HCC by 70% (RR = 0.30, 95%CI: 0.21-0.43). The administration of 300-599 cDDD and 30-299 cDDD of statins decreases the risk by 29% (RR = 0.71; 95%CI: 0.55-0.91) and 43% (RR = 0.57; 95%CI: 0.40-0.82), respectively (Figure 2C)[50,51].

Publication bias and quality assessment

A funnel plot and Egger's test were used to ascertain publication bias. There was no evidence of significant publication bias among the studies included in our final analysis (Figure 3). Using the Cochrane risk of bias tool, all studies were determined to have a low risk of bias.

Risk of bias assessment

Based on MINORS criteria for non-randomized studies, the quality of studies was classified as poor (score ≤ 5), fair (score 6-10), or high quality (score ≥ 11), as described previously[52]. All the studies were rated as high quality. The quality assessment of the studies is summarized in Supplementary Table 1.

Table 1 Baseline characteristics of included studies

Ref.	Study design	Age (years)	Total patients (*n*)	Males/females	Statin users	Non-users	Cirrhosis	No cirrhosis	Follow-up (years)
Zou *et al*[51]	Cohort	52.1 ± 14.7	272430	126804/145626	73384	199046	34257	238173	727390 person-year
Pinyopornpanish *et al*[50]	Cohort	59 ± 10.4	1072	432/640	440	632	950	122	4326 person-year
German *et al*[49]	Case-control	64.3 ± 13.1	102	66/36	40	62	93	9	Not applicable
Lee *et al*[48]	Cohort	52.7 (41.6-64.4)	18080	9502/8578	6382	11698	0	18080	6.32 (3.04-10.10)

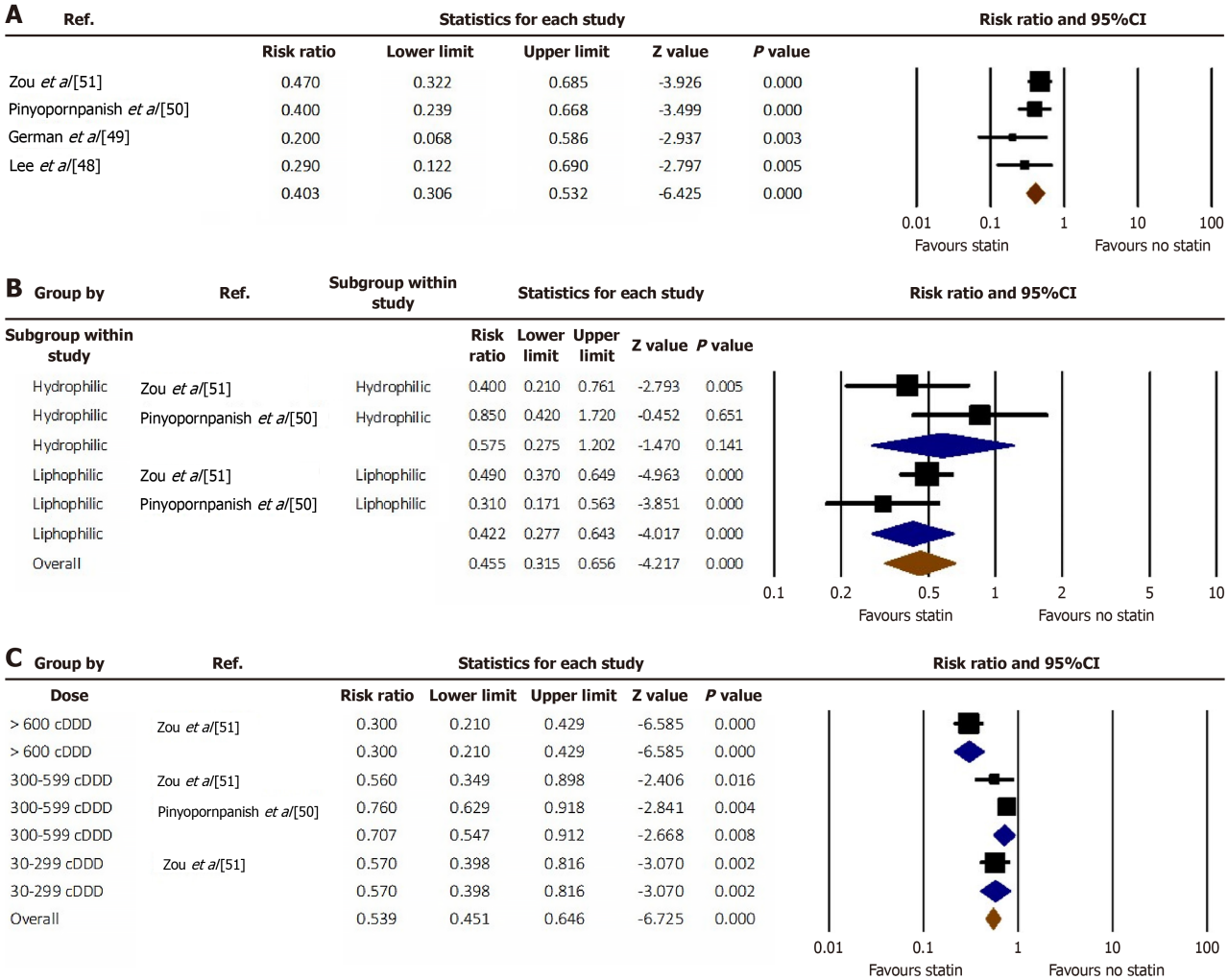

Figure 2 Forest plot. A: The risk difference of hepatocellular carcinoma among MASLD patients between statin users versus non-users; B: The risk difference of hepatocellular carcinoma among users of lipophilic versus hydrophilic statins; C: The dose-dependent risk reduction of hepatocellular carcinoma among statin users. cDDD: Cumulative defined daily doses.

DISCUSSION

This meta-analysis has comprehensively assessed the impact of statin use on HCC risk in patients with MASLD. We included four observational studies. Our findings are summarized as follows: (1) Statin use reduces the risk of HCC in patients with MASLD; (2) Lipophilic statins are more potent in lowering the risk of HCC compared to hydrophilic statins; and (3) The risk reduction with cDDD of statin follows a U-shaped curve.

Liver cancer is a major contributor to the global cancer burden, and its incidence rate has increased in recent decades. According to the Global Cancer Statistics, HCC of the liver parenchyma is the sixth most frequently diagnosed cancer worldwide, with approximately 865269 new cases reported in 2022[53]. With a 5-year survival rate of only 18%, liver

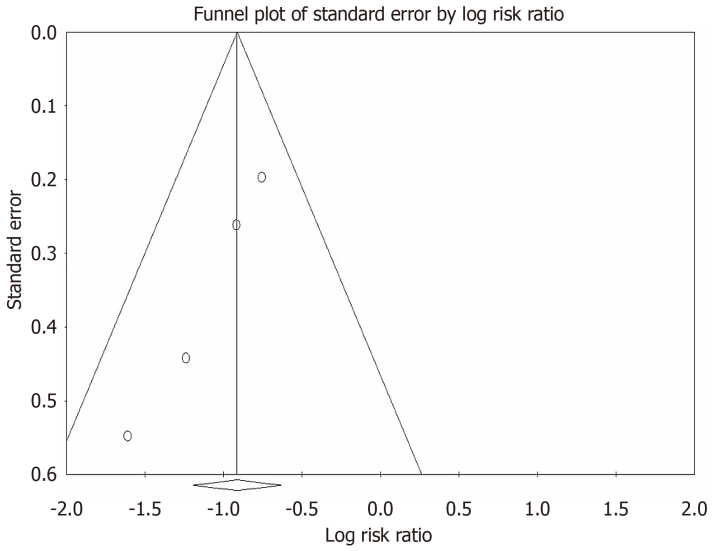

Figure 3 Funnel plot for publication bias.

cancer constitutes the third most common cause of cancer-related mortality worldwide, with over 757948 deaths in 2022 [53,54]. MASLD is the fastest-growing cause of HCC in several countries, including the United States, the United Kingdom, and France[55]. Global MASLD-HCC case numbers are projected to increase significantly due to the rapidly rising MASLD prevalence rates[56,57]. This warrants a concerted effort to reduce the incidence of HCC in MASLD by exploring the impact of lifestyle modification and chemoprevention[58,59]. Current clinical evidence indicates a growing interest in the chemopreventive role of statin therapy[60].

Our results show that statin therapy significantly reduces the risk of HCC in MASLD patients. Statin use has previously been linked to a lower risk of HCC in the general population[61]. These agents have also resulted in a similar clinical benefit in patient populations with diabetes and cirrhosis[62,63]. However, the data regarding chemopreventive effects of statins for MASLD-HCC has remained inconclusive. MASLD is closely associated with metabolic syndrome, which consists of diabetes mellitus, hypertension, dyslipidemia, and obesity. These conditions lead to systemic inflammation and raise the HCC risk, likely by activating oncogenic pathways[64]. Lipid accumulation in MASLD causes a number of cellular derangements, including lipotoxicity, endoplasmic reticulum stress, and the generation of reactive oxygen species leading to DNA damage resulting in oncogenesis[65,66]. The antineoplastic effect of statin occurs through both hydroxymethylglutaryl-CoA reductase-dependent and independent pathways. Clinical evidence shows that lipid-lowering agents other than statins have fewer anticancer effects[67]. Despite their established safety in MASLD, statins remain underused in this patient population[68-70]. Therefore, our meta-analysis highlights the importance of statin usage in patients with MASLD in the context of HCC prevention.

We noted that lipophilic statins were associated with a higher reduction in the risk of HCC among MASLD patients. The use of hydrophilic statins had no significant effect on HCC risk reduction. A Swedish prospective cohort study also reported an association between lipophilic statins and reduced 10-year HCC incidence and death among patients with viral hepatitis-related chronic liver disease[71]. In contrast, two meta-analyses reported that the beneficial effect of statins in lowering the risk of HCC was similar for both hydrophilic and lipophilic statins[72,73]. Pre-clinical studies revealed the effectiveness of lipophilic statins in preventing viral replication, potentiating antiviral therapy, and stimulating antitumor immunity compared to hydrophilic statins[74]. Kato *et al*[75] described reduced surface expressions of anion transporter proteins in hepatocytes during inflammation and carcinogenesis, preventing hydrophilic statins from penetrating the cells. Lipophilic statins readily diffuse across the cell membrane and induce potent antitumor effects through G0/G1 cell cycle arrest and suppression of the Ras/Raf/MEK/ERK pathway[75].

The higher doses of statin (cDDD > 600) correlated with a greater risk reduction of HCC in our analysis. The beneficial effects plummeted with the cDDD of 300-599 but increased again with the lower cDDD. Congruent to our findings, Tsan *et al*[76] found that a high dose duration of the statin product was associated with greater hepatoprotective effects in the hepatitis B cohort. Similarly, a meta-analysis showed increasing cDDD of statins resulted in HCC risk reduction in general as well as at-risk non-MASLD populations, confirming a dose-dependent effect[72]. A retrospective study from the United States based on 9135 chronic hepatitis C patients also substantiated a dose-dependent reduction in incident cirrhosis and HCC[77]. A nationwide, nested case-control study from the Republic of Korea found a dose-dependent risk reduction, with doses greater than 720 cDDD showing greater clinical effectiveness[78]. In a Taiwanese study, statin usage also dose-dependently reduced the incidence of HCC, decompensation, and death in cirrhosis patients[79]. On the other hand, a few studies have revealed that a higher dose of statin has no significant benefit over a lower dose in HCC prevention[80,81]. However, these studies had limitations such as a small sample size and inclusion of patients with several different types of cancer. Therefore, further population-based studies are warranted to determine the dose effect of statins on MASLD-HCC prevention.

Our meta-analysis has several strengths. We examined the use of statins specifically in patients with MASLD, avoiding the heterogeneity of patient populations with other high-risk liver conditions. We conducted a detailed literature search of all the major databases and a manual search of the bibliographies of the included studies. Furthermore, three investigators searched and screened the databases separately, and a fourth reviewer approved the final studies included in the analysis. Our meta-analysis consists of four studies, but the combined total number of patients was sufficiently large. Finally, we used a random-effects model to provide a more conservative pooled estimate. This meta-analysis is unique as it offers pooled evidence regarding HCC risk stratified by solubility status and doses of statins in patients with MASLD. Therefore, it may provide guidance for future clinical trial design that would form the basis for deriving an effective HCC prevention strategy in these patients. A recent literature review found that statins had the strongest clinical evidence currently available among all the chemopreventive agents for MASLD-HCC[82]. While the effect of statins requires further evaluation, an expert panel supports their regular use for HCC prevention in patients with MASLD[83].

Limitations

The small number of included studies constituted a major limitation. Moreover, the non-randomized, observational nature of the studies could result in bias due to flaws in study selection criteria, design, and the presence of confounding factors, including the concurrent use of other drugs, comorbidities, activity status, and genetic predisposition. It could potentially make it difficult to prove that statins alone were responsible for the protective effect against HCC in these patients. However, an updated meta-analysis has recently indicated that only statin use was significant for HCC chemoprevention in subgroup analyses accounting for concurrent drugs such as aspirin and metformin[84]. Large-scale randomized prospective trials are required to further evaluate the effects of statins on HCC prevention in the MASLD population.

CONCLUSION

This systematic review and meta-analysis evaluates the difference in HCC risk between MASLD patients on statin treatment and those who did not receive these medications. Based on the available data, our findings conclude that the use of statins lowers the risk of HCC in patients with MASLD. Lipophilic statins are found to be more potent in reducing the risk of HCC compared to hydrophilic statins. The reduction in risk with cDDD of statin follows a U-shaped curve. Further reliable research with a rigorous study design is required to confirm our results in the future.

FOOTNOTES

Author contributions: Tarar ZI, Farooq U, Inayat F, Basida SD, Ibrahim F, and Gandhi M concepted and designed the study, participated in the acquisition of data, interpretation of results, writing of the original draft, and critical revisions of the important intellectual content of the final manuscript; Nawaz G, Afzal A, Chaudhary AJ, Kamal F, and Ali AH contributed to the analysis and interpretation of results and drafting of the manuscript; Ghouri YA reviewed, revised, and improved the manuscript by suggesting pertinent modifications; all authors critically assessed, edited, and approved the final manuscript and are accountable for all aspects of the work.

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LETTER TO THE EDITOR

Comprehensive analysis of the impact of primary percutaneous coronary intervention on patients with ST-segment elevation myocardial infarction

Ayrton I Bangolo, Nikita Wadhwani

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Abstract

This comprehensive analysis by Saeed and Faeq investigates the impact of primary percutaneous coronary intervention (pPCI) on mortality among patients with ST-segment elevation myocardial infarction (STEMI) at the Erbil Cardiac Center. Analyzing data from 96 consecutive STEMI patients, the study identified significant predictors of in-hospital mortality, emphasizing the critical impact of time of hospital arrival post-symptom onset on overall prognosis. Findings indicate that factors such as atypical presentation, cardiogenic shock, chronic kidney disease, and specific coronary complications are associated with higher mortality rates. The study underscores the necessity of prompt medical intervention for improving survival outcomes in STEMI patients, especially in the high-risk subgroup. This research offers valuable insights into optimizing STEMI management and enhancing patient survival rates through effective and timely pPCI.

Key Words: ST-segment elevation myocardial infarction; Primary percutaneous coronary intervention; Mortality predictors; Timely hospital arrival; Cardiogenic shock

Core Tip: This study by Saeed and Faeq reveals the significant impact of timely primary percutaneous coronary intervention on mortality reduction in ST-segment elevation myocardial infarction patients. The analysis showed that chronic kidney disease, specific culprit coronary lesions, and an atypical presentation characterized by presence of either syncope, cardiogenic shock, or ventricular arrhythmias on arrival were predictive of post-percutaneous coronary intervention mortality.

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TO THE EDITOR

In this letter, we present our comments on the article by Saeed and Faeq[1]. This article addresses a critical aspect of cardiovascular emergency care, focusing on the outcomes of primary percutaneous coronary intervention (pPCI) in patients with ST-segment elevation myocardial infarction (STEMI) treated at the Erbil Cardiac Center.

RESULTS/DISCUSSION

The study[1] is premised on the assertion that reduction in time from symptom onset to hospital arrival can significantly improve prognosis of STEMI patients. The authors' hypothesis is evidently a strength of this study owing to the extensive body of medical literature supporting this assertion. It, therefore, stands to reason that the current guidelines endorse a door-to-balloon time of less than 90 minutes, as shorter times significantly correlate with reduced 1-year mortality. The authors analyze data from 96 patients with STEMI to tease out factors predictive of inpatient mortality and 30-day mortality post-discharge. Of the 96 patients, 92.7% (89) presented with typical ischemic chest pain, whereas, the remainder had an atypical presentation comprising syncope, ventricular arrhythmias, cardiogenic shock (CS), or cardiac arrest. Patients with certain culprit lesions and an atypical presentation on arrival had markedly higher mortality.

Several studies have shown that moderate to severe chronic kidney disease (CKD) is associated with adverse outcomes after uncomplicated primary PCI[2-5]. The authors[1] arrived at a similar conclusion, however, the findings lacked granularity. Differentiating mortality rates across different stages of CKD would have ascribed greater predictive power to this variable and offered insight into the impact of renal dysfunction, making it a robust parameter in risk-stratification of pPCI patients. Authors also mentioned that higher serum creatinine was statistically associated with a higher risk for mortality. This is analogous to the conclusion drawn for the association between presence of CKD and mortality previously. It is important to note here that serum creatinine, per se, is a flawed surrogate marker as it is affected by lean body mass, dietary protein intake, anabolic drug/bodybuilding supplement consumption, *etc*. Reliance on other parameters (namely cystatin C), that are less prone to such fallacies, could help address the aforementioned shortcomings. Also, it would be interesting to know the rationale for the analysis that compares varying amounts of white cell count with inpatient and 30-day mortality rates.

Pre-procedural A1c levels didn't show a statistically significant relationship with mortality in this study[1]. Historically, higher A1c values have been associated with increased risk for major adverse cardiovascular events, but outcomes on mortality are mixed with some studies demonstrating higher mortality even with stringent glycemic control[3,4]. Although the authors state that CS on presentation was an adverse prognostic marker, the timing of development of new in-hospital CS events, and early *vs* late mechanical circulatory support initiation is unclear. These estimates have been shown to be independent prognostic markers in some studies[6,7]. Therefore, inclusion of this information could have been conducive to the overall analyses. Also, subgroup analyses involving patients with atypical presentation who are inherently at heightened risk for adverse overall outcomes, or conduction of a multivariate analysis that adjusts for the potential confounding effect of baseline illness severity could engender a more reliable assessment of prognostic predictors in this cohort.

Presence of anemia did not confer higher mortality in this study[1]. Notably, all participants had a hemoglobin level greater than 11 g/dL. This interesting observation is in line with the contemporary data [TRICS, REALITY, MINT] surrounding transfusion thresholds in acute coronary syndromes, which hints at improvement in cardiovascular outcomes with liberal transfusion strategy as opposed to restrictive strategy[8-10]. Patients in this study[1] were above the transfusion threshold of 10 g/dL and were optimized from a hematocrit standpoint, potentially explaining the negative association between anemia ("mild") and mortality.

The study[1] also elucidates survival outcomes associated with location of culprit lesions. Left circumflex, left anterior descending (LAD), and obtuse marginal lesions conferred higher inpatient mortality, whereas only LAD lesions resulted in higher 30-day mortality post-discharge. Triple-vessel disease accounted for the majority of inpatient deaths ($P < 0.001$). Left main stem disease was also significantly associated with inpatient mortality ($P < 0.05$). Notably, 30-day mortality post-discharge was noted in patients with triple vessel disease only ($P < 0.05$). It would have been interesting to analyze the relationship between type of vascular access (radial *vs* femoral) and stent used with incident mortality. Studies have shown that transfemoral access (TFA) is associated with statistically greater need for critical care support, and 30-day mortality in patients with cardiogenic shock. Transradial approach yields successful PCI outcomes with lesser incidence of adverse events when compared with TFA[11]. Additionally, newer generation drug eluting stents (DES) are associated with lower mortality when compared with older generation DES[4].

Since patients with atypical symptomology have suboptimal short and long - term outcomes, it is important to note that this often involves prolonged out-of-hospital cardiac arrest times or ventricular dysrhythmias with resultant adverse neurological sequelae[12]. This, in turn, is tied to the paucity or lack of volunteer training in cardiopulmonary resu-

scitation (CPR) and automated external defibrillator (AED) use, which varies widely across different jurisdictions. Studies have shown that implementation of a structured response plan can double survival rates if bystanders are well-trained and equipped to utilize AED in an accurate and timely fashion. Therefore, universal AED access and meticulous CPR training of laymen is indubitably paramount to improving survival rates, especially for the high-risk subset. Postperfusion STEMI patients are routinely triaged to cardiac intensive care units. However, it seems that stable patients may not necessarily require intensive care treatment. Clinicians should predicate their decision on the overall clinical risk profile and the anticipation of need for critical care treatments. Several predictive variables can assist in risk-stratifying postperfusion patients, allowing for timely institution of appropriate therapies along with optimal use of resources.

Overall, the authors[1] did a commendable job at determining prognostic variables in a sizable cohort of STEMI patients undergoing pPCI. Their findings further underscore the need for early risk-stratification of high-risk subsets and concomitant correction of metabolic derangements and organ dysfunction. Optimization of survival rates in this cohort requires prompt institution of modalities namely renal replacement, blood transfusion, mechanical circulatory support, inotropes, mechanical ventilation, *etc.*, based on the overall risk profile of the patient as discussed herein.

FOOTNOTES

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LETTER TO THE EDITOR

Familial hypercholesterolemia: Current limitations and future breakthroughs

Ze Xiang, Jia-Rui Li, Wei-Min Wan, Shu-Hui Li, Jian Wu

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Abstract

Familial hypercholesterolemia (FH) is characterized by elevated low-density lipoprotein cholesterol levels due to genetic mutations, presenting with xanthomas, corneal arch, and severe cardiovascular diseases. Early identification, diagnosis, and treatment are crucial to prevent severe complications like acute myocardial infarction. Statins are the primary treatment, supplemented by Ezetimibe and proprotein convertase subtilisin/kexin type 9 inhibitors, though their effectiveness can be limited in severe cases. Over 90% of FH cases remain undiagnosed, and current treatments are often inadequate, underscoring the need for improved diagnostic and management systems. Future strategies include advancements in gene testing, precision medicine, and novel drugs, along with gene therapy approaches like AAV-mediated gene therapy and clustered regularly interspaced short palindromic repeats. Lifestyle modifications, including health education, dietary control, and regular exercise, are essential for managing FH and preventing related diseases. Research into FH-related gene mutations, especially *LDLR*, is critical for accurate diagnosis and effective treatment.

Key Words: Familial hypercholesterolemia; Genetic mutations; Treatment; Limitations; Breakthroughs

Core Tip: Familial hypercholesterolemia (FH) is a genetic disorder characterized by significantly elevated levels of plasma low-density lipoprotein cholesterol, often leading to severe cardiovascular conditions such as acute myocardial infarction. Early detection, diagnosis, and treatment are crucial for improving patient outcomes. Despite growing awareness, over 90% of the estimated 30 million global FH cases remain undiagnosed, and many patients lack adequate treatment. Current management primarily involves statins, with additional therapies like Ezetimibe and proprotein convertase subtilisin/kexin type 9 inhibitors, though effectiveness varies, particularly in homozygous FH cases. Advancements in gene testing and precision medicine are essential for better understanding and treating FH. Future strategies include gene therapy and novel lipid-lowering drugs, alongside lifestyle modifications and genetic diagnosis for early intervention and improved prognosis.

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TO THE EDITOR

Familial hypercholesterolemia (FH) refers to a disease characterized by a significant increase in plasma low-density lipoprotein cholesterol (LDL-C) levels caused by genetic mutations[1]. FH patients have clinical manifestations such as skin and tendon xanthoma or corneal arch and may also experience cardiovascular involvement, even leading to acute myocardial infarction and other diseases in severe cases[2]. FH affects about 10 million people worldwide and is mainly a heterozygous form. This condition is usually caused by mutations in the LDL receptor protein gene (*LDLR*), but mutations in the apolipoprotein B (*APOB*) and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes have also been implicated[3]. Of note, 17% to 33% of patients with clinically diagnosed monogenic hypercholesterolemia have no genetic cause found at known loci[4]. In recent years, the incidence of FH has been increasing year by year, and there has been growing attention towards this condition. However, despite these developments, more than 90% of the estimated 30 million people worldwide with FH remain undiagnosed. Therefore, early identification, diagnosis, and treatment of FH are particularly important.

The serum cholesterol level of FH patients is significantly increased, and the incidence of atherosclerotic coronary artery disease is markedly enhanced[5]. Severe cases can lead to acute myocardial infarction and other diseases[6]. At present, there is a lack of effective methods for the identification and long-term management of FH patients. Besides, shockingly, as many as one-third of patients do not receive any form of treatment. And the current treatment options for FH are not ideal. Once a patient is diagnosed with FH, early initiation of lipid-lowering therapy is recommended[7]. Regarding treatment approaches, both domestic and foreign guidelines recommend statins as the cornerstone therapy for FH management[8]. Additionally, the cholesterol inhibitor Ezetimibe or PCSK9 inhibitors can be added to statin therapy if necessary. Other commonly prescribed medications include lomitapide, CETP inhibitors, and other agents. Surgical interventions such as liver transplantation and partial ileal bypass are currently not widely employed in clinical practice but hold potential for future development[9]. In the future, it is necessary to strengthen the screening of FH patients and further establish a sound FH patient management system, so as to achieve early detection, early diagnosis and early treatment to improve the prognosis of the disease.

With advancements in gene testing and precision medicine technology over time, more attention has been given to studying FH-related gene mutations among individuals affected by this condition[10]. However, approximately 50% of identified mutations in FH patients are still classified as variants of unknown significance, which includes *LDLR* gene mutation classification. It is highly significant to study and evaluate the functional impact of *LDLR* gene mutations on the treatment outcomes for FH patients. *LDLR* gene mutations have been divided into five functional groups[11], and the common mutations with diagnostic value for FH are different in different countries or regions[12-15]. Up to now, more than 4000 kinds of *LDLR* mutations have been found worldwide (University College London LDLR FH database (www.ucl.ac.uk/Ldlr). And identification of *LDLR* mutations plays a crucial role in the accurate diagnosis and targeted treatment of FH. For example, the presence and type of *LDLR* mutations can affect the lipid profile and response to lipid-lowering therapy in Brazilian patients with heterozygous FH, and patients carrying null mutations have a poor prognosis [16]. Miltiadous *et al*[17] studied the efficacy of atorvastatin 20 mg/day for 12 weeks in 49 patients with heterozygous FH, divided into two groups, class V and II, based on *LDLR* mutations. Patients with class V mutations had relatively lower baseline LDL-C values and better response to statin therapy. Studies have demonstrated that *LDLR* mutations are associated with higher serum LDL-C levels and the incidence of cardiovascular disease is increased in patients with extremely high LDL-C[18]. For refractory homozygous FH patients with *LDLR* deletion mutations, adding cholesterol inhibitors or PCSK9 inhibitors to statin therapy has limited or even ineffective effects. Foreign guidelines suggest using class 4 lipid-lowering drugs such as Lomitapide, Mipomersen or Evolocumab in such cases; however, their potential side effects and economic considerations need to be taken into account[19]. Therefore, there is an urgent need for a safe and cost-effective new drug along with novel therapeutic strategies to effectively reduce LDL-C levels. Gene therapy for FH is still in the exploratory phase and primarily involves adeno-associated viruses (AAVs), AAV-mediated gene therapy, and clustered regularly interspaced short palindromic repeats (CRISPR) gene therapy. The clinical applicability of these approaches requires further investigation[20]. Nonetheless, due to the demanding requirements associated with *in vitro*

cell model research methods used for this purpose, it becomes challenging to widely implement them across various settings[10]. Moreover, there are variations observed when predicting functional effects and clinical.

Through health education, dietary control, lifestyle improvement, and regular exercise, the regulation of body lipid and lipoprotein metabolism can effectively achieve early prevention and treatment of FH and related cerebrovascular diseases. A randomized controlled clinical trial evaluated the effect of an individualized lifestyle intervention based on the transtheoretical model of health behavior change on disease management in patients with FH. The results showed a significant reduction in body mass index, LDL-C and blood pressure, and an improvement in treatment adherence in the intervention group[21]. Meta-analyses have shown cholesterol-lowering effects on FH by the additional addition of plant sterols or stanols to a low-cholesterol diet, or by the reduction of triglycerides by supplementation with omega-3 fatty acids[22].

Genetic diagnosis enables early detection and diagnosis of FH, while future advancements in personalized medicine combined with gene editing hold promising potential for curing an increasing number of rare diseases and significantly enhancing patients' quality of life. Currently, the disconnect between clinical diagnosis and interpretation of genetic results between medical staff and patients leads to underdiagnosis and treatment of FH. Phenotypic features combined with genetic diagnosis enable early detection and diagnosis of FH; however, even with early diagnosis and lipid-lowering therapy, FH patients with null *LDLR* mutations tend to be at increased risk. Personalized medicine combined with gene editing is expected to cure more and more rare diseases and significantly improve the quality of life of patients[23]. In the future, it is still necessary to refine LDL-C management according to *LDLR* mutation types, and improve the diagnosis and treatment of FH around the world through the formulation of national screening programs, new drug development, personalized lifestyle intervention, and awareness education.

FOOTNOTES

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