ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2024 July 21; 30(27): 3264-3360

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

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World Journal of Gastroenterology

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NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

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PUBLICATION DATE
July 21, 2024

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World Journal of Gastroenterology

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World J Gastroenterol 2024 July 21; 30(27): 3264-3267

DOI: 10.3748/wjg.v30.i27.3264

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Effects of excess high-normal alanine aminotransferase levels in relation to new-onset metabolic dysfunction-associated fatty liver disease: Clinical implications

Giovanna McGinty, Robert Przemioslo

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade E
Novelty: Grade C
Creativity or Innovation: Grade D
Scientific Significance: Grade C

P-Reviewer: Ebraheim LLM

Received: March 11, 2024
Revised: May 29, 2024
Accepted: June 21, 2024
Published online: July 21, 2024
Processing time: 121 Days and 14.3 Hours

Giovanna McGinty, Department of Gastroenterology, North Bristol Trust, Southmead Hospital, Bristol BS10 5NB, United Kingdom

Robert Przemioslo, Department of Gastroenterology and Hepatology, North Bristol Trust, Southmead Hospital, Bristol BS10 5NB, United Kingdom

Corresponding author: Giovanna McGinty, MBChB, MSc, Doctor, Department of Gastroenterology, North Bristol Trust, Southmead Hospital, Southmead Road, Bristol BS10 5NB, United Kingdom. giovanna.sheiybani@nhs.net

Abstract

In this editorial, we comment on the article by Chen *et al* recently published in 2024. We focus the debate on whether reducing the upper limit of normal of alanine aminotransferase (ALT) would effectively identify cases of fibrosis in metabolic-dysfunction associated fatty liver disease (MAFLD). This is important given the increasing prevalence of MAFLD and obesity globally. Currently, a suitable screening test to identify patients in the general population does not exist and most patients are screened after the finding of an abnormal ALT. The authors of this paper challenge the idea of what a normal ALT is and whether that threshold should be lowered, particularly as their study found that 83.12% of their study population with a diagnosis of MAFLD had a normal ALT. The main advantages of screening would be to identify patients and provide intervention early, the mainstay of this being changing modifiable risk factors and monitoring for liver fibrosis. However, there is not enough suitable therapeutic options available as of yet although this is likely to change in the coming years with more targets for therapy being discovered. Semaglutide is one example of this which has demonstrated benefit with an acceptable side effect profile for those patients with MAFLD and obesity, although studies have not yet shown a significant improvement in fibrosis regression. It would also require a huge amount of resource if a reduced ALT level alone was used as criteria; it is more likely that current scoring systems such as fibrosis-4 may be amended to represent this additional risk. Currently, there is not a good argument to recommend widespread screening with a reduced ALT level as this is unlikely to be cost-effective. This is compounded by the fact that there is a significant heterogeneity in what is considered a normal ALT between laboratories. Although studies previously have suggested a more pragmatic approach in screening those over the age of 60, this is

likely to change with the increasing incidence of obesity within the younger age groups. The main message from this study is that those who have hypercholesterolemia and high body metabolic index should have these risk factors modified to maintain a lower level of ALT to reduce the risk of progression to fibrosis and cirrhosis.

Key Words: Alanine aminotransferase; Metabolic-dysfunction associated fatty liver disease; Metabolic syndrome; Fibrosis; Cirrhosis; Semaglutide

Core Tip: Alanine aminotransferase (ALT) is a surrogate marker for metabolic-dysfunction associated fatty liver disease (MAFLD) but is not specific for histological inflammation. The rationale to reduce the upper limit of normal for ALT to help identify more cases of MAFLD remains controversial. It is more important to identify patients who may display elements of the metabolic syndrome and support modifying these to maintain a lower level of ALT. This will become increasingly important as more targets for therapy are identified that may justify treating these patients early to prevent progression to fibrosis.

Citation: McGinty G, Przemioslo R. Effects of excess high-normal alanine aminotransferase levels in relation to new-onset metabolic dysfunction-associated fatty liver disease: Clinical implications. *World J Gastroenterol* 2024; 30(27): 3264-3267
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3264.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3264

INTRODUCTION

Metabolic-dysfunction associated fatty liver disease (MAFLD) is the most common liver disease worldwide with a current prevalence of around 25% in the Western world and rising[1]. Obesity, type 2 diabetes mellitus (T2DM), hypertension and dyslipidemia are established risk factors for the development of MAFLD. Elevation in alanine aminotransferase (ALT) is an associated biomarker of the metabolic syndrome[2]. It also has an association with serum triglycerides, plasma fasting glucose and body metabolic index. The prevalence of MAFLD has increased considerably in the last few years and will continue to increase due to the increasing prevalence of obesity and the metabolic syndrome. The morbidity related to MAFLD is also significant: It can increase the risk of cardiovascular disease and increase the cancer-related mortality[3]. This rising incidence that has been observed and the parallel with obesity will likely cause an increase in the aforementioned causes of morbidity.

ALT has previously been shown to be an independent predictor of the development of fibrosis and is a marker of hepatocellular injury[4,5]. However, ALT is not specific for histological inflammation, nor can it help determine whether cirrhosis is likely to be present[6]. MAFLD has been shown to occur in the presence of a normal ALT[7]. It is often the case that advanced fibrosis is seen in patients with normal ALT in MAFLD. Currently, there is no recommendation to screen for MAFLD in the asymptomatic population although this has gained more attention in recent times[8]. A recent study by Lomonaco *et al*[9] suggests we should be more proactive in screening patients with T2DM since the authors found that 15% of patients of those with T2DM had F2 or higher fibrosis. It is not known whether there is a level of ALT where we should consider screening for MAFLD. The data from Chen *et al*[10] sought to answer this question.

In the study by Chen *et al*[10], the authors reported on a retrospective and prospective population-based cohort study in China. A similar study has been performed in the pediatric population to help determine an optimal cut-off for ALT [11]. The patients were aged over 18-year-old and were selected over a consecutive period of 3 years between January 2017 and December 2019. 3553 participants were found to be eligible from their initial pool of 7817 participants. The authors used a combination of physical measurements, laboratory test results, Doppler ultrasound measurements and established diagnostic criteria for MAFLD.

The authors used a receiver operating characteristic curve with the maximum value of the Youden index to determine the ALT cut-off point, in line with other similar studies[12,13]. The cumulative effect of this was found to be significant compared to those that only had a single occurrence of an ALT > 18.5 U/L. It has been shown in a prospective study by Gawrieh *et al*[14] that advanced fibrosis and steatohepatitis increases in frequency when ALT increases from 20 U/L to 39 U/L. The authors of the study by Chen *et al*[10] were able to demonstrate that 83.12% of the participants with MAFLD had a normal ALT. They also concluded that the risk of developing MAFLD is related to a persistently high ALT leading to a cumulative effect. This supports other studies that have found that a persistently raised ALT can lead to significant fibrosis from MAFLD[15,16].

SHOULD WE BE SCREENING FOR MAFLD AT A LOWER ALT?

As of 2022, MAFLD affected one-third of the global population[17]. The diagnosis of MAFLD is made by the presence of

hepatic steatosis and the presence of one or more of the elements of the metabolic syndrome[18]. Hepatocellular damage releases ALT, with MAFLD being the most common cause of asymptomatic elevation of transaminase levels. With a significant morbidity associated with this, the most important being cardiovascular disease, we should be examining whether a suitable screening test exists to pick up those asymptomatic patients that would benefit from early intervention.

The Chen *et al*[10] study provides the argument that the cut-off for abnormal ALT should be reduced to 18.5 U/L, which is lower than the level typically used to start investigating for the presence of MAFLD in clinical practice, at least in the United Kingdom. If this definition was adopted widely, investigating individuals with this level of ALT would require a significant amount of extra resource to manage the workload of identifying and managing these patients. This should be balanced with the cost related to health-related complications that can eventually occur, such as hospitalization from cardiovascular events, decompensated liver disease and hepatocellular carcinoma. It has been argued previously that the demographic most likely to benefit from screening for MAFLD would be those aged older than 60 due to the increased mortality in this age group[19]. However, this is likely to change with the increasing incidence of obesity seen at younger ages[20]. There has been some debate on whether screening should be employed, on which patients and how. The American Association for the Study of Liver Diseases argue that there is simply not a cost-effective method to do this routinely and should only be performed in specific population groups[21].

There is a lack of effective pharmacological solutions to managing MAFLD. The mainstay of management currently is in the managing of metabolic risk factors and working closely with endocrinologists and cardiologists as necessary to help prevent disease progression. However, the landscape of pharmacological treatments is changing and much research is ongoing to establish new targets for therapy[22]. Semaglutide, a glucagon-like peptide-1 receptor agonist which is used to treat diabetes and obesity, has shown some promising initial results for treating MAFLD whilst also being well-tolerated. However, there have not been enough large randomized controlled trials to date to suggest that it significantly reverses biochemistry or causes fibrosis regression[23].

The current practice is that ALT is usually combined with other markers to give a predictor of fibrosis to which a clinician may investigate further by magnetic resonance imaging, transient elastography or liver biopsy. Fibrosis-4 and enhanced liver fibrosis scores are the most widely used non-invasive screening tests[24] and it may be in time, the ALT level that contributes to this is lowered to reflect the evidence that MAFLD can occur in the presence of a traditionally "normal" ALT. However, it is not clear currently that doing this routinely would be cost-effective or lead to a lower mortality necessarily given the lack of treatment options that exist currently.

CONCLUSION

A cumulative higher level of ALT, whether that is upper limit of normal or above this, can present an additional risk factor for the development of MAFLD. There is currently no consensus on how we should screen asymptomatic patients for MAFLD due to lack of therapeutic options and being able to prevent disease progression consistently. The question is not whether MAFLD can be diagnosed with a normal ALT: It is what we define as a normal ALT. This study suggests that MAFLD can occur at a lower level than we would typically flag as "abnormal". The high prevalence and morbidity should give us the motivation to keep searching the answers to these questions. As clinicians, we should be more astute to those that may have typical risk factors but have a normal ALT; perhaps we should have a lower threshold for screening these patients. It is likely to be more important to support patients in changing modifiable risk factors to maintain a lower level of ALT.

FOOTNOTES

Author contributions: McGinty G and Przemioslo R contributed, designed the overall concept and contributed to writing, editing and review of the literature.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: United Kingdom

ORCID number: Giovanna McGinty 0000-0003-3953-5275.

S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Zheng XM

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World Journal of Gastroenterology

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World J Gastroenterol 2024 July 21; 30(27): 3268-3272

DOI: 10.3748/wjg.v30.i27.3268

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

What aspects do we overlook in the rehabilitation of patients with inflammatory bowel disease?

Benil Nesli Ata, Sibel Eyigor

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade C, Grade C

Novelty: Grade A, Grade B, Grade B

Creativity or Innovation: Grade A, Grade B, Grade C

Scientific Significance: Grade A, Grade B, Grade B

P-Reviewer: Liu W; Wang YD; Wu L

Received: March 15, 2024

Revised: June 5, 2024

Accepted: June 28, 2024

Published online: July 21, 2024

Processing time: 117 Days and 15.9 Hours

Benil Nesli Ata, Department of Physical Medicine and Rehabilitation, Izmir City Hospital, Izmir 35530, Türkiye

Sibel Eyigor, Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Ege University, Izmir 35100, Türkiye

Corresponding author: Benil Nesli Ata, MD, Doctor, Department of Physical Medicine and Rehabilitation, Izmir City Hospital, Sevket Ince Mah. 2148/11 Sk No. 1/11 Bayrakli/Izmir 35530, Turkiye. drbenilnesli@gmail.com

Abstract

In this editorial, we comment on the article by Stafie *et al*. Inflammatory bowel disease (IBD) constitutes a cluster of chronic and progressive inflammatory disorders affecting the digestive system. IBD can impede an individual's capacity to perform daily activities, hinder work productivity, limit physical capabilities, and negatively impact medical outcomes. Although physical activity and structured exercise programs are becoming increasingly important in many chronic inflammatory diseases, they are not being sufficiently implemented in IBD patients. Effective prevention of future disability and drug dependence in IBD patients requires timely diagnosis and treatment of musculoskeletal problems, including sarcopenia, as well as decreased muscle strength, aerobic capacity, and bone mineral density. To improve treatment outcomes for IBD patients, it is crucial to develop individualized rehabilitation programs tailored to their unique needs. Equally critical is the active participation of pertinent departments in this process. It is imperative to highlight the significance of creating a personalized rehabilitation program with a multidisciplinary approach in IBD management.

Key Words: Inflammatory bowel disease; Physical activity; Disability; Sarcopenia; Structured exercise; Rehabilitation

Core Tip: Effectively managing clinical issues in inflammatory bowel disease (IBD) patients is critical to reducing the risk of long-term disability and facilitating optimal health outcomes. This includes addressing concerns such as decreased bone mineral density, muscle weakness, limited aerobic capacity, and sarcopenia. Customized structured exercise programs should be provided for patients in accordance with their individual needs, considering factors such as joint involvement, frailty, fatigue, and disease activity. Healthcare providers should adopt a multidisciplinary approach to provide personalized exercise recommendations, educate patients, and address misconceptions. This approach improves quality of life, minimizes complications associated with IBD, and enhances treatment success.

Citation: Ata BN, Eyigor S. What aspects do we overlook in the rehabilitation of patients with inflammatory bowel disease? *World J Gastroenterol* 2024; 30(27): 3268-3272
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3268.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3268

INTRODUCTION

Inflammatory bowel disease (IBD) is an umbrella term that encompasses two disease entities, Crohn's disease and ulcerative colitis, which are chronic, progressive inflammatory disorders of the digestive system. This disease imposes a significant burden on healthcare systems, leading to substantial annual healthcare expenditures. IBD is a condition that can lead to a variety of primary and secondary symptoms that can include diarrhea, abdominal pain, urgency, fatigue, frailty, anorexia, and depression[1]. Secondary symptoms significantly impact an individual's daily routine, work productivity, academic performance, physical abilities, medical outcomes, and overall quality of life (QOL). Young patients with IBD are seldom advised to engage in physical activity, which can result in a sedentary lifestyle due to concerns regarding potential complications[2].

OVERLOOKED ASPECTS IN INFLAMMATORY BOWEL DISEASE REHABILITATION

It is crucial to distinguish between the terms "structured exercise" and "physical activity" because they are frequently used interchangeably. Structured exercise and physical activity are two distinct types of activities that exhibit physiological differences. Structured exercise is a specialized and comprehensive program aimed at enhancing the functions of specific body systems, such as cardiovascular endurance and muscular strength. However, physical activity encompasses any form of movement that results in an increase in energy expenditure. Numerous chronic illnesses are believed to be exacerbated by inadequate physical activity[3,4]. The "BE-FIT-IBD" study found that 42.9% of IBD patients were physically inactive, despite their awareness of the importance of physical activity in managing the disease. Fear of disease flare-ups, patients' inadequate knowledge about exercise, and inadequate guidance from healthcare providers regarding physical activity are significant factors contributing to this lack of physical activity[5]. Physical inactivity has negative effects on IBD patients, including comorbidities such as cardiovascular disease, sarcopenia, osteoporosis, and mental health disorders. Physical rehabilitation and structured exercise are insufficiently implemented in the IBD population despite providing statistically significant improvements in disease activity, health outcomes, physical function, functional capacity, and overall QOL[6].

Currently, there is a lack of a comprehensive guide for healthcare practitioners or a detailed evidence-based physical rehabilitation protocol for IBD[7]. It is recommended that IBD patients engage in structured exercise under medical supervision until such time that they are deemed capable of doing so independently[8]. Inpatient rehabilitation programs can improve the physical mobility of IBD patients after they reach a state of medical stability[9]. When oxygen capacity is impaired due to anemia or dehydration, structured exercise and physical rehabilitation programs should be planned to include elements such as tempo, workload distribution, and rest intervals[10]. Employing suitable rehabilitation strategies and structured exercises can mitigate complications, such as gastrointestinal bleeding, that may develop due to excessive exercise[4]. There is evidence that shows aerobic exercise regimens can provide cardiovascular and psychosocial benefits for adult IBD patients with mild to moderate disease who are in clinical remission[11].

Despite exhibiting body compositions that are comparable to those of healthy controls, IBD patients in remission exhibit reduced muscle strength and power[12]. This may be due to impaired nutrient absorption, weight loss, decreased physical activity, and the upregulation of cytokines, which leads to muscle atrophy. Reduced muscle strength and function lead to functional deficits. Resistance exercise, when prescribed appropriately, aids muscle growth and strength by stimulating the release of biologically active myokines[13]. Populations with a higher risk of sarcopenia, malnutrition, being female, or being older have higher rates of complications. The early assessment of lower extremity and core strength can play a vital role in identifying individuals with IBD who are at risk of preclinical disability. Thus, timely diagnosis and treatment of musculoskeletal problems in these patients can effectively prevent future dependence and disability[14].

Both aerobic and resistance exercise training have been scientifically validated as effective approaches for improving cardiovascular health as well as muscle mass and strength. Numerous chronic conditions, including cardiovascular disease, diabetes, and rheumatoid arthritis, are believed to be impacted by insufficient physical activity. Interleukins (IL),

leukocytes, tumor necrosis factor, and visceral fat play a significant role in the pathogenesis and progression of IBD[15]. The potential benefits of exercise for individuals with IBD are linked to its anti-inflammatory properties. Physical activity is recognized for its ability to reduce inflammation by releasing anti-inflammatory myokines, such as IL-6, into the circulation in large quantities during exercise[16]. Furthermore, exercise may contribute to positive outcomes by reducing adipose tissue in the abdominal and intestinal regions[17]. Patients with IBD have concerns about engaging in physical exercise, unlike healthy individuals. While several studies have demonstrated the safety and effectiveness of physical activity in IBD patients, these studies primarily included patients with mild disease or those in remission[18].

The most common extra-intestinal symptom in IBD patients is articular involvement, with a prevalence of 17%-39% [19]. Although it is often characterized by involvement of axial joints, it may also be associated with peripheral arthritis such as synovitis and/or dactylitis and/or enthesopathy. IBD-related arthritis rehabilitation aims to prevent disability and deformity. Physical therapy is crucial for enhancing spinal mobility to prevent spinal deformities that could impair breathing and cause disability.

Patients with IBD have a higher risk of fractures compared to the general population. Adequate nutrition and a combination of progressive low-impact and resistance exercise training are important for preventing and treating osteoporosis[3].

IBD patients exhibit alterations in body composition due to the catabolic state caused by chronic inflammation and the use of treatments such as corticosteroids. Therefore, IBD patients frequently develop sarcopenia. The prevalence of sarcopenia is reported to be 52% and 37% in Crohn's disease and ulcerative colitis, respectively. However, accurately determining the precise prevalence is challenging[20]. Sarcopenia exerts a significant impact on QOL, duration of hospitalization, surgical outcomes, and mortality. This is an important consideration in the management of IBD, as it impacts both prognosis and treatment. Although sarcopenia is defined as age-related loss of muscle mass, it is known that it can also occur as a result of chronic illness, inactivity, and inflammation[21]. Research suggests that patients with sarcopenia may benefit from personalized resistance and aerobic exercise regimens[22].

Prehabilitation is a multimodal (nutritional, exercise, and psychological) intervention that aims to increase functional ability in the presence of impending physical stress (*e.g.*, surgery). While most studies have concentrated on cancer patients, this strategy could have a significant impact on IBD patients with refractory disease awaiting elective surgery [23].

The epidemiology of IBD varies significantly across different regions of the globe. Although IBD is more prevalent in Western societies, which are characterized by higher socioeconomic status and improved health conditions, there has been a significant rise in the incidence and prevalence of IBD in developing countries like Türkiye[24].

In developed countries, the average rate of non-adherence to treatment is 50%, and in developing countries, this figure is even higher. Furthermore, IBD has been associated with an increase in disease activity, relapse, diminished response to medical agents, higher morbidity and mortality, and increased health expenditure[25]. To address these concerns, telehealth services may be employed, which are both feasible and acceptable in terms of patient education, evaluation of treatment compliance, and emphasizing the need for personalized rehabilitation programs. More comprehensive research is necessary to utilize these systems for improving healthcare access in developing nations[26].

CONCLUSION

In IBD patients, several factors such as reduced bone mineral density, diminished muscle strength, decreased aerobic capacity, and sarcopenia are often not adequately screened or treated during medical evaluation. A multidisciplinary approach helps address the comorbidities accompanying IBD and reduces subsequent morbidity and mortality. Furthermore, personalized rehabilitation programs can help alleviate the economic burden and frequency of disease exacerbations that are associated with the condition. Therefore, it is imperative that healthcare professionals prioritize these factors and ensure that they are addressed appropriately to improve the overall health outcomes of patients. To obtain optimal results, it is essential to collaborate with a team of specialized professionals, including a gastroenterologist, surgeon, rheumatologist, and physiatrist. This editorial aims to underline the significance of developing a specific rehabilitation program for IBD patients and assessing them from a comprehensive perspective. This is crucial in mitigating morbidity and mortality along with tackling the issue of physical inactivity among IBD patients, as was highlighted in the main article by Stafie *et al*[27].

FOOTNOTES

Author contributions: Eyigor S designed the overall concept and outline of the manuscript; Ata BN contributed to the discussion and design of the manuscript; Eyigor S and Ata BN contributed to the writing and editing of the manuscript, illustrations, and review of the literature.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: Türkiye

ORCID number: Benil Nesli Ata 0000-0003-0900-0069; Sibel Eyigor 0000-0002-9781-2712.

S-Editor: Li L
L-Editor: A
P-Editor: Zhang XD

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World Journal of Gastroenterology

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World J Gastroenterol 2024 July 21; 30(27): 3273-3277

DOI: 10.3748/wjg.v30.i27.3273

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Novel insights into autophagy in gastrointestinal pathologies, mechanisms in metabolic dysfunction-associated fatty liver disease and acute liver failure

Tsvetelina Velikova, Milena Gulinac

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Liu J

Received: March 21, 2024
Revised: May 26, 2024
Accepted: June 24, 2024
Published online: July 21, 2024
Processing time: 111 Days and 18.2 Hours

Tsvetelina Velikova, Milena Gulinac, Medical Faculty, Sofia University St Kliment Ohridski, Sofia 1407, Bulgaria

Milena Gulinac, Department of General and Clinical Pathology, Medical University of Plovdiv, Plovdiv 4002, Bulgaria

Corresponding author: Milena Gulinac, MD, PhD, Academic Research, Department of General and Clinical Pathology, Medical University of Plovdiv, 15A Vassil Aprilov Blvd, Plovdiv 4002, Bulgaria. mgulinac@hotmail.com

Abstract

In this editorial, we comment on three articles published in a recent issue of *World Journal of Gastroenterology*. There is a pressing need for new research on autophagy's role in gastrointestinal (GI) disorders, and also novel insights into some liver conditions, such as metabolic dysfunction-associated fatty liver disease (MAFLD) and acute liver failure (ALF). Despite advancements, understanding autophagy's intricate mechanisms and implications in these diseases remains incomplete. Moreover, MAFLD's pathogenesis, encompassing hepatic steatosis and metabolic dysregulation, require further elucidation. Similarly, the mechanisms underlying ALF, a severe hepatic dysfunction, are poorly understood. Innovative studies exploring the interplay between autophagy and GI disorders, as well as defined mechanisms of MAFLD and ALF, are crucial for identifying therapeutic targets and enhancing diagnostic and treatment strategies to mitigate the global burden of these diseases.

Key Words: Gastrointestinal diseases; Autophagy; Metabolic dysfunction-associated fatty liver disease; High-normal alanine aminotransferase level; Silent information regulator sirtuin 1; Acute liver failure

Core Tip: Exploration of autophagy's role in gastrointestinal (GI) disorders, as well as metabolic dysfunction-associated fatty liver disease (MAFLD) and acute liver failure (ALF), is imperative for advancing diagnostic and treatment strategies in gastroenterological diseases. Despite advancements, understanding autophagy's intricate mechanisms in these conditions remains incomplete. Further research into MAFLD's diagnostic markers and treatment modalities, considering its hepatic steatosis and metabolic dysregulation, is crucial. Elucidating diagnostic and therapeutic approaches for ALF, a severe hepatic dysfunction, is essential. Investigating autophagy's implications in diagnosing and treating GI disorders, MAFLD, and ALF is pivotal for improving patient outcomes.

Citation: Velikova T, Gulinac M. Novel insights into autophagy in gastrointestinal pathologies, mechanisms in metabolic dysfunction-associated fatty liver disease and acute liver failure. *World J Gastroenterol* 2024; 30(27): 3273-3277
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3273.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3273

INTRODUCTION

In recent years, there is a growing body of evidence for the critical role of autophagy in gastrointestinal (GI) health and disease. Autophagy, a fundamental process in the cells, involved in the breakdown and recycling of impaired or malfunctioning cellular elements, has emerged as a critical player in maintaining GI homeostasis and responding to various pathological conditions. However, despite significant advancements in our understanding of autophagy, numerous questions remain unanswered regarding its intricate mechanisms and implications in GI disorders[1].

One area of particular interest is elucidating the molecular mechanisms underlying metabolic dysfunction-associated fatty liver disease (MAFLD), previously referred to as nonalcoholic fatty liver disease (NAFLD). As a multifaceted disorder encompassing both hepatic steatosis and metabolic dysregulation, MAFLD represents a significant public health concern worldwide. Despite its prevalence and impact on global health, the precise mechanisms driving the progression from simple steatosis to more severe liver pathology, such as nonalcoholic steatohepatitis (NASH) and acute liver failure (ALF), remain incompletely understood[2].

Furthermore, the pathogenesis of ALF, a life-threatening condition characterized by sudden and severe hepatic dysfunction, is poorly understood, posing significant challenges in its management and treatment. While autophagy has been implicated in ALF pathophysiology, its precise role and potential therapeutic implications require further investigation[3].

Thus, there is an urgent need for innovative research and studies aimed at unraveling the intricate interplay between autophagy, MAFLD, and ALF. By elucidating the underlying mechanisms and signaling pathways involved in these complex GI disorders, novel therapeutic targets may be identified, paving the way for developing more effective diagnostic and treatment strategies to improve patient outcomes and alleviate the global burden of GI-related diseases.

This is why we focus on three particular papers published in the recent issue of the *World Journal of Gastroenterology* in this editorial.

AUTOPHAGY IN GASTROINTESTINAL DISEASES

The minireview by Chang *et al*[4], published in the *World Journal of Gastroenterology*, examined descriptively and in detail the role of autophagy in GI diseases. Even though the studies in the field of apoptosis, *i.e.*, programmed cell death, are excessively numerous, there is still a lack of those concerning autophagy processes. Therefore, this article sheds light on the future of science and could be an excellent basis for further research in basis science and clinical practice. We choose to include this minireview in our editorial because it is presented and distinguished by its relevance.

It is well-known that autophagy has been discovered to serve a supportive function in non-life-threatening GI conditions such as intestinal ischemia-reperfusion (I/R) injury, inflammatory bowel disease (IBD), and motility GI disorders[1].

However, autophagy encourages the membrane localization of the occludin protein, a key component of tight junctions (TJ) responsible for enhancing the TJ barrier. This mechanism potentially safeguards against the loss of barrier function induced by inflammation[5].

Additionally, dysfunction in autophagy has the potential to disrupt intestinal barrier and initiate an intestinal inflammation culminating in chronic condition. Genome-wide association studies identified certain mutations in autophagy-related genes linked to IBD, including *ATG16L1*, *ULK1*, *NOD2*, *LRRK2*, and *IRGM*[6,7].

The minireview also highlights that the mechanisms of autophagy are discovered not only in intestinal I/R injury but also in GI motility disorders and GI cancers (*i.e.*, gastric colorectal). Furthermore, Chang *et al*[4] discussed the role of autophagy in the onset and progression of GI cancer and drug resistance. Numerous studies have revealed that various natural compounds can induce autophagy, exerting anti-cancer effects. However, one of the major drawbacks of Chang *et al*'s[4] paper is that they focused relatively insufficiently on the autophagy and GI drugs. In fact, they mentioned mainly some herbs while did not include relevant papers on the mutual relationship of autophagy and GI drugs.

It is worth mentioning that several studies solidified the connection between autophagy and the normal functions of GI cells. Furthermore, morphological studies have yielded data on pro-survival function of autophagy in benign GI diseases. However, in pathological states, the role of autophagy may vary, potentially influenced by the degree of the process or other factors. Consequently, further research on the autophagy involvement in GI tumors is imperative to unravel these questions and hypothesis.

EXCESS HIGH-NORMAL ALT LEVELS AND NEW-ONSET MAFLD

This retrospective cohort study by Chen *et al*[8], published in the recent issue of the *World Journal of Gastroenterology*, is exceptionally well presented and valuable because, although it deals with one of the most common degenerations in the world, its relationship to excess high-normal ALT (ehALT) levels is understudied. The authors also described MAFLD in detail, including a morphological perspective.

This article is fascinating, and the idea in such a detailed study is extremely promising and practical for clinicians. The cohort included 3553 patients presented at four consecutive health examinations for four years. Of practical significance is that 83.13% of MAFLD patients exhibited normal ALT levels. However, the MAFLD incidence demonstrated a consistent linear increase in the cumulative elevated ALT (ehALT) group.

Since MAFLD is characterized by the presence of both NAFLD and metabolic dysfunction and encompasses conditions such as overweight or obesity, type 2 diabetes, or other metabolic disorders, the diagnosis relies on liver biopsy, imaging examinations, or blood tests for biomarkers indicating fatty liver[9,10].

In line with this, a recent study has suggested that normal ALT levels serve as a significant biomarker for predicting NAFLD. Moreover, it has been found that NASH or advanced fibrosis can be diagnosed in a considerable proportion of NAFLD patients, ranging from 37.5% to 59%, who have normal ALT levels. The authors' previous research has also indicated a correlation between a typical ALT trajectory and the risk of developing new-onset MAFLD, as observed in the cohort study. Based on these findings, the authors suggested that a specific ALT level, particularly a long-term high-normal ALT level, may be related with an increased hazard ratio for new-onset MAFLD development[11-14].

This study by Chen *et al*[8] utilized a population-based cohort to investigate the cumulative impact of elevated ALT levels and the risk of new-onset MAFLD. A key strength of this study lies in its assessment of optimal reference range for ALT and the utilization of various methods to determine the cumulative values of elevated ALT levels and weight cumulative impact.

On the contrary to prior research that predominantly focused on single ALT measurements or ALT trajectories, this study took a novel approach by reflecting the long-term quantitative cumulative impact of ALT employing a lifespan methodology. By doing so, the study aimed to understand better the association between elevated ALT levels and the risk of developing MAFLD. However, there are some limitations to this study, *i.e.*, the follow-up of the patients was relatively short, and the proportion of participants with consistently elevated ALT levels was low. Still, they are not fatal and can inform future research. Additionally, future randomized controlled trials are warranted to explore the efficacy of various lifestyle interventions, such as weight loss through dietary modifications and physical exercise, in improving long-term ALT levels among individuals with high-normal levels. Understanding the potential of these interventions in preventing MAFLD could provide valuable insights into disease prevention strategies. Based on all the study's strengths and weaknesses, we consider the paper to be reliable and the data are of utmost significance.

NOVEL INSIGHTS INTO MECHANISMS OF ACUTE LIVER FAILURE

The other paper of significance in the current issue of the *World Journal of Gastroenterology*, acknowledged by us, is the original paper by Zhou *et al*[15].

This article is extremely well presented and valuable, even though the article included a small number of patients and healthy subjects. The subject of this article is one of the most common causes of high mortality among patients with liver disease. These authors further discussed two interrelated processes, ferroptosis and pyroptosis, considering them as silent information regulators of sirtuin 1 (SIRT1) in the process of mediated deacetylation affecting apoptosis, cellular senescence, metabolism, oxidative stress and inflammation. The aim of this study was clearly stated. Still, we believe that further studies on a larger number of patients are needed to acquire practical applicability.

The authors explored the role of ferroptosis and pyroptosis in mouse ALF model (utilizing lipopolysaccharide/D-galactosamine-induced ALF). Their findings revealed that activation of the SIRT1 mitigated ALF by modulating the p53/glutathione peroxidase 4 (GPX4)/gasdermin D pathway, which facilitated the crosstalk between ferroptosis and pyroptosis. By elucidating the upstream regulatory mechanisms, our study established a connection between ferroptosis and pyroptosis in ALF. These results hold promise for identifying potential therapeutic targets for ALF.

Chen *et al*[16] discovered that the levels of p53 remained unaffected despite the loss of ACSL4 and GPX4, and p53-induced ferroptosis occurred independently of GPX4. However, increased GPX4 activity decreased p53 transcription in contrast to the Western blot findings. The described discrepancy between mRNA and protein levels implies that other processes protein levels: post-transcriptional regulation, translational effectiveness, and post-translational modifications. It is speculated that lower translational efficiency may be balanced by heightened transcriptional activity[16]. Nonetheless, the mechanism behind p53 transcription and translation differences remains unclear.

SIRT1 has been widely recognized for its defending role in nutrient deprivation, DNA repair, aging, oxidative stress, and inflammation[17,18]. Research suggests that suppressed SIRT1 negatively impacts pyroptosis (GSDMD) and exacerbates acute pro-inflammatory responses in the liver[19]. This observation aligns with another research indicating that SIRT1 is downregulated in APAP-induced hepatotoxicity[20]. Resveratrol, a small-molecule SIRT1 activator, used as a therapeutic agent demonstrates a protection against mouse liver ischemia-reperfusion injury[21,22].

The study by Zhou *et al*[15] used mainly mouse and cell models but not clinical settings. Although their research demonstrated reduced expression of SIRT1 in human ALF liver tissue, the effectiveness of SIRT1 activators in treating acute liver injury and failure remains uncertain. We have to mention the major limitation to this study: The authors focused only on cellular and mouse models, but not clinical studies. Further investigations are warranted to assess the safety and efficacy of SIRT1 activators in clinical settings. Comprehensive studies are required to evaluate the potential benefits and risks associated with SIRT1 activation as a therapeutic approach for ALF, ultimately paving the way for informed clinical decisions and developing novel treatment strategies.

CONCLUSION

The three discussed studies serve as a wake-up call, bringing attention to the complex mechanisms of autophagy in GI disorders, revealing how this knowledge could be employed in clinical practice. While the first article highlights the mechanisms of autophagy in GI disorders, further research is required to translate these findings into clinical practice. Regarding the observational study by Chen *et al*[18], we agree that persistent ehALT levels increase the risk of new-onset MAFLD development in all patients, therefore the detection and active measurements to reduce ehALT levels may prevent MAFLD. As for ALF, similar to MAFLD, both conditions present the significant challenges in diagnosis and treatment, and clinical and fundamental studies are required. However, the original data on SIRT1 activation for attenuation of ferroptosis and pyroptosis induced by lipopolysaccharide/D-galactosamine by suppressing the p53/GPX4/GSDMD signaling in ALF, is a significant breakthrough in the current knowledge. Innovative research is crucial to unraveling their underlying mechanisms and identifying novel therapeutic targets. Such efforts will improve patient outcomes and reduce the global burden of GI-related diseases.

FOOTNOTES

Author contributions: Velikova T and Gulinac M wrote the paper; Velikova T revised the paper; All authors approved the final version of the manuscript before submission.

Supported by the European Union-NextGenerationEU, through The National Recovery and Resilience Plan of The Republic of Bulgaria, No. BG-RRP-2.004-0008.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: Bulgaria

ORCID number: Milena Gulinac 0000-0001-7970-9378.

S-Editor: Li L
L-Editor: Ma JY
P-Editor: Cai YX

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2024 July 21; 30(27): 3278-3283

DOI: 10.3748/wjg.v30.i27.3278

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Gastric cystica profunda: Another indication for minimally invasive endoscopic resection techniques?

Harjot K Bedi, Douglas Motomura, Neal Shahidi

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Ji Y

Received: March 29, 2024
Revised: June 7, 2024
Accepted: June 24, 2024
Published online: July 21, 2024
Processing time: 103 Days and 14.7 Hours

Harjot K Bedi, Douglas Motomura, Neal Shahidi, Department of Medicine, University of British Columbia, Vancouver V6Z 2K5, BC, Canada

Corresponding author: Neal Shahidi, MD, PhD, Assistant Professor, Department of Medicine, University of British Columbia, 770-1190 Hornby Street, Vancouver V6Z 2K5, BC, Canada. nealshahidi@gmail.com

Abstract

Gastric cancer presents a significant global health burden, as it is the fifth most common malignancy and fourth leading cause of cancer mortality worldwide. Variations in incidence rates across regions underscores the multifactorial etiology of this disease. The overall 5-year survival rate remains low despite advances in its diagnosis and treatment. Although surgical gastrectomy was previously standard-of-care, endoscopic resection techniques, including endoscopic mucosal resection and endoscopic submucosal dissection (ESD) have emerged as effective alternatives for early lesions. Compared to surgical resection, endoscopic resection techniques have comparable 5-year survival rates, reduced treatment-related adverse events, shorter hospital stays and lower costs. ESD also enables *en bloc* resection, thus affording organ-sparing curative endoscopic resection for early cancers. In this editorial, we comment on the recent publication by Geng *et al* regarding gastric cystica profunda (GCP). GCP is a rare gastric pseudotumour with the potential for malignant progression. GCP presents a diagnostic challenge due to its nonspecific clinical manifestations and varied endoscopic appearance. There are several gaps in the literature regarding the diagnosis and management of GCP which warrants further research to standardize patient management. Advances in endoscopic resection techniques offer promising avenues for GCP and early gastric cancers.

Key Words: Early gastric cancer; Endoscopy; Endoscopic submucosal dissection; Gastric cystica profunda

Core Tip: Gastric cystica profunda is a rare pseudotumour with risk of progression to gastric cancer. In addition to endoscopic visual assessment, endoscopic ultrasound and computed tomography of the abdomen should be used to investigate the depth and lymph node invasion depending on the lesion morphology. Endoscopic resection, specifically endoscopic submucosal dissection, can be an effective management strategy.

Citation: Bedi HK, Motomura D, Shahidi N. Gastric cystica profunda: Another indication for minimally invasive endoscopic resection techniques? *World J Gastroenterol* 2024; 30(27): 3278-3283
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3278.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3278

INTRODUCTION

Gastric cancer represents a significant global health challenge, ranking as the fifth most common malignancy and the fourth leading cause of cancer deaths worldwide in 2020[1,2]. The International Agency for Research on Cancer projected a rise in the annual burden of gastric cancer to 1.8 million new cases and approximately 1.3 million deaths by 2040 globally[3]. There exists considerable global variation in gastric cancer incidence, with the highest rates observed in Asia and Eastern Europe, and a predilection for men over women. Sung *et al*[1] suggested a 40-fold difference in gastric cancer incidence between Eastern Asia (*e.g.* Japan and Mongolia, which have the highest incidence) and North America and North Europe (which have the lowest incidence). This variability is likely influenced by multiple factors, including environmental, genetic, dietary, and infectious elements, such as variations in *Helicobacter pylori* (*H. pylori*) prevalence and virulence[1].

The overall 5-year survival rate for gastric cancer patients globally stands at approximately 20%-30%[4]. However, notably higher survival rates, such as 67% in Japan and 69% in Korea, have been observed, largely attributed to robust screening programs facilitating detection of early gastric cancer (EGC) and their treatment[5,6]. Gastric cancers can be categorized based on their topography, with the majority occurring in the distal stomach (non-cardia), often associated with *H. pylori*, while those located in the proximal stomach (cardia) tend to correlate with increased alcohol consumption, smoking, and obesity[7-9].

The Japanese Society for Gastroenterological Endoscopy defines EGC as cancer that does not invade gastric layers deeper than the submucosa, regardless of lymph node involvement[10]. Histopathological exam is the gold standard for diagnosis of early EGC[11]. However, with improved endoscopic technologies, including white light endoscopy, magnifying endoscopy with narrow band imaging, and chromoendoscopy, the detection, optical analysis, and diagnosis of EGCs has improved[12]. Computed tomography (CT) or endoscopic ultrasound (EUS) are used for staging and determination of presence of lymph nodes. Gastrectomy with lymph node dissection was the standard of treatment for EGC with a 5-year overall survival rate of > 97%[13]. However, endoscopic resection techniques, including endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD), have emerged as accepted standards, particularly for patients without lymph node involvement or those meeting absolute indications per Japanese guidelines[13,14].

ENDOSCOPIC RESECTION TECHNIQUES

EMR, pioneered in Japan in the 1990s, initially targeted early gastric lesions detected through screening efforts and community awareness, proving effective as an *en bloc* resection technique for lesions under 15 mm[15]. However, larger lesions necessitated piecemeal resection, hindering margin evaluation. Thus, ESD was developed in the early 2000s and enabled *en bloc* resection of larger lesions (≥ 20 mm) by using an electrosurgical knife facilitating dissection in the submucosal plane[15]. A retrospective cohort study comparing ESD and gastrectomy for EGC or severely dysplastic lesions found that gastrectomy was associated with significantly longer operative time (265 minutes *vs* 89.6 minutes, $P < 0.001$) and length of hospital stay (9.9 days *vs* 3.0 days, $P < 0.001$)[16]. There was no difference in mortality, but gastrectomy was also associated with higher overall complication rate[16]. Jeon *et al*[17] compared ESD with surgery for EGC demonstrated similar 5-year overall survival rates (96.5% *vs* 99.1%, $P = 0.125$), and disease-specific survival rates (100% *vs* 99.1%). However, the disease-free survival rate (90.3% *vs* 98.0%, $P = 0.002$), and recurrence-free survival rates (95.1% *vs* 98.0%, $P < 0.001$) were lower in ESD compared to surgery[17]. A retrospective analysis comparing EMR *vs* gastrectomy for intramucosal gastric cancer found no difference in the risk of death (HR 1.30, 95%CI: 0.87-2.23) or recurrence (HR 1.18, 95%CI: 0.22-6.35)[18]. EMR was associated with shorter length of hospital stay (median 8 days *vs* 15 days, $P < 0.001$), and cost of care ($2049 *vs* $4042, $P < 0.001$), compared to surgery[18]. However, EMR was associated with a significantly higher risk of metachronous gastric cancers (HR 6.72, 95%CI: 2.00-22.58) which were also treated with EMR without any effect on overall survival[18]. Two additional retrospective studies comparing endoscopic resection *vs* surgery for patients with EGC found comparable 5-year overall survival[14,19]. There were higher rates of metachronous gastric cancers in patients treated with endoscopic resection which were successfully treated with endoscopic resection [14,18,19]. In summary, endoscopic resection techniques offer safe, effective, and minimally invasive alternatives to surgical resection, with comparable 5-year survival rates, lower adverse event rates, shorter hospital stays, and reduced costs[13,14,20].

Endoscopic resection techniques, including ESD and EMR, have different strengths in management of EGCs. In a meta-analysis comparing ESD and EMR for EGC, ESD was superior to EMR for "*en bloc*" and histological complete resection (OR = 9.69, $P < 0.001$ and OR = 5.66, $P < 0.001$, respectively)[21]. ESD is also associated with lower recurrence rates (OR = 9.69, $P < 0.001$[21]). However, ESD is also associated with higher complications, including higher perforation rates (OR = 0.09, $P < 0.001$) and a longer operating time (mean difference = 1.73, $P = 0.005$) with no difference in adverse events related to bleeding[21]. Additionally, a Japanese multicenter prospective cohort study compared 5-year overall survival in patients with EGC which were categorized by tumour differentiation, tumour staging, size, and ulceration and received endoscopic resection, with ESD in 99.6% and EMR in the remaining patients[22]. They reported a 5-year overall survival rates of 89% (95%CI: 88.3%-89.6%) in patients undergoing endoscopic resection which was similar to those who underwent surgical resection for EGC. Additionally, there was no difference in hazard ratio between the various characterizations of EGC, affirming the efficacy of these techniques[22]. A prospective study evaluated ESD of gastrointestinal lesions in 10 centers from the United States of America and Canada and found high proportions of *en bloc*, R0 and curative resections in 91.5%, 84.2%, and 78.3%, respectively[23]. These proportions are similar to proportions identified in a meta-analysis and systematic review, of 95%, 89%, and 82% for *en bloc*, R0 and curative resections, respectively, in Eastern studies[24]. Daoud *et al*[24] did report lower proportions of all resection markers in studies from Western countries. However, all included studies pre-dated the prospective study by Draganov *et al*[23], likely representing an improvement in ESD techniques after its adoption in Western countries. In a recent article, Kim[25] proposed that quality indicators, such as *en bloc*, complete, and curative resection rates of > 95%, > 90% and > 80%, respectively, should be implemented by all endoscopists for ESD for EGC to improve patient outcomes.

Recognizing the effectiveness of endoscopic resection, the Japanese Gastroenterological Endoscopy Society (JGES) established it as a safe and effective strategy, outlining absolute and expanded criteria for ESD[26]. Both the absolute and expanded indication lesions are presumed to have a < 1% risk of lymph node metastasis. This approach has been adopted by the American Society of Gastrointestinal Endoscopy (ASGE) and the European Society of Gastrointestinal Endoscopy (ESGE) for managing EGC in Western populations[27,28].

GASTRIC CYSTICA PROFUNDA: WORKUP AND MANAGEMENT

In this issue, Geng *et al*[29] described their outcomes for the endoscopic management of gastric cystica profunda (GCP); which is a rare, gastric lesion initially identified by SCOTT and Payne[30]. The etiology of GCP remains unclear, with some studies suggesting associations with prior gastric insults, such as surgery or endoscopic procedures, with others finding no such correlation[31-34]. Only 12.5% of patients were noted to have a previous endoscopic or surgical treatment, suggesting a lack of association between previous gastric insults with GCP development[29]. In addition, geographic variation of GCPs is heterogenous worldwide, with most reports of GCP diagnosis and management from China[29,31,35], and only a few reports from Japan[36,37], Turkey[34], Pakistan[38], and United States[39,40]. Clinical presentations also vary and may include epigastric abdominal pain, belching, regurgitation, gastrointestinal bleeding, weight loss, or anorexia, though many patients remain asymptomatic and are diagnosed incidentally during endoscopy; such as in 63.5% of patients in Geng *et al*[29] study. They performed a single-center retrospective review of 104 patients with GCP who underwent endoscopic resection[29]. Endoscopically, GCP manifests as a tumour-like gastric lesion or polyp, characterized histologically by cystic dilations of the gastric glands and hyperplasia of connective tissue extending into the submucosa of the stomach[41]. Geng *et al*[29] demonstrated that 99% of GCP exhibited intraluminal growth and 74% had regular morphology. Most studies report GCP presenting as submucosal lesions, mimicking gastrointestinal stromal tumour[42], with fewer reports of GCP presenting with gastrointestinal bleeding[43] or malignancy[44].

Diagnosing GCP can be challenging, as biopsies obtained during endoscopy generally capture only the superficial mucosa. EUS may aid in identifying GCPs and determining their depth of invasion, with multiple anechoic cysts in the submucosa being a consistent EUS finding[39]. Although EUS is reliable for investigation of depth of most small and non-ulcerated gastric lesions, it has been shown to have poor accuracy determining the depth of gastric lesions greater than 20mm, those with ulceration, and/or non-flat morphology, which usually make up the expanded criteria outlined by JGES[45,46]. CT of the abdomen can assist in identifying associated lymph nodes and metastasis[47]. Despite the availability of various diagnostic techniques, there are no clear guidelines on the approach to diagnosing GCP. It is, therefore, reasonable that in a patient where GCP is suspected, EUS should be performed, for histopathology and depth of invasion evaluation. Moreover, CT should be performed, specifically in the presence of high-risk lesions, such as those with ulceration, irregular morphology, diameter > 20 mm, or IIc morphology. Although the role of optical assessment has not been established for GCP, Geng *et al*[29] found that irregular and ulcerated lesions in the cardia, IIa + IIc morphology were shown to be significantly associated with GCP with EGC compared to those without gastric cancer. Multidisciplinary team discussion, ideally at centers with expertise in minimally invasive endoscopic resection techniques and involving endoscopists, surgeons, radiologists, and pathologists, is recommended to optimize patient management.

Despite their benign nature, GCPs harbour malignant potential. Geng *et al*[29] reported a 59.6% association between GCP and EGC, consistent with 66.67% reported in a previous study[48]. Consequently, resection is warranted, with endoscopic resection preferred over surgical gastrectomy, particularly for benign lesions lacking deeper invasion. While no trials have compared various endoscopic resection techniques specifically for GCPs, ESD likely supersedes EMR due to its high rate of *en bloc* and histologically complete resection, especially given the risk of malignant potential[14,20]. Jiang *et al*[49] compared curative resection rates, rates of local recurrence, metachronous gastric cancers, and mortality in patients who underwent gastric ESD for GCP compared to gastric ESD for other indications and did not find any difference in their retrospective review. Almost 77% of patients in the aforementioned study underwent ESD. They

reported *en bloc* and complete resection in 91.3% of cases. Five cases had recurrence, although the initial resection modality was not specified. We suggest ESD for resection of GCP, especially larger lesions with irregular morphology or lateral spreading, as ESD facilitates more thorough post-resection assessment of surrounding and deeper layers to ensure complete resection, making it preferable despite its longer procedural time and higher risk of perforation compared to EMR. ESD can also enable resections of gastric lesions in patients who are comorbid or too frail for surgery, therefore, expanding its role in management of GCP and EGC[50].

CONCLUSION

A dearth of literature exists regarding the diagnosis and management of GCPs, likely attributed to their low incidence, with the majority of reported cases and series originating from the Eastern regions. This discrepancy could reflect underdiagnosis in Western populations rather than geographic prevalence variations. Consequently, additional studies elucidating endoscopic appearances and features on EUS or CT would enhance diagnostic accuracy. ESD should be the management of choice for EGCs given their superiority in *en bloc*, complete and curative resection over EMR, and equivalent success in both Eastern and Western settings. Moreover, centralizing endoscopic GCP management in expert centers could optimize patient outcomes. Integrating GCP management into the endoscopic resection criteria for gastric lesions outlined by ASGE, ESGE, and JGES would promote standardized management protocols.

FOOTNOTES

Author contributions: Bedi HK designed and drafted the article; Motomura D and Shahidi N performed critical revision of the article for important intellectual content; Shahidi N approved the final article.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Country of origin: Canada

ORCID number: Harjot K Bedi 0000-0002-1328-9152; Neal Shahidi 0000-0002-4536-0515.

S-Editor: Qu XL
L-Editor: A
P-Editor: Zheng XM

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World Journal of Gastroenterology

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World J Gastroenterol 2024 July 21; 30(27): 3284-3289

DOI: 10.3748/wjg.v30.i27.3284

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Pro and anti-inflammatory diets as strong epigenetic factors in inflammatory bowel disease

Adele Rostami, Kristen White, Kamran Rostami

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade B
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Ye X

Received: April 7, 2024
Revised: June 16, 2024
Accepted: June 28, 2024
Published online: July 21, 2024
Processing time: 95 Days and 1.9 Hours

Adele Rostami, Digestive Health Clinic, Palmerston North 4410, New Zealand

Kristen White, Digestive Health Clinic & Kōtare Wellness Ltd, Palmerston North 4410, New Zealand

Kamran Rostami, Department of Gastroenterology, Palmerston North Hospital, Palmerston North 4442, New Zealand

Corresponding author: Kamran Rostami, FRACP, MD, PhD, Doctor, Research Scientist, Department of Gastroenterology, Palmerston North Hospital, 50 Ruahine Street, Roslyn, Palmerston North 4442, New Zealand. kamran.rostami@midcentraldhb.govt.nz

Abstract

Inflammatory bowel disease (IBD) is the consequence of a complex interplay between environmental factors, like dietary habits, that alter intestinal microbiota in response to luminal antigens in genetically susceptible individuals. Epigenetics represents an auspicious area for the discovery of how environmental factors influence the pathogenesis of inflammation, prognosis, and response to therapy. Consequently, it relates to gene expression control in response to environmental influences. The increasing number of patients with IBD globally is indicative of the negative effects of a food supply rich in trans and saturated fats, refined sugars, starches and additives, as well as other environmental factors like sedentarism and excess bodyweight, influencing the promotion of gene expression and increasing DNA hypomethylation in IBD. As many genetic variants are now associated with Crohn's disease (CD), new therapeutic strategies targeting modifiable environmental triggers, such as the implementation of an anti-inflammatory diet that involves the removal of potential food antigens, are of growing interest in the current literature. Diet, as a strong epigenetic factor in the pathogenesis of inflammatory disorders like IBD, provides novel insights into the pathophysiology of intestinal and extraintestinal inflammatory disorders.

Key Words: Inflammatory bowel disease; Epigenetic; Anti-inflammatory diet; Immunogenetics; Microbiome; Polymeric diet; Elemental diet

Core Tip: This editorial highlights the implication of environmental factors, in particular diet, as epigenetic factors in pathogenesis of inflammatory bowel disease (IBD). The concept of epigenetic factors involved in the genesis of IBD brings new insight into the identified risk factors and future targeted approaches, as a guide to the prevention and treatment of IBD.

Citation: Rostami A, White K, Rostami K. Pro and anti-inflammatory diets as strong epigenetic factors in inflammatory bowel disease. *World J Gastroenterol* 2024; 30(27): 3284-3289
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3284.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3284

INTRODUCTION

Epigenetic represent alteration in an organism's phenotype that persists through mitosis and meiosis[1], for example how the environment can change the way the body reads DNA sequences without changing the DNA itself. These factors are mainly involved in regulating innate and adaptive immunity, as well as maintaining intestinal epithelial barrier function [2]. The term "epigenetics" was introduced in 1942 by Waddington[3], to explain how a phenotype might be produced by the interaction between genes and environmental influences.

DIET AS EPIGENETIC FACTOR IN INFLAMMATORY BOWEL DISEASE

As highlighted in a study by Marangoni *et al*[4] published in *World J Gastroenterol*[4], DNA methylation is an essential remodeling process in the control of genetic information, which contributes to the epigenetics by regulating gene expression. DNA methylation and histone modifications are two central epigenetic mechanisms that impact gene transcription and cell fate. The authors emphasize the role of diet, gut microbiota composition, and exercise in activation and modification of epigenetic mechanisms through the individual's genetic inheritance[4,5]. An anti-inflammatory diet, by acting on gut microbiota composition, can induce phenotype changes through gene expression without changing the genetic sequence. Nutrition is a powerful convertible factor, acting directly on DNA methylation pathways. For example, diets deficient in methyl donors and proteins may cause global DNA hypomethylation, or high-fat diets may result in changes in DNA methylation[6].

Nutrition affects the epigenetic regulation of DNA methylation in several possible epigenetic pathways: Mainly, by altering the substrates and cofactors that are necessary for proper DNA methylation; additionally, by changing the activity of enzymes regulating the one-carbon cycle; and lastly, by playing a role in several possible mechanisms related to DNA demethylation activity[7].

Histone modifications are highly dynamic and respond to various environmental cues, such as dietary compounds, and have been found to alter the epigenome which impacts gene expression. A pro-inflammatory diet disrupts the balance between histone acetyltransferase and histone deacetylase activities, and when this balance is disrupted, it has a repressive action on the gene expression regulation network in cancer and inflammation[8,9].

Marangoni *et al*[4] also highlights the impact of non-coding RNA molecules that play a crucial role in the gene transcription and translation by non-coding RNAs[4]. Non-coding RNAs are RNAs not involved in protein translation, and they are divided into two categories by size, which include short and long non-coding RNAs[10]. They have essential roles in epigenetic modifications, regulating gene expression and chromatin remodeling. It is also envisaged that silencing of repeats in the genome is mediated by small RNAs[11].

In a study by Glória *et al*[12], DNA global hypomethylation profile[12] was increased in rectal mucosa of active and inflamed ulcerative colitis (UC) patients, supporting epigenetic and kinetic changes that might predispose these individuals to develop colorectal neoplasms[12]. This explains why inflammatory bowel disease (IBD) is at high risk for developing malignancy long term. Dysbiosis, following exposure to an inflammatory diet and other environmental factors, leads to the activation of IBD genes *via* hypomethylation and histone modification. Westernized dietary risk factors like deficiencies in micronutrients, and being rich in ultra-processed foods, additives, and emulsifiers[13] are implicated in reducing bacterial diversity and promoting an inflammatory response[4,14] (Figure 1).

The DNA methylation is a widely studied, heritable epigenetic alteration in animals, involving the covalent transfer of a methyl group to the C-5 position of the cytosine ring of DNA-by-DNA methyltransferases. Gene expression will inactivate, either because proteins bind to the methylated cytosine phosphate guanin island and initiate DNA compensation and inactivation, or methylation itself blocks the DNA sequence and transcription factors are unable to bind[15].

The consequences of dysbiosis are often systemic immune dysregulations in the form of pathogenic autoantibodies *via* activation of chronic inflammatory cells that ultimately result in a wide range of clinical manifestations, including skin rashes and arthritis in inflammatory conditions[16].

An emerging body of evidence suggests nutritional epigenetics as a novel mechanism underlying gene–diet interactions. The bioactive compounds of nutrients impart advantageous environments, such as homeostatic inflammation, through differential gene expression[17]. Dietary habits therefore shape the gut microbiota and influence the interaction with the immune system depending on the diet component. For instance, medical nutrition therapy using an elemental

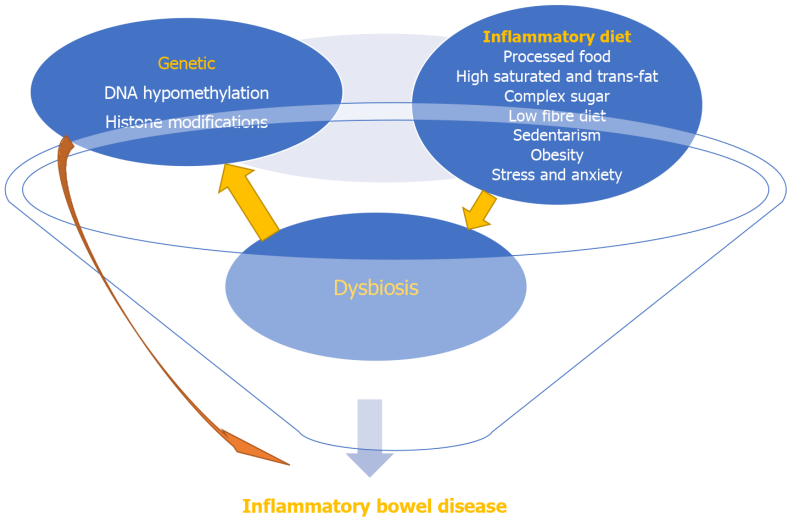

Figure 1 Epigenetic factors turning the inflammatory bowel disease genes on.

formula in conjunction with a LOFFLEX[18,19] diet in active Crohn's disease (CD), restores the intestinal microflora by preventing the growth of potentially pathogenic bacteria, thus promoting an anti-inflammatory action. However, this is not valid with polymeric formula due to its exponentially high fat (long chain triglycerides which is considered colitogenic)[20], refined sugar content[21] and other antigenic components, such as whole protein casein and soy[22], with colitogenic and antigenic properties[23]. Based on studies and evidence above, polymeric diet seems unsafe to use in active CD (Figure 2).

Environmental factors like industrialization, urbanization and antibiotic/nonsteroidal anti-inflammatory drugs usage are not only involved in the development of Inflammatory bowel disease, but also the course of prognosis and severity of IBD manifestation. These factors are implicated in rising incidence of CD in newly industrialized countries like Africa, Asia, and South America[24]. Environmental factors promote histone methylation or histone demethylation resulting in epigenetic modifications, that have the power to reduce or bolster gene expression, especially because of altering chromatin structure[2,25].

A histone is a protein that helps to comprise the structure of chromatin, which is composed of DNA-wrapped protein octamers[2]. Various amino acids on the histone tails, namely lysine, arginine, serine, and threonine, are epigenetically altered by enzymes, which then influences if a gene is accessible for binding by transcription factors and the RNA polymerase II machinery[15].

A rise in IBD in the Western world during the 20th century has been reported[26]. In Asia, Africa, and Latin America, few IBD were reported in the 20th century[26], but this has steadily increased during the 21st century[24] and does not appear to have peaked yet[27]. Environmental exposures associated with the westernization of societies are found to be the primary factor for these trends[28]; especially early life exposures that can alter the diversity, composition and function of intestinal microbiome that may lead to the development of IBD later in life[28].

In addition to diet, other environmental factors described in the literature include mode of childbirth, breastfeeding, urban environments, air pollution, and use of antibiotics/contraception and nonsteroidal anti-inflammatory drugs[29-31]. This may explain why one member of identical twins develops IBD and the other member may stay IBD-free despite sharing the same genetic background. Epigenetic modifications influenced by environmental factors, might help to understand the increasing IBD incidence.

Where patients have genetic susceptibility for IBD, modulating these environmental exposures can potentially prevent the development of IBD in the future[27].

For instance, diets high in fibre[32] and low in ultra-processed foods[13,33], exercise and mindfulness are all protective against gut inflammation and can potentially turn off the manifestation of disease in genetically predisposed individuals.

In a consensus statement produced by a group of experts from the Organization for the Study of Inflammatory Bowel disease, recommendations also include screening, at diagnosis and during flare-ups, for a patient's mental well-being and excluding psychosocial stressors and symptoms of depression and anxiety[34]. Regular physical exercise and healthy weight maintenance, as well as screening for obesity and nutritional deficiencies[34], are also advised. Tobacco smoke and long term, frequent use of high dose non-steroidal anti-inflammatory drugs should be avoided. Breastfeeding is encouraged[34], as breastfeeding for more than 12 months was found to be 7 times more protective against the development of UC[27]. As reported by Ng *et al*[24], a New Zealand study found breastfeeding to be protective against both UC and CD[24]. Evidence-based anti-inflammatory diets, in form of nutrition therapy of active IBD, should be encouraged[34], as this plays a significant role in removing possible food antigens with pathogenic potential in IBD and improves the long-term prognosis[18].

Westernized diets high in trans and saturated fats, refined carbohydrates and animal proteins are found to cause an imbalance in gut microflora with an increase in pathogenic bacteria[35]. Ultra-processed foods, additives and emulsifiers can also increase inflammatory mechanisms through increased intestinal permeability and a reduction in bacterial diversity[14]. In contrast, fresh, whole foods produce short chain fatty acid (SCFA) bacteria, that promote gut health and

Elemental	Polymeric
Simple amino acids – absorbed through simple diffusion → no antigen presentation to lamina propria	Whole proteins – in the form of **casein (milk)** and **soy** increases rate of transcytosis → **increase antigen load to lamina propria** → inflammation
Monosaccharides	**Polysaccharides - corn syrup *via*** depletion of luminal short-chain fatty acids
Lower fat content – makes up 2%-3% of calories	**Higher fat content** – makes up **30% of calories** (long chain triglycerides) ↑Faecal endotoxin ↓Mucin production
Restore the Microbiota	Colitogenic
Polymeric diet seems to be unsafe in active Crohn's disease	

Figure 2 Elemental *vs* polymeric.

protect against malignancy[14].

We should emphasize that similarly, high fibre diets also produce SCFA through the fermentation of dietary fibre by gut bacteria and have been shown to reduce inflammation, modulate gut microbiota, improve gut barrier function and thus protect against IBD[32,36].

Studies on smoking and antibiotic use in the western world compared to Asia, have demonstrated that variation of populations and ethnicity may make some people more vulnerable to certain environmental factors, whereas, in contrast, other environmental factors may be preventative in others[27].

CONCLUSION

Epigenetic mechanisms mediate the interactions between genome and environment. Pro and anti-inflammatory diets are strong epigenetic factors implicated in IBD pathogenesis and treatment. Future studies are needed to enrich our insight into the manipulation of environmental factors for the prevention and optimal treatment of IBD.

FOOTNOTES

Author contributions: Rostami A, White K and Rostami K reviewed the literature and wrote the editorial.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: New Zealand

ORCID number: Kamran Rostami 0000-0002-2114-2353.

Corresponding Author's Membership in Professional Societies: NZSG, 397.

S-Editor: Li L
L-Editor: A
P-Editor: Zheng XM

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2024 July 21; 30(27): 3290-3303

DOI: 10.3748/wjg.v30.i27.3290

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Case Control Study

Targeted metabolomics study of fatty-acid metabolism in lean metabolic-associated fatty liver disease patients

Pei-Qi Sun, Wen-Min Dong, Yi-Fu Yuan, Qin Cao, Xiao-Yan Chen, Li-Li Guo, Yuan-Ye Jiang

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C, Grade C

Novelty: Grade A, Grade B, Grade B

Creativity or Innovation: Grade A, Grade B, Grade B

Scientific Significance: Grade B, Grade B, Grade B

P-Reviewer: Capone D, Italy; Soriano-Ursúa MA, Mexico; Sukocheva OA, Australia

Received: January 22, 2024

Revised: May 9, 2024

Accepted: June 6, 2024

Published online: July 21, 2024

Processing time: 170 Days and 20.7 Hours

Pei-Qi Sun, Yi-Fu Yuan, Qin Cao, Xiao-Yan Chen, Yuan-Ye Jiang, Department of Gastroenterology, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200062, China

Wen-Min Dong, Department of Pharmacy, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai 201399, China

Li-Li Guo, Central Laboratory, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China

Co-first authors: Pei-Qi Sun and Wen-Min Dong.

Co-corresponding authors: Li-Li Guo and Yuan-Ye Jiang.

Corresponding author: Yuan-Ye Jiang, Doctor, Doctor, Department of Gastroenterology, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, No. 164 Lanxi Road, Shanghai 200062, China. yuanye1014@126.com

Abstract

BACKGROUND

The annual incidence of metabolic-associated fatty liver disease (MAFLD) in China has been increasing and is often overlooked owing to its insidious characteristics. Approximately 50% of the patients have a normal weight or are not obese. They are said to have lean-type MAFLD, and few studies of such patients are available. Because MAFLD is associated with abnormal lipid metabolism, lipid-targeted metabolomics was used in this study to provide experimental evidence for early diagnosis and pathogenesis.

AIM

To investigate the serum fatty-acid metabolic characteristics in lean-type MAFLD patients using targeted serum metabolomic technology.

METHODS

Between January and June 2022, serum samples were collected from MAFLD patients and healthy individuals who were treated at Shanghai Putuo District Central Hospital for serum metabolomics analysis. Principal component analysis and orthogonal partial least squares-discriminant analysis models were developed, and univariate analysis was used to screen for biomarkers of lean-type

MAFLD and analyze metabolic pathways. UPLC-Q-Orbitrap/MS content determination was used to determine serum palmitic acid (PA), oleic acid (OA), linoleic acid (LA), and arachidonic acid (AA) levels in lean-type MAFLD patients.

RESULTS

Urea nitrogen and uric acid levels were higher in lean-type MAFLD patients than in healthy individuals ($P < 0.05$). Alanine transaminase and cholinesterase levels were higher in lean-type MAFLD patients than in healthy individuals ($P < 0.01$). The expression of high-density lipoprotein and apolipoprotein A-1 were lower in lean-type MAFLD patients than in healthy individuals ($P < 0.05$) and the expression of triglycerides and fasting blood glucose were increased ($P < 0.01$). A total of 65 biomarkers that affected the synthesis and metabolism of fatty acids were found with $P < 0.05$ and variable importance in projection > 1". The levels of PA, OA, LA, and AA were significantly increased compared with healthy individuals.

CONCLUSION

The metabolic profiles of lean-type MAFLD patients and healthy participants differed significantly, yielding 65 identified biomarkers. PA, OA, LA, and AA exhibited the most significant changes, offering valuable clinical guidance for prevention and treatment of lean-type MAFLD.

Key Words: Lean-type metabolic-associated fatty liver disease; Targeted serum metabolomics; Fatty acids; Principal component analysis; Orthogonal partial least squares-discriminant analysis

Core Tip: This study targeted the serum metabolomics of healthy individuals and metabolic-associated fatty liver disease (lean-type MAFLD), screened biomarkers and related metabolic pathways, and conducted targeted quantitative analysis of their specific biomarkers with the aim of providing experimental evidence for the early diagnosis and pathogenesis of lean-type MALFD.

Citation: Sun PQ, Dong WM, Yuan YF, Cao Q, Chen XY, Guo LL, Jiang YY. Targeted metabolomics study of fatty-acid metabolism in lean metabolic-associated fatty liver disease patients. *World J Gastroenterol* 2024; 30(27): 3290-3303
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3290.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3290

INTRODUCTION

The annual prevalence of metabolic-associated fatty liver disease (MAFLD) in China has been increasing, with the current rate exceeding 30%[1-4]. Owing to subtle early manifestations that often go unnoticed, approximately 20% of MAFLD patients progress to metabolic-associated steatohepatitis, fibrosis, cirrhosis, and liver cancer[5,6]. MAFLD is closely associated with obesity and type 2 diabetes, but approximately 40.8% of MAFLD patients have a body mass index (BMI) that does not meet the criteria for overweight or obesity[7-9]. This condition is commonly referred to as lean or nonobese-type MAFLD. Compared with other ethnic groups, the prevalence of metabolic disorders is higher in Asians with lower BMIs[10].

Because studies of lean-type MAFLD are limited, its pathogenesis and optimal treatment are not clear. Patients with lean-type MAFLD are at increased risk of progressing to fatty liver inflammation and liver fibrosis, with an incidence of 30%, and it is closely associated with metabolic dysfunction[11]. Metabolomics is a high-throughput detection method widely used for disease diagnosis and in mechanistic investigations[12]. In this study, we used ultra-high-performance liquid chromatography-tandem mass spectrometry with an electrospray ionization quadrupole trap analyzer to identify serum metabolic markers that distinguished lean-type MAFLD patients from healthy individuals. We aimed to identify metabolic pathways specific to those markers and to conduct a targeted investigation of the metabolites and pathways that were significantly changed in lean-type NAFLD. This study aimed to provide experimental evidence for the early diagnosis and pathogenesis of lean-type MAFLD.

MATERIALS AND METHODS

Study participants

Between January 2022 and June 2022, 20 patients diagnosed with lean-type MAFLD were recruited from the gastroenterology department of the Central Hospital of Putuo District, Shanghai. The control group included 20 healthy volunteers who were recruited after physical examination. General information and clinical data, including a complete

blood count, liver function, renal function, and a lipid profile, were collected for the analysis. The research protocol was approved by the Ethics Committee of the Central Hospital of Putuo District (Affiliated to the Putuo Hospital of Shanghai University of Traditional Chinese Medicine; Approval No. PTEC-R-2020-29-1). All the enrolled patients provided informed consent before participating in the study.

Diagnostic criteria

MAFLD was diagnosed following the clinical criteria included in the 2010 Guidelines for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease of the Chinese Society of Hepatology, Chinese Medical Association[11]. The criteria for MAFLD were: (1) No history of excessive alcohol consumption or an equivalent ethanol intake of < 140 g per week for men (< 70 g per week for women); (2) Absence of specific diseases, including viral hepatitis, drug-induced liver disease, hepatolenticular degeneration, autoimmune liver diseases that can cause fatty liver, or use of total parenteral nutrition; and (3) Histopathological changes in liver biopsy specimens consistent with the pathological diagnosis criteria for fatty liver disease.

MAFLD was defined by: (1) Liver imaging findings consistent with the diagnosis of diffuse fatty liver and exclusion of other causes; and (2) Manifestations related to metabolic syndrome with persistently elevated levels of serum alanine transaminase (ALT), aspartate transaminase, or gamma-glutamyl transferase for > 6 months. Individuals with abnormal enzyme profiles and a fatty liver on imaging and return to normal or improve after weight loss and decreased insulin resistance fulfill the diagnostic criteria of MAFLD. The diagnostic criteria for MAFLD included a BMI of < 23 for lean-type, 23 ≤ BMI < 28 for overweight-type, and ≥ 28 for obese-type.

Inclusion criteria

Patients who met the following criteria were included in the study: (1) Between 16 years and 75 years of age, regardless of sex; (2) Meeting the diagnostic criteria for lean-type MAFLD according to Western medical standards[13]; (3) Having complete and reliable clinical data, biochemical tests, and specimen collection; and (4) Providing consent to participate.

Exclusion criteria

The exclusion criteria were: (1) Presence of concomitant liver-extrinsic fibrotic diseases, including systemic lupus erythematosus, rheumatic diseases, renal failure, chronic obstructive pulmonary disease; (2) Presence of severe primary diseases related to cardiovascular, cerebrovascular, urinary, renal, hematopoietic systems, malignant tumors, other serious complications, or psychiatric disorders; (3) Presence of thyroid disorders, including hyperthyroidism, hypothyroidism, subclinical hypothyroidism, and Hashimoto's thyroiditis; and (4) Lack of complete clinical data relevant to the study.

Materials

Ultra-high-performance liquid chromatography (Ultimate 3000; Thermo Fisher Scientific, Waltham, MA, United States); high-resolution mass spectrometry (Orbitrap Elite; Thermo Fisher Scientific); cryogenic high-speed centrifuge (1730R; Mr. Gene GmbH, Regensburg, Germany); ultrapure water system (Milli-Q; Merck Biotechnology Shanghai Co., Ltd., Shanghai, China); multi-tube vortex oscillator (VX-II; Beijing Tajin Technology Co., Ltd., Beijing, China); methanol (HPLC grade, Batch No.: O0621152; China National Pharmaceutical Group Chemical Reagent Co., Ltd., Shanghai, China); methyl tert-butyl ether (analytical grade, Batch No. 20210227; China National Pharmaceutical Group Chemical Reagent Co., Ltd.); formic acid (chromatography grade, Batch No.: D1290265; Shanghai ANPEL Scientific Instrument Co., Ltd., Shanghai, China); ammonium acetate (chromatography grade, Batch No. BCBR1129V; Shanghai ANPEL Scientific Instrument Co., Ltd.); isopropanol (chromatography grade, Batch No. V589K144; Shanghai ANPEL Scientific Instrument Co., Ltd.); acetonitrile (chromatography grade, Batch No. K30217281 Shanghai ANPEL Scientific Instrument Co., Ltd.).

Metabolomics study

Serum handling: The study included 20 patients with lean-type MAFLD and 20 healthy volunteers. Morning fasting venous blood (10 mL) was collected and allowed to stand at 4 °C for 2 h before centrifuging at 3000 rpm for 15 minutes. Serum (50 μL) was collected from the upper layer. Serum samples were mixed with 200 μL methanol and vortexed for complete extraction. After low-temperature centrifugation at 14000 rpm and 4 °C for 10 minutes, the supernatant was transferred to a sample vial for analysis.

Chromatographic conditions: Column: C_{18} chromatographic column (Hypersil Gold C_{18}, 100 mm × 2.1 mm, 1.9 μm); flow rate: 0.3 mL/min; column temperature: 40 °C; mobile phase composition: A: Pure water + 0.1% formic acid and B: Acetonitrile + 0.1% formic acid. Gradient elution program: 0-2 minutes, 95% A; 2-12 minutes, 5%-95% A; 12-15 minutes, 5%-95% A; 15-17 minutes, 5%-95% A.

Mass spectrometric conditions: Positive ion mode: Heater temperature: 300 °C; sheath gas flow rate: 45 psi; auxiliary gas flow rate: 5 L/min; sweep gas flow rate: 0.3 L/min; electrospray voltage: 3.0 kV; capillary temperature: 350 °C; S-Lens RF level: 30%. Negative ion mode: Heater temperature 300 °C; sheath gas flow rate: 45 psi; auxiliary gas flow rate: 5 L/min; sweep gas flow rate: 0.3 L/min; electrospray voltage: 3.2 kV; capillary temperature: 350 °C; S-Lens RF level: 60%.

Fatty-acid targeted metabolomics

Chromatographic conditions: Column: Acquity UPLC BEH C_8 column (2.1 mm × 100 mm, 1.7 μm) (Waters Corp., Milford, MA, United States); column temperature: 40 °C; flow rate: 0. 35 mL/min; mobile phase: water (0.1% formic acid):

acetonitrile (0.1% formic acid); gradient elution program: 1 minute, 50% B; 1-5 minutes, 50%-80% B; 5-6.5 minutes, 80%-95% B; 6.5-10 minutes, 95% B.

Tandem mass spectrometry (MS/MS detection): The serum concentration of fatty acids and their metabolites were determined using ultra-high-performance liquid chromatography (H-Class; Waters Corp.) coupled with triple quadrupole mass spectrometry (6500; AB SCIEX, Framingham, MA, United States). MS/MS data were collected using deuterated arachidonoylethanolamide (AEA-d8), deuterated oleoylethanolamide (OEA-d4), deuterated linoleoylethanolamide (LEA-d4), and deuterated oleic acid (OA-d9) as internal standards. Analytes, including AEA, 2-arachidonoyl glycerol ester, palmitoylethanolamide (PEA), OEA, LEA, 2-arachidonoylglycerol (2-AG), 1-palmitoyl glycerol (1-PG), 1-oleoyl glycerol (1-OG), and 1-linoleoyl glycerol (1-LG), were detected in the positive electrospray ionization mode, and arachidonic acid (AA), stearic acid, palmitic acid (PA), OA, and linoleic acid (LA) were detected in the negative mode. The optimized operational conditions were: ion spray voltage of + 5500 V in positive mode and -4500 V in negative mode, ion source temperature of 550 °C. Nitrogen was used as the collision gas. The ion pairs and related internal standards for multiple reaction monitoring are shown in Table 1.

Sample preparation: A volume of 30 μL of serum was combined with mixed internal standards, followed by successive addition of 500 cL of methyl tert-butyl ether, 150 μL methanol, and 140 μL ultrapure water. The mixture was vortexed for 1 minute and then centrifuged at 4 °C for 10 minutes (3000 rpm). The upper layer was collected, concentrated, and dried before reconstitution with 100 μL acetonitrile. The resulting supernatant were transferred to a sample vial for further analysis.

Standard and internal standard preparation: Precisely weighed amounts of AEA, LEA, PEA, OEA, 2-AG, 1-LG, 1-PG, 1-OG, AEA-d8, OEA-d4, and LEA-d4 were prepared at concentrations of 1250, 500, 250, 125, 50, 25, 12. 5, 5, 1, and 0. 5 ng, respectively. OA, PA, AA, and LA standards were prepared at concentrations of 5, 10, and 20 μg/mL, respectively, including internal standards AA-d8 (10 μg/mL) and OA-d9 (1 μg/mL).

Data processing and statistical methods: Peak alignment, retention time correction, and peak area were calculated using LC-MS software. Accurate molecular weights and MS/MS spectra were used for the identification and database retrieval of the metabolites. Unsupervised principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were used for multidimensional statistical analysis. Enrichment analysis of significantly altered metabolic pathways was performed using the Kyoto Encyclopedia of Genes and Genomes database. All other data were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, United States). Normally distributed quantitative data were reported as means ± SD. Comparison of quantitative data among groups was performed with analysis of variance if the data satisfied the normality and homogeneity of variance assumptions; otherwise, the Wilcoxon non-parametric test was used. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical data analysis

Twenty lean-type MAFLD patients and 20 healthy individuals were included in this study. Differences in general characteristics, including sex, age, or BMI observed in lean-type MAFLD patients and healthy individuals were not significant (Table 2). Routine blood tests revealed a significant difference between the white blood cell counts of the lean-type MAFLD patients and healthy controls ($P < 0.01$). Renal function test results revealed significant differences in urea nitrogen, uric acid, and creatinine levels in the lean-type MAFLD patients and healthy controls ($P < 0.01$). The results of liver function tests, including cholinesterase and ALT levels in the lean-type MAFLD patients and healthy controls, were significantly different ($P < 0.01$). The glucose metabolism tests found a significant difference of fasting blood glucose level between lean-type MAFLD patients and healthy controls ($P < 0.01$). Blood lipid analysis revealed significant differences in high-density lipoprotein (HDL), triglycerides, and apolipoprotein A1 (APOA-1) level between lean-type MAFLD patients and healthy controls ($P < 0.01$).

LC-MS quality control results

The total ion chromatogram of quality control samples of sera from lean-type MAFLD patients is shown in Figure 1. The overlapping chromatograms indicated excellent instrument stability and consistent retention times, validating the reliability of the analytical data.

PCA analysis

Patient serum metabolomics data were analyzed using Compound Discover software, followed by normalization. An unsupervised PCA model was constructed using Simca-P 14.0 and used to compare the overall profiles of individual samples in both positive and negative detection modes. In both positive and negative modes, samples from lean-type MAFLD patients clustered together and were clearly differentiated from the control group samples (Figure 2). That indicated a pronounced differences in the levels of metabolites found in lean-type MAFLD patients and healthy controls. The PCA model of the positive mode had an R^2X of 0. 698 and Q^2 of 0. 497, and for the negative mode the R^2X was 0. 6794 and a Q^2 of 0. 566. These results confirmed that PCA data model was able to elucidate variations in metabolites among samples.

Table 1 Ion pairs of analytes and internal standards

ID	Q1	Q3	DP	CE	CXP
AEA-d8	356.100	294.000	64.94	19.09	18.93
AEA	348.000	62.000	56.00	42.00	10.00
2-AG	379.000	287.400	163.86	20.05	13.09
OEA	326.400	309.200	195.32	21.31	29.01
OEA-d4	330.600	66.100	132.66	21.55	12.58
LEA	324.400	62.100	130.26	17.76	11.28
PEA	300.300	62.100	124.00	19.83	10.20
LEA-d4	328.100	66.000	133.80	20.98	8.59
1-LG	355.300	338.400	32.21	10.73	18.49
1-OG	357.300	339.300	145.00	13.20	24.00
1-PG	331.200	313.500	155.00	12.70	19.00
LA	325.200	279.100	-10.11	-8.72	-25.70
OA	327.200	281.300	-47.00	-10.50	-10.00
PA	301.200	255.200	-58.00	-14.00	-15.00
AA	303.100	259.000	-73.90	-16.14	-13.02
OA-d9	336.300	290.300	-14.34	-9.32	-7.94

1-LG: 1-linoleoyl glycerol; 1-OG: 1-oleoyl glycerol; 1-PG: 1-palmitoyl glycerol; 2-AG: 2-arachidonoylglycerol; AA: Arachidonic acid; AEA: Arachidonoylethanolamide; AEA-d8: Deuterated arachidonoylethanolamide; LA: Linoleic acid; LEA: Linoleoylethanolamide; LEA-d4: Deuterated linoleoylethanolamide; OA: Oleic acid; OA-d9: Deuterated oleic acid; OEA: Oleoylethanolamide; OEA-d4: Deuterated oleoylethanolamide; PA: Palmitic acid; PEA: Palmitoylethanolamide.

OPLS-DA analysis

OPLS-DA was used to comprehensively analyze and compare differences in the metabolite data collected from lean-type MAFLD patients and healthy controls. In both positive and negative modes, the lean-type MAFLD patients and healthy controls were completely separated in the OPLS-DA plots (Figure 3). The corresponding R^2Y and Q^2 values in the positive and negative modes were 0.957 and 0.962 and 0.954 and 0.921, respectively. To further validate the robustness of the model, a permutation test with 200 iterations was conducted, and it revealed no signs of data overfitting. This confirmed the good fit and predictive ability of the model, with statistically significant results.

Differential metabolic pathway screening

Following OPLS-DA analysis, differential metabolites were selected by a combination of variable importance in projection (VIP) values and *t*-tests. The selection criteria were set to satisfy VIP > 1 and $P < 0.05$. The selected metabolites were cross-referenced against the HMDB database. Ultimately, 65 potential differentially expressed metabolites were identified in lean-type MAFLD patients. Of those, 33 were identified in the positive mode and 32 in the negative modes (Figure 4). Metabolic pathway enrichment analysis of the identified metabolites with MetaboAnalyst 5.0 found that the 65 metabolites were associated with pathways involving unsaturated fatty acid biosynthesis, LA metabolism, fatty acid degradation, and ether lipid metabolism. Significant differences were found in the pathways of unsaturated fatty-acid biosynthesis and LA metabolism (Figure 5). A targeted metabolomic analysis of fatty acids was performed to gain a deeper understanding of the significance of fatty-acid metabolism in lean-type MAFLD patients.

Fatty-acid targeted metabolomics standard curve and chromatogram

To construct the standard curve, an increasing concentration (μg/mL) series of mixed fatty-acid standards was sequentially injected, with the peak area plotted on the Y-axis. The linear range and correlation coefficients (r) are listed in Table 3. The results show that the standard curve had excellent linearity and was suitable for the quantitative detection of fatty acids in the samples. The multiple reaction monitoring chromatograms of the samples and standards are shown in Figures 6 and 7, and the results indicate that there was no interference from other matrices in the content detection of the samples.

Changes in fatty-acid content in lean-type MAFLD patients

Changes in the fatty-acid content of lean-type MAFLD patients and healthy individuals are shown in Table 4. The PA, OA, LA, and AA levels in lean-type MAFLD patients were 3.41 ± 0.84, 2.63 ± 1.45, 2.42 ± 1.18, and 2.45 ± 1.21 μg/mL, respectively, and were significantly higher than those in the control group ($P < 0.05$).

Table 2 General characteristics and clinical indicators of lean metabolic-associated fatty liver disease patients

Indicator	Lean MAFLD group, *n* = 20	Control group, *n* = 20	Statistical value	*P* value
Male/female sex	5/15	0/20	$\chi^2 = 3.657$	0.056
Age in years	52.75 ± 15.17	44.95 ± 12.11	$t = 1.798$	0.08
Weight in kg	60 ± 6.12	54.8 ± 5.64	$t = 2.793$	0.008[b]
BMI in kg/m²	22.65 (21.52, 22.80)	21.25 (20.05, 21.80)	$Z = 3.196$	0.001[b]
White blood cells as × 10^9/L	7.25 ± 2.08	5.31 ± 1.06	$t = 3.715$	0.001[b]
Red blood cells as × 10^{12}/L	4.49 ± 0.63	4.23 ± 0.34	$t = 1.616$	0.117
Hemoglobin in g/L	134.20 ± 20.78	125.70 ± 13.58	$t = 1.531$	0.134
Hematocrit in %	39.22 ± 5.84	36.67 ± 3.27	$t = 1.703$	0.097
Platelets as × 10^9/L	261.25 ± 69.41	228.80 ± 45.42	$t = 1.749$	0.088
Mean platelet volume in fL	10.35 ± 1.21	10.96 ± 1.06	$t = -1.693$	0.099
Neutrophils in %	55.03 ± 11.49	51.53 ± 6.33	$t = 1.192$	0.243
Lymphocytes in %	35.01 ± 11.49	38.25 ± 6.39	$t = -1.104$	0.278
Monocytes in %	7.53 ± 1.8773	6.71 ± 0.93	$t = 1.75$	0.091
Mean red cell volume in fL	87.90 (84.5, 90.58)	87.85 (86.08, 89.95)	$Z = -0.298$	0.766
Mean red cell hemoglobin in pg	30.25 (28.63, 31.13)	30.55 (29.25, 31.28)	$Z = -0.69$	0.49
Mean red cell hemoglobin concentration in g/L	341.00 (337.00, 349.75)	346.00 (337.50, 351.00)	$Z = -0.895$	0.371
Red cell volume distribution width in %	12.90 (12.33, 13.63)	12.50 (12.13, 13.08)	$Z = -1.178$	0.239
Eosinophils in %	1.80 (0.75, 2.40)	1.95 (1.53, 3.33)	$Z = -0.934$	0.35
Basophils in %	0.50 (0.40, 0.70)	0.60 (0.40, 0.70)	$Z = -0.849$	0.396
Creatinine in μmol/L	66.25 ± 17.65	57.20 ± 6.99	$t = 2.132$	0.043[a]
Urea nitrogen in mmol/L	5.55 (4.35, 6.98)	4.65 (3.78, 5.38)	$Z = -2.274$	0.023[a]
Uric acid in μmol/L	350.50 (296.25, 450.00)	269.00 (236.75, 310.00)	$Z = -3.044$	0.002[b]
Total bilirubin in μmol/L	9.00 (11.00, 13.00)	9.00 (10.00, 11.00)	$Z = -1.186$	0.236
Direct bilirubin in μmol/L	1.66 (1.88, 2.30)	1.56 (1.62, 1.92)	$Z = -1.556$	0.120
Alkaline phosphatase in U/L	69.00 (59.25, 89.75)	61.50 (51.00, 82.50)	$Z = -1.448$	0.148
Cholinesterase in U/L	9214.95 ± 1232.29	7059.20 ± 1260.51	$t = 5.469$	0.001[b]
Alanine aminotransferase in U/L	17.00 (11.00, 35.75)	9.50 (6.25, 16.00)	$Z = -3.022$	0.003[b]
Aspartate aminotransferase in U/L	24.00 (20.00, 33.75)	20.50 (17.25, 26.75)	$Z = -1.64$	0.101
γ-glutamyl transferase in U/L	36.00 (21.50, 45.50)	20.50 (16.50, 34.75)	$Z = -1.719$	0.086
Total protein in g/L	73.05 ± 5.93	72.15 ± 4.04	$t = 0.561$	0.578
Albumin in μmol/L	42.10 ± 3.37	41.55 ± 2.98	$t = 0.547$	0.588
High-density lipoprotein in mmol/L	1.07 (0.98, 1.26)	1.40 (1.27, 1.59)	$Z = -3.882$	0.001[b]
Low-density lipoprotein in mmol/L	3.48 ± 0.72	3.40 ± 1.10	$t = 0.255$	0.800
Triglycerides in mmol/L	2.68 (1.37, 4.20)	1.09 (0.94, 1.39)	$Z = -3.598$	0.001[b]
Total cholesterol in mmol/L	5.19 ± 1.07	5.06 ± 1.28	$t = 0.366$	0.716
Apolipoprotein A1 in g/L	1.35 (1.22, 1.56)	1.55 (1.36, 1.74)	$Z = -2.153$	0.031[a]
Apolipoprotein B in g/L	0.99 ± 0.22	0.94 ± 0.32	$t = 0.559$	0.579
Glycated albumin in %	12.60 (10.90, 14.45)	13.00 (12.53, 14.18)	$Z = -0.717$	0.473
Fasting blood glucose in mmol/L	5.40 (5.03, 6.05)	4.90 (4.63, 5.15)	$Z = -3.052$	0.002[b]

[a]$P < 0.05$, lean metabolic-associated fatty liver disease group *vs* control group.

[b]*P* < 0.01, lean metabolic-associated fatty liver disease group *vs* control group.
MAFLD: Metabolic-associated fatty liver disease.

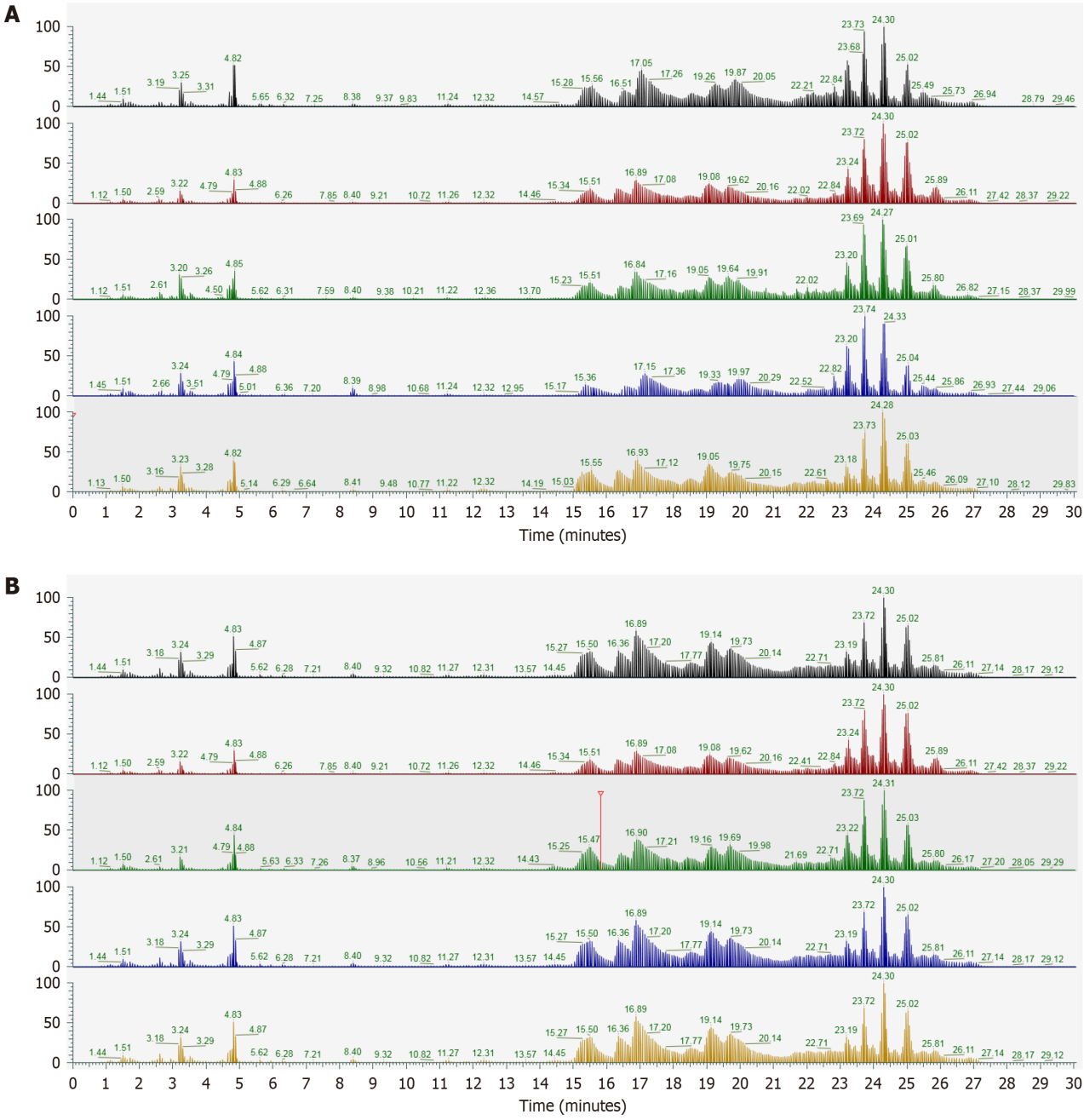

Figure 1 Total ion chromatogram. A: Negative mode; B: Positive mode.

DISCUSSION

Obesity and type 2 diabetes were prevalent in MAFLD patients, and approximately 40.8% of MAFLD patients had BMIs below threshold for overweight or obese MAFLD (< 23 kg/m^2), referred to as lean-type MAFLD. According to epidemiological surveys, lean-type MAFLD accounts for 25.8% of all MAFLD cases globally, but accounts for 44.3% of the MAFLD cases in China[14]. Owing to its subtle onset, mild, or nonspecific symptoms, the diagnosis of lean-type MAFLD is challenging and is often diagnosed during routine liver function testing or imaging examination. Current research predominantly focuses on obese-type MAFLD, with limited studies on lean-type patients. This study used serum metabolomics to investigate biomarkers characteristic of lean-type MAFLD patients and performed a quantitative analysis.

In this study, the lean-type MAFLD patients had BMIs of < 23 kg/m^2, and had higher BMIs and body mass than healthy lean participants. Compared with healthy controls, lean-type MAFLD patients had significantly elevated white

Table 3 Fatty-acid standard curve and linear range

No.	Name	Linear	Range in μg/mL	*r*
1	Arachidonic acid	y = 0.0087x - 0.0053	0.1-5	0.9963
2	Oleic acid	y = 0.0272x - 0.0425	0.1-5	0.9914
3	Palmitic acid	y = 0.0074x + 0.0036	0.1-5	0.9940
4	Linoleic acid	y = 0.0341x - 0.0821	0.1-5	0.9919

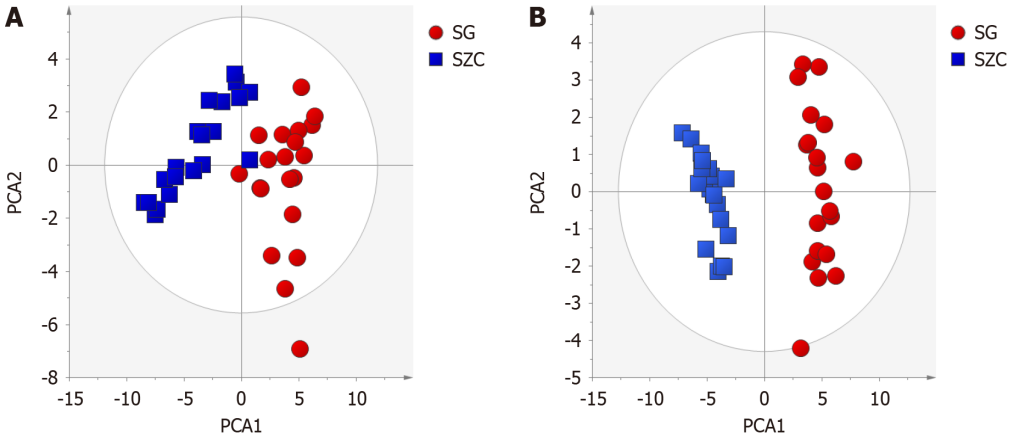

Figure 2 Principal component analysis. A: Positive mode; B: Negative mode. SG: Lean metabolic-associated fatty liver disease (metabolic-associated fatty liver disease); SZC: Healthy lean individuals.

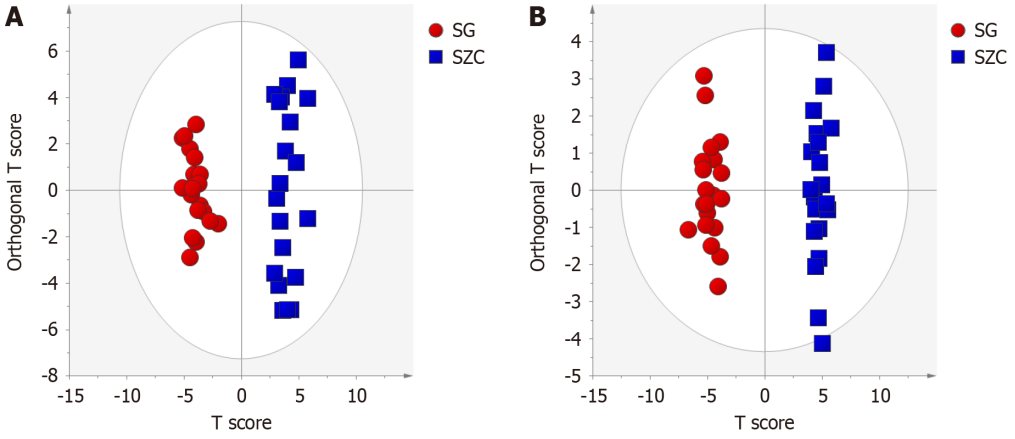

Figure 3 Orthogonal partial least squares discriminant analysis. A: Positive mode; B: Negative mode. SG: Lean-type metabolic-associated fatty liver disease (metabolic-associated fatty liver disease); SZC: Lean healthy individuals.

blood cell counts ($P < 0.01$). Additionally, significant differences were observed in liver and kidney function indexes and lipid profile components, such as urea nitrogen, uric acid, creatinine, cholinesterase, ALT, fasting blood glucose, HDL, triglycerides, and APOA-1 ($P < 0.01$).

ALT is present in hepatic cell cytoplasm, where increased intracellular triglycerides in provide sufficient reactive substrates for lipid peroxidation, thereby affecting the activity of antioxidant enzymes. This leads to increased oxidative stress. When liver cells are damaged, intracellular enzymes are released into the blood, causing ALT to spill over from liver cells into the extracellular space, resulting in increased ALT in the peripheral blood. Therefore, ALT levels reflect the integrity of liver cells. In this study, ALT and triglyceride levels were significantly higher in lean-type MAFLD patients than they were in healthy lean controls ($P < 0.01$). Accumulation of excess lipids in liver cells is a crucial factor that leads to hepatocyte degeneration and inflammation. Therefore, effective lipid transport and reduction in lipid synthesis may be crucial for the prevention and treatment of fatty liver disease. APOA-1, the primary apolipoprotein in HDL, is involved in cholesterol transport[15]. Mice with APOA-1 deficiency cannot form normal HDL particles, resulting in decreased cholesterol transport to liver tissue and cholesterol accumulation[16], which is consistent with our finding that APOA-1 levels were lower in lean-type MAFLD patients than in lean healthy controls, whereas triglyceride levels were signi-

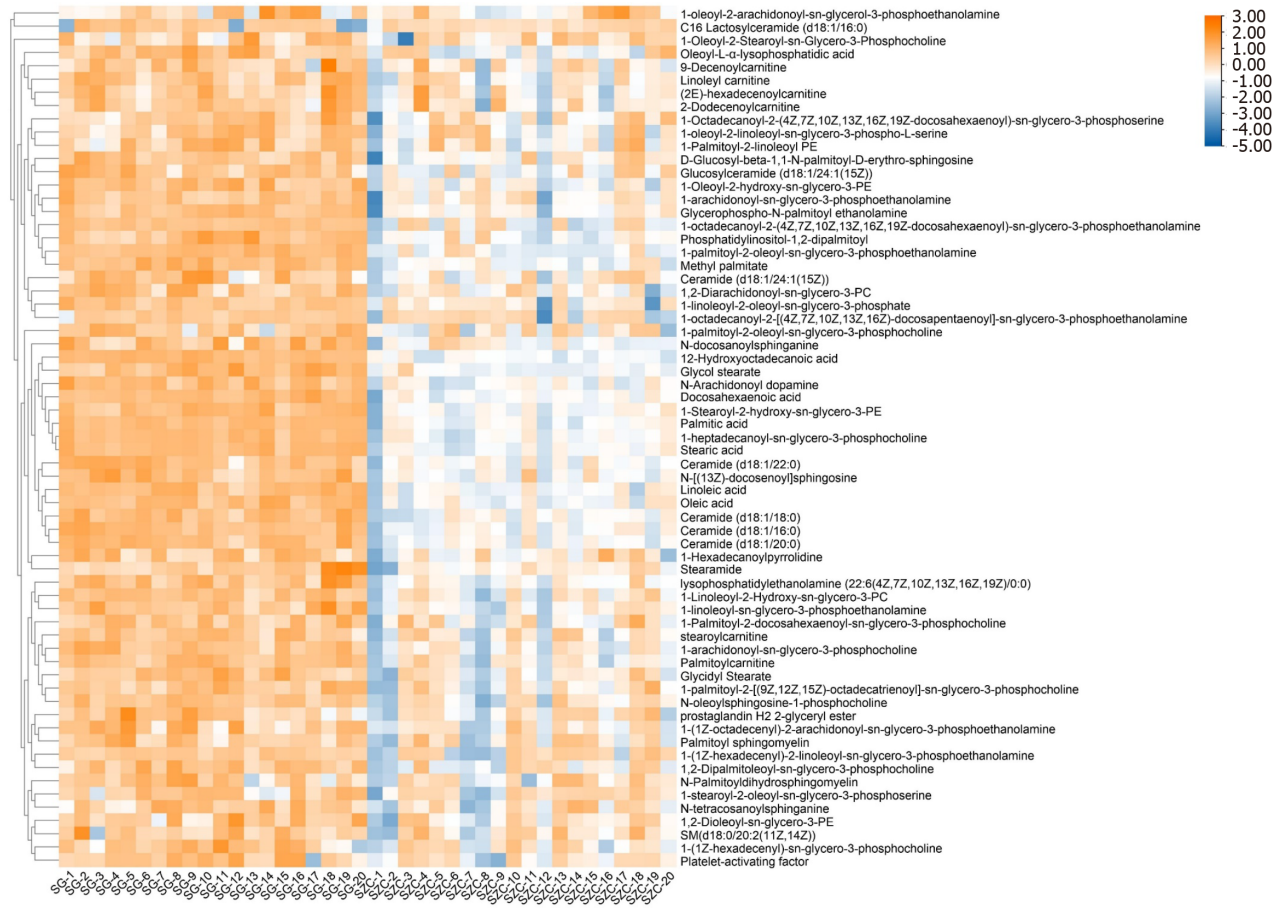

Figure 4 Heatmap of differential metabolites.

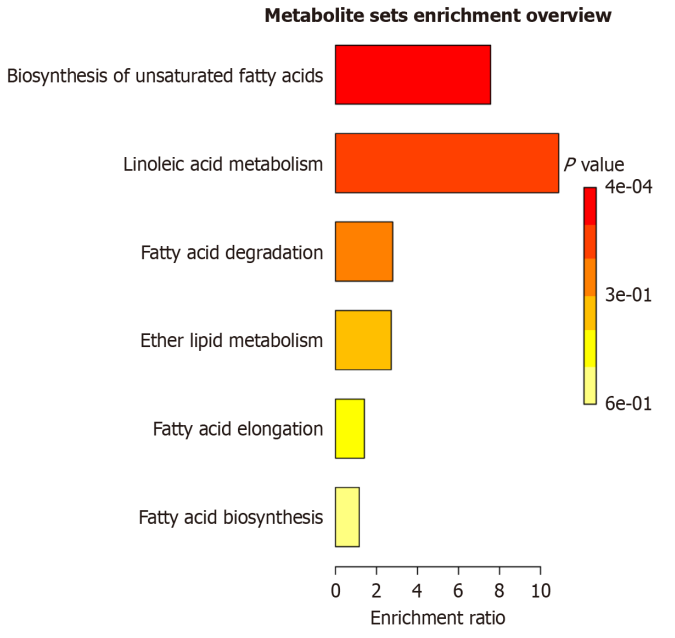

Figure 5 Metabolic pathway enrichment analysis.

ficantly increased ($P < 0.05$). Cholinesterase is an enzyme secreted into the bloodstream by liver cells. A cross-sectional analysis of 5384 individuals found elevated serum cholinesterase concentration in patients with fatty livers, which is consistent with our results[17].

Metabolomics is a high-throughput detection method that reflects disease status by the overall biochemical phenotype. It allows the examination of changes in endogenous metabolites at the macroscopic level following exposure to biological

Table 4 Changes in fatty-acid content in lean metabolic-associated fatty liver disease patients, *n* = 6

No.	Name	Lean MAFLD patients	Normal individuals
1	Palmitic acid	3.41 ± 0.84[a]	1.53 ± 0.85
2	Oleic acid	2.63 ± 1.45[a]	1.26 ± 0.65
3	Linoleic acid	2.42 ± 1.18[a]	1.30 ± 0.49
4	Arachidonic acid	2.45 ± 1.21[a]	1.24 ± 0.56

[a]$P < 0.05$.

Data are mean ± SD in μg/mL. MAFLD: Metabolic-associated fatty liver disease.

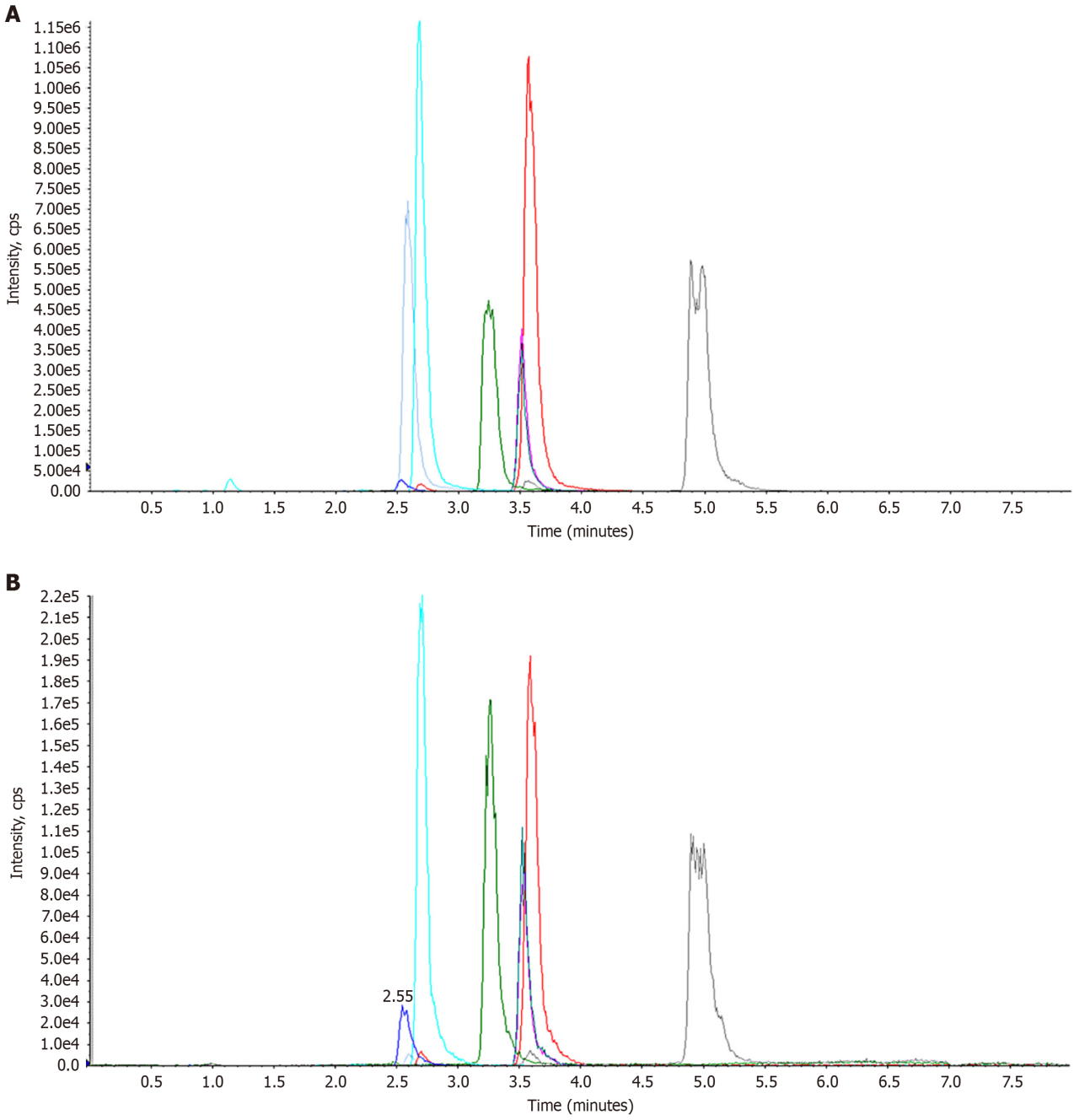

Figure 6 Standard material and sample total ion chromatograms. A: Standard material; B: Sample total.

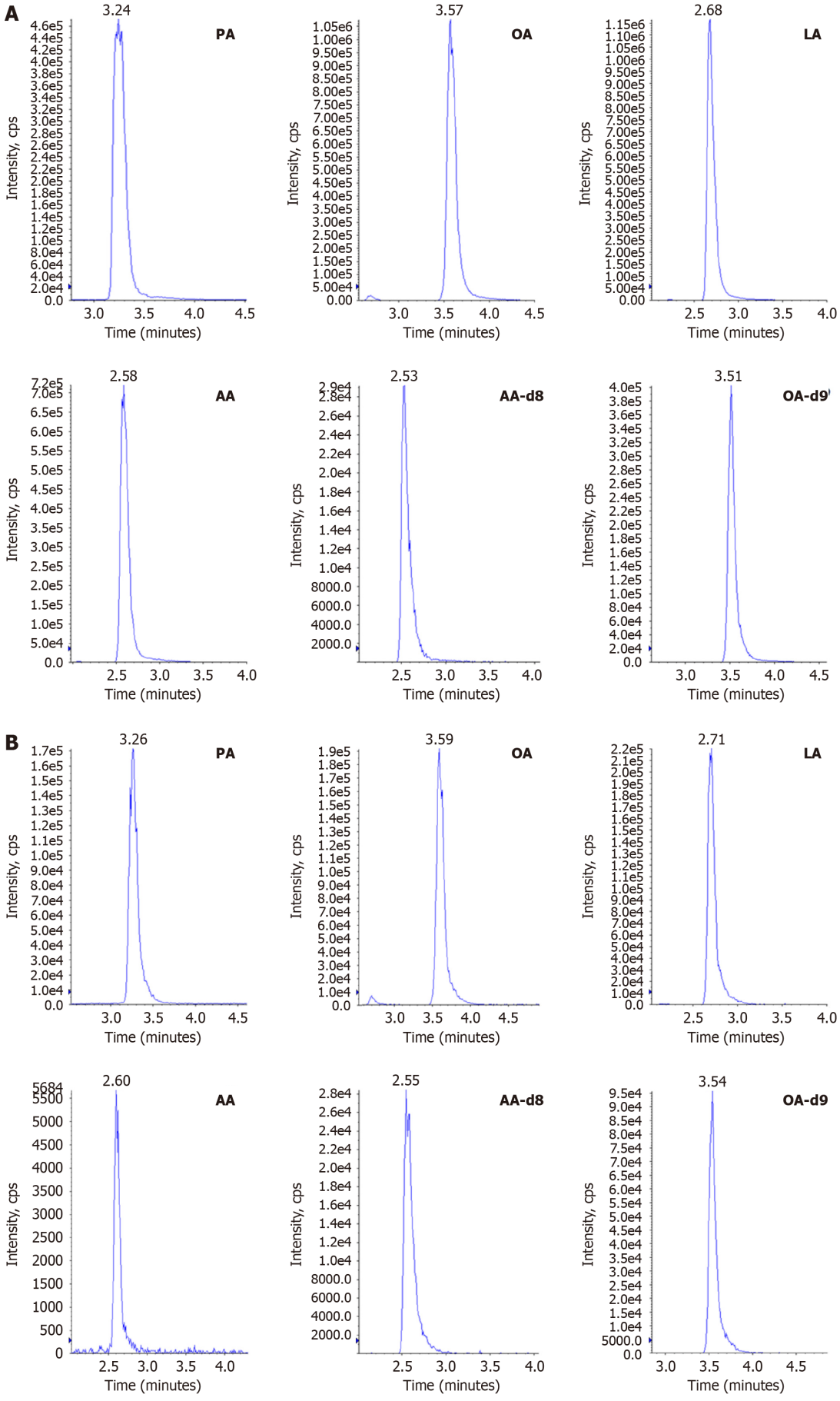

Figure 7 Multiple reaction monitoring chromatograms of palmitic acid, oleic acid, linoleic acid, arachidonic acid, and internal standards.
A: Standards; B: Samples. PA: Palmitic acid; OA: Oleic acid; LA: Linoleic acid; AA: Arachidonic acid; OA-d9: Deuterated oleic acid.

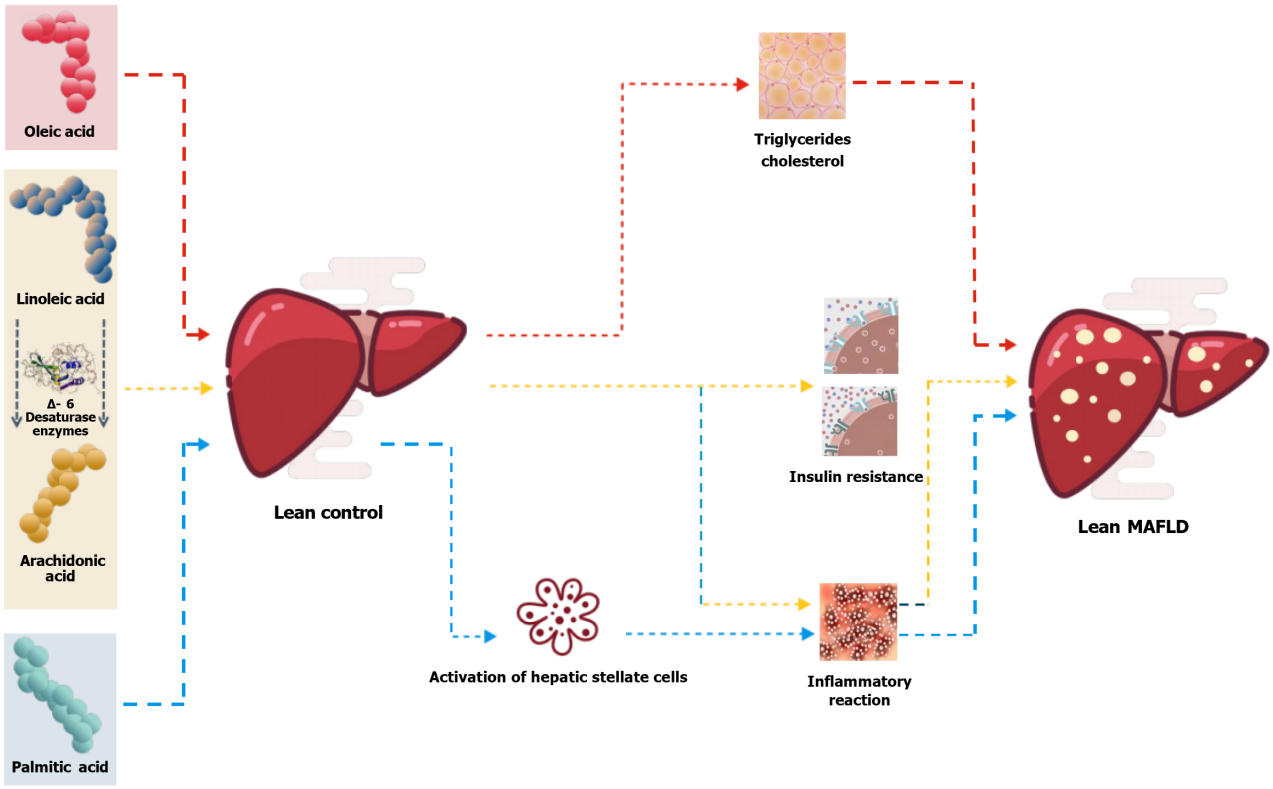

Figure 8 Effects of fatty acids on metabolic-associated fatty liver disease. Through serum targeted metabolomics studies, we measured four specific biomarkers. The preliminary conclusion was that the transformation of lean control individuals to lean metabolic-associated fatty liver disease (MAFLD) may affect the Oleic acid, linoleic-arachidonic acid and palmitic acid pathways. Linoleic acid is converted to arachidonic acid by Δ-6 desaturase, as shown in the figure.

stimuli. By analyzing comprehensive metabolic profiles, it is possible to identify disease-associated metabolites and reveal their metabolic pathways. In our study, serum metabolomics and liquid chromatography coupled with triple quadrupole mass spectrometry were used to investigate biomarkers specicic to lean-type MAFLD. Using a VIP > 1 and $P < 0.05$" as selection criteria, 65 biomarkers specific to lean-type MAFLD patients were identified in both positive and negative modes. These biomarkers were primarily concentrated in pathways related to fatty-acid, AA, and ether lipid metabolism.

Additionally, quantitative lipidomic analysis of four specific biomarkers revealed significant increases in the serum level of PA, OA, LA, and AA in lean-type MAFLD patients ($P < 0.05$), which were 2.23, 2.09, 1.86, and 1.98 times higher, respectively, than those in healthy individuals. FAs are components of triglycerides. Approximately 60 types of fatty acids have been identified in the plasma and tissues. However, only a few of them can be absorbed and used by the human body[18]. The homeostasis of body fatty acids ensures normal functioning.

FAs have a crucial role in lipid metabolism, but studies of their role in lean-type MAFLD patients are lacking. Current research indicates a close association between fatty acids and metabolic disorders, in which elevated levels of PA, palmitoleic acid, and LA are positively correlated with the onset and progression of MAFLD[19]. A study by Gambino *et al*[20] compared the changes in serum free FAs in MAFLD patients and in healthy controls, and reported significant increases in the serum level of free LA, OA, and AA. A plasma lipidomics study by Puri *et al*[21] reported significantly higher levels of PA, OA, and LA in MAFLD and nonalcoholic steatohepatitis patients, than in healthy individuals, but found no significant changes in AA. These findings align broadly with our research findings and, to a certain extent, reflect the serum levels of PA, OA, LA, and AA in lean-type MAFLD patients.

AA is a fatty-acid present in the cell membrane and is involved in cellular signal transduction during various inflammatory responses[22]. Abnormalities of FA metabolism disrupt the balance between the release and uptake of FAs in serum, leading to increased FA generation and reduced re-esterification capability. This eventually causes the accumulation of serum FAs, resulting in lipotoxicity and subsequent damage to the cardiovascular, endocrine, and digestive systems[23]. These studies indicate that abnormal FA metabolism in lean-type MAFLD patients significantly increases the likelihood of developing metabolic disorders. Based on existing research, we believe that elevated serum levels of PA and OA are directly associated with the occurrence of lean-type MAFLD, and that increased LA and AA levels are linked to the progression of MAFLD. In addition, PA promotes hepatic stellate cell activation, increases extracellular matrix deposition in MAFLD rats[24], induces podocyte apoptosis[25], and contributes to inflammation[26]. OA is a monounsaturated FA and is the preferred substrate for synthesizing triglycerides and cholesterol esters, and can induce hepatic cell steatosis and enhance tumor invasiveness[27]. LA is an essential FA that cannot be synthesized in the body and must be obtained from dietary sources. LA is converted to AA enzymes, such as Δ-6 desaturase (Figure 8).

CONCLUSION

Serum-targeted metabolomics found that fatty-acid metabolism was impaired in lean-type MAFLD patients. The biomarkers identified in this study potentially provide insights into the treatment of lean-type MAFLD. The study results warrant further investigation but are limited by the small sample size.

ACKNOWLEDGEMENTS

Special thanks to each of the authors for their contributions to this manuscript.

FOOTNOTES

Author contributions: Guo LL and Jiang YY conceived and designed the study; Jiang YY supervised the work, Guo LL contributed to the writing of the revision draft, they are the co-corresponding authors of this manuscript; Sun PQ and Dong WM performed the research, they contributed to this study equally; Sun PQ acquired patient and samples; Dong WM contributed to the writing of the manuscript; Yuan YF, Cao Q, and Chen XY analyzed the data and wrote the manuscript; All authors have read and approved the final manuscript.

Supported by Shanghai Natural Science Foundation, No. 22ZR1455900; Shanghai Putuo District Health System Science and Technology Innovation Project Key Project, No. ptkwws202201; and Shanghai Putuo District Xinglin Excellent Youth Talent Training Program, No. ptxlyq2201.

Institutional review board statement: An ethics review form is available for this study (Approval No: PTEC-A-2018-49-1).

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

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: We consent to data sharing.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Country of origin: China

ORCID number: Wen-Min Dong 0000-0001-9135-6974; Li-Li Guo 0009-0008-7885-4686; Yuan-Ye Jiang 0000-0002-4979-4206.

S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Zheng XM

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World Journal of Gastroenterology

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World J Gastroenterol 2024 July 21; 30(27): 3304-3313

DOI: 10.3748/wjg.v30.i27.3304

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Clinical Trials Study

Vonoprazan-amoxicillin dual therapy for *Helicobacter pylori* eradication in Chinese population: A prospective, multicenter, randomized, two-stage study

Xue-Ping Huang, Yi-Juan Liu, Shao-Wei Lin, Yan-Feng Shao, Feng Qiu, Qing-Wu Qiu, Zhang-Kun Xu, Jin-Xian Chen, Liang-Huo Chen, Zhen-Qun Lin, Wen-Hua Dai, Ming-Qing Zhang, Qi Jiang, Zhong-Qin Xiao, Xian-Xing Cheng, Xiang-Fei Zhang, Wen-Bin You, Wei Chen, Long-Qin Li, Wei-Xing Lin, Yong-Fu Wang, Fu-Jin Lai, Long-Qun Chen, Zhong-Hua Huang, Wen-Qi Zheng, Jin-Qi Wei, Zhi-Hui Lin

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade B

Scientific Significance: Grade A, Grade B

P-Reviewer: Kansu A; Khalil G

Received: March 22, 2024

Revised: June 12, 2024

Accepted: June 25, 2024

Published online: July 21, 2024

Processing time: 111 Days and 4.6 Hours

Xue-Ping Huang, Zhi-Hui Lin, Department of Gastroenterology, Shengli Clinical Medical College of Fujian Medical University, Fuzhou 350000, Fujian Province, China

Xue-Ping Huang, Zhi-Hui Lin, Department of Gastroenterology, Fujian Provincial Hospital, Fuzhou 350001, Fujian Province, China

Yi-Juan Liu, Department of Gastroenterology, The First Affiliated Hospital of Fujian Medical University, Fuzhou 350000, Fujian Province, China

Shao-Wei Lin, School of Public Health, Fujian Medical University, Fuzhou 350000, Fujian Province, China

Yan-Feng Shao, Department of Gastroenterology, The Third People's Hospital of Fujian Province, Fuzhou 350000, Fujian Province, China

Feng Qiu, Department of Gastroenterology, Fujian Provincial Hospital North Brance Fujian Provincial Geriatric Hospital, Fuzhou 350000, Fujian Province, China

Qing-Wu Qiu, Department of Gastroenterology, Xiaoao Hospital, Fuzhou 350000, Fujian Province, China

Zhang-Kun Xu, Department of Gastroenterology, The General Hospital of Fujian Energy Group, Fuzhou 350000, Fujian Province, China

Jin-Xian Chen, Department of Gastroenterology, The Second Hospital of Zhangzhou, Zhangzhou 363000, Fujian Province, China

Liang-Huo Chen, Department of Gastroenterology, Anxi Country Hospital, Quanzhou 362000, Fujian Province, China

Zhen-Qun Lin, Department of Gastroenterology, Zhangzhou Municipal Hospital of TCM, Zhangzhou 363000, Fujian Province, China

Wen-Hua Dai, Department of Gastroenterology, Zhangpu Hospital, Zhangzhou 363000, Fujian Province, China

Ming-Qing Zhang, Department of Gastroenterology, The Affiliated Dongnan Hospital of Xiamen University, Zhangzhou 363000, Fujian Province, China

Qi Jiang, Department of Gastroenterology, First Hospital of Nanping City, Nanping 353000, Fujian Province, China

Zhong-Qin Xiao, Department of Gastroenterology, Fujian Nanping Second Hospital, Nanping 353000, Fujian Province, China

Xian-Xing Cheng, Department of Gastroenterology, Wuyishan Municipal Hospital, Nanping 353000, Fujian Province, China

Xiang-Fei Zhang, Department of Gastroenterology, Fuzhou Changle District Hospital, Fuzhou 350000, Fujian Province, China

Wen-Bin You, Wei Chen, Department of Gastroenterology, Changle City Second Hospital, Fuzhou 350000, Fujian Province, China

Long-Qin Li, Department of Gastroenterology, Jinjiang Municipal Hospital, Quanzhou 362000, Fujian Province, China

Wei-Xing Lin, Department of Gastroenterology, Fuding Hospital Affiliated to Fujian University of Traditional Chinese Medicine, Ningde 352000, Fujian Province, China

Yong-Fu Wang, Department of Gastroenterology, Liancheng Hospital, Longyan 364000, Fujian Province, China

Fu-Jin Lai, Department of Gastroenterology, Xiapu County Hospital, Ningde 352000, Fujian Province, China

Long-Qun Chen, Department of Gastroenterology, Jinjiang Second Hospital, Quanzhou 362000, Fujian Province, China

Zhong-Hua Huang, Department of Gastroenterology, The First Hospital of Putian City, Putian 351100, Fujian Province, China

Wen-Qi Zheng, Department of Gastroenterology, Putian Fude Hospital, Putian 351100, Fujian Province, China

Jin-Qi Wei, Department of Gastroenterology, The 5th Affiliated Hospital of Sun Yat-sen University, Zhuhai 519000, Guangdong Province, China

Co-first authors: Xue-Ping Huang and Yi-Juan Liu.

Co-corresponding authors: Jin-Qi Wei and Zhi-Hui Lin.

Corresponding author: Zhi-Hui Lin, MD, PhD, Chief Doctor, Department of Gastroenterology, Shengli Clinical Medical College of Fujian Medical University, No. 134 Dongjie, Gulou District, Fuzhou 350000, Fujian Province, China. fjslyylzh@fjmu.edu.cn

Abstract

BACKGROUND

The efficacy of Vonoprazan-amoxicillin dual therapy (VAT) in the treatment of *Helicobacter pylori* (*H. pylori*) is controversial.

AIM

To evaluate the efficacy of VAT in the Chinese population.

METHODS

This prospective, multicenter, randomized, open-label, and two-stage study was conducted at 23 centers in Fujian, China (May 2021-April 2022). *H. pylori*-infected patients were randomized to bismuth quadruple therapy (BQT), BQT-Vonoprazan (BQT-V), seven-day VAT (VAT-7), ten-day VAT (VAT-10), and fourteen-day VAT (VAT-14) groups. The primary endpoint was the *H. pylori* eradication rate. The secondary endpoint was the frequency of adverse events. This study was registered with the Chinese Clinical Trial Registry, ChiCTR2100045778.

RESULTS

In the first stage, VAT-7 and BQT-V groups were selected for early termination because less than 23 among 28 cases were eradicated. In the second stage, the eradication rates for BQT, VAT-10, and VA-14 were 80.2% [95% confidence interval (95%CI): 71.4%-86.8%], 93.2% (86.6%-96.7%), 92.2% (85.3%-96.0%) in the intention-to-treat (ITT) analysis, and 80.9% (95%CI: 71.7%-87.5%), 94.0% (87.5%-97.2%), and 93.9% (87.4%-97.2%) in the per-protocol analysis. The ITT analysis showed a higher eradication rate in the VAT-10 and VAT-14 groups than in the BQT group (P = 0.022 and P = 0.046, respectively). The incidence of adverse events in the VAT-10 and VAT-14 groups was lower than in the BQT group (25.27% and 13.73% *vs* 37.62%, respectively; P < 0.001).

CONCLUSION

VAT with a duration of 10 or 14 days achieves a higher eradication rate than the BQT, with a more tolerable safety profile in *H. pylori*-infected patients in Fujian.

Key Words: *Helicobacter pylori*; Vonoprazan; Amoxicillin; Dual therapy; Bismuth quadruple therapy

Core Tip: Our study was a prospective, multicenter, randomized, two-stage study for *Helicobacter pylori* (*H. pylori*) eradication in Chinese population. We found that a daily dose of 20 mg of Vonoprazan was sufficient to eradicate *H. pylori*. We also found that compared to bismuth quadruple therapy, Vonoprazan-amoxicillin dual therapy with a duration of 10 days or 14 days, rather than 7 days, achieved higher eradication rates and that the safety profile of this dual therapy was more tolerable and manageable in Chinese patients. These results will guide further research and clinical practice for *H. pylori* eradication.

Citation: Huang XP, Liu YJ, Lin SW, Shao YF, Qiu F, Qiu QW, Xu ZK, Chen JX, Chen LH, Lin ZQ, Dai WH, Zhang MQ, Jiang Q, Xiao ZQ, Cheng XX, Zhang XF, You WB, Chen W, Li LQ, Lin WX, Wang YF, Lai FJ, Chen LQ, Huang ZH, Zheng WQ, Wei JQ, Lin ZH. Vonoprazan-amoxicillin dual therapy for *Helicobacter pylori* eradication in Chinese population: A prospective, multicenter, randomized, two-stage study. *World J Gastroenterol* 2024; 30(27): 3304-3313
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3304.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3304

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral-shaped, Gram-negative bacterium affecting approximately 50% of the global population[1]. However, the *H. pylori* eradication rate has decreased to less than 80% in some countries, primarily prompted by increasing antibiotic resistance[2]. Recent international guidelines recommend bismuth quadruple therapy (BQT) or non-BQT as the first-line treatment for *H. pylori* in regions with high *H. pylori* clarithromycin (CLA) resistance[3,4]. However, these quadruple therapy regimens have some disadvantages, including high cost, numerous adverse reactions, and poor treatment compliance due to the use of numerous drugs. These drawbacks have prevented the widespread implementation of BQT in the treatment of *H. pylori* infection. As a result, there is increased interest in developing effective and easier treatment options.

Unge *et al*[5] first reported the use of dual therapy in 1989, consisting of a combination of proton pump inhibitors (PPIs) and amoxicillin (AMX), to eradicate *H. pylori*. However, the eradication rate of this regimen was only 55%-75% at the standard dosage and frequency rate, and the efficacy was low[6,7]. As a result, dual therapy was gradually replaced by triple therapy. In recent years, some studies have attempted to improve dual therapy by increasing the dosage and frequency of the drugs. The results show that high-dose dual therapy has achieved good efficacy and fewer adverse reactions[8,9].

For the eradication of *H. pylori*, maintaining a near-neutral gastric pH during treatment is critical to the success of the dual therapy regimen. Vonoprazan, a new type of potassium-competitive acid blocker, has been proven to have a stronger and more durable effect on the inhibition of gastric acid secretion than other PPIs. Therefore, Vonoprazan is expected to be more effective than other PPIs when used in combination with AMX to treat *H. pylori* infection. Several randomized studies in Japan[10], China[11], Europe, and the United States[12] have demonstrated the effectiveness of Vonoprazan-amoxicillin dual therapy (VAT) in eradicating *H. pylori*. However, the duration and dosages of VAT regimen are still controversial, and large multicenter randomized studies on this regimen in the Chinese population are still lacking.

This study aimed to evaluate the safety and efficacy of different dosages and duration of treatment with VAT regimens. The goal was to provide a safer and more effective regimen to be used in clinical practice to optimize treatment outcomes for patients in China with *H. pylori* infections.

MATERIALS AND METHODS

Patient selection

The inclusion criteria included patients with *H. pylori* infection, treatment naive, and if they are 18 to 70 years old, no use of antibiotics, bismuth, or traditional Chinese medicine four weeks prior to treatment, no use of PPIs or histamine type 2 receptor antagonists (H2 blockers) two weeks prior to treatment, and informed and consenting to participate in the study. Exclusion criteria included: The presence of serious medical conditions, such as severe heart, lung, or kidney dysfunction, compromised immune functioning, allergies to medications used in the study, mental illness, communication difficulties, pregnancy or lactation, and any organic diseases, such as gastrointestinal tumors and gastrointestinal hemorrhage.

Study design

This was a prospective, multicenter, randomized, open-label and phase II study according to Simon's two-stage optimal design. This study was conducted on patients with *H. pylori* infection in the Gastroenterology Departments of 23 centers from May 2021 to April 2022 in Fujian province, Southeast China. Esomeprazole-amoxicillin dual regimen was abandoned at the beginning of the clinical trial because esomeprazole could not be obtained in Fujian hospital for medical policy adjustment reasons. Patients were randomly assigned 1:1:1:1:1 to five study groups. Simon's two-stage optimal design was used for each group, which was performed according to the following hypothesis: The minimum eradication rate was set at 80%, and the expected eradication rate was set at 90%, with a one-tailed type I error = 5%, and power equal to 80%. In the first stage, 28 eligible patients were entered into each group. If fewer than 23 of 28 patients responded to a treatment, that treatment group would be valid for early termination. Otherwise, an additional 69 patients would be accrued to a maximum size of 97. If more than 83 patients achieved an objective response after the completion of the second stage, the treatment group was considered worthy of further investigation. The probability of early termination was 0.69.

Randomization was done centrally with a random number-generating system and an interactive internet and voice-response system. Clinicians and patients were not masked to treatment assignment. Treatment continued unless intolerable or unsafe adverse effects developed, or consent was withdrawn. A demographic data and medical history were recorded for each patient, including: Age, sex, medical history, coffee, tea, and alcohol consumption, and smoking habits. Coffee or tea consumption was defined as consumption of one or more cups per day. Alcohol consumption was defined as consuming more than 50 g of alcohol per day in the past six months. Smoking was defined as smoking at least one pack of cigarettes per week. Patients with duodenal and/or gastric ulcers were defined as having peptic ulcers, while those without ulcers were defined as having non-ulcer dyspepsia. The esomeprazole-amoxicillin dual regimen was abandoned at the beginning of the clinical trial because esomeprazole could not be obtained at Fujian Hospital due to medical policy adjustments, as presented in Supplementary Table 1.

Diagnosis and treatment regimens for H. pylori infection

Patients were diagnosed with *H. pylori* infection by using 13C-/14C- urea breath test (13C-UBT/14C-UBT), or rapid urease test (San Qiang Bio & Che, Fujian, China). After being diagnosed with *H. pylori* infection, patients were randomly assigned (1:1:1:1:1) to five treatment groups: BQT group (ilaprazole 10 mg, bismuth potassium citrate 220 mg, amoxicillin 1000 mg, and clarithromycin 500 mg, twice daily, for 14 days); BQT-V group (Vonoprazan 20 mg, once daily, bismuth potassium citrate 220 mg twice daily, amoxicillin 1000 mg, twice daily, and clarithromycin 500 mg, twice daily, for 14 days); VAT-7 group (Vonoprazan 20 mg, twice daily, and amoxicillin 1000 mg, three times daily, for 7 days); VAT-10 group (Vonoprazan 20 mg, twice daily, and amoxicillin 1000 mg, three times daily, for 10 days); and VAT-14 group (Vonoprazan 20 mg, once daily, and amoxicillin 1000 mg, three times daily, for 14 days). Eprazole and Vonoprazan were suggested to be taken half an hour before meals, while CLA and AMX were suggested to be taken postprandially.

The study drug information was as follows: ilaprazole (Group Lizhu Pharmaceutical Factory), Vonoprazan (Takeda), amoxicillin (Zhejiang Jinhua Kangenbei Biopharmaceutical Co. LTD), clarithromycin (Guangdong East Sunshine Pharmaceutical Co. LTD), and bismuth potassium citrate (Yuekang Pharmaceutical Group Co. LTD).

Study outcomes

The primary endpoint in this study was the *H. pylori* eradication rate assessed by 13C-urea or 14C-urea breath tests, at least 6 weeks after completing the regimen. The secondary endpoints were the severity and frequency of adverse events, and the rate of clinical symptom remission. The adverse events were recorded in a questionnaire by investigators for 14 days following the commencement of therapy, and symptom severity was evaluated according to a four-point scale system: None, mild (discomfortable or annoying, but not interfering with daily life), moderate (discomfort sufficient to interfere with daily life), and severe (discomfort resulting in discontinuation of eradication therapy)[13]. Patients were asked to recall all unused medications and empty bags to assess compliance.

Statistical analysis

The primary analyses were determined by intention-to-treat (ITT) and per-protocol (PP) analyses. The ITT analysis was defined to include all randomized patients. The patients who were lost to follow-up or who did not undergo 13C-/14C-urea breath tests were considered as treatment failures in the ITT analysis. The PP analysis included patients who achieved > 80% drug compliance and underwent urea breath testing. Drug compliance was recorded in a specific questionnaire form by patients. Efficacy and safety analyses were performed on the intent-to-treat population. Response rates were reported with exact two-sided 95% confidence intervals. Differences among groups were analyzed using Pearson's χ^2 test for categorical variables, and ANOVA for continuous variables, with post hoc analysis using the Bonferroni method. All *P* values were two sided and were considered statistically significant if the *P* value was < 0.05. All analyses were computed using the R V.3.5.2 software (R Foundation for Statistical Computing, Vienna, Austria).

Statement of ethics

This study was approved by the independent Ethics Committees of the Fujian Provincial Hospital (Approval No. K2020-006-02). This study is registered with the Chinese Clinical Trial Registry, ChiCTR2100045778. All subjects signed written informed consents prior to receiving treatment. This study was carried out in accordance with the guidelines of the Declaration of Helsinki and the Consolidated Standards of Reporting Trials (CONSORT).

RESULTS

Patient enrolment and demographic data

The study flowchart is shown in Figure 1. A total of 362 patients were enrolled within the 1-year study period. In the first stage, 28 patients were enrolled in each group. The numbers of cases eradicated in the VAT-7 group and BQT-V group were 19 and 21, respectively. Since the proportion of responses was not sufficiently high to recommend this regimen to go to the next step in the clinical trial, this group was selected for early termination. The numbers of cases eradicated in the other three groups were 24 for BQT, 26 for VAT-10, and 25 for VAT-14, respectively, which were all greater than 23, therefore these groups were selected for further study. At the second stage, 222 patients were enrolled and randomly assigned to three treatment groups. Demographic data of these participants are presented in Table 1. There was a significant difference in gender. There was no significant difference in age, diagnosis, cigarette smoking, or alcohol, coffee, and tea consumption amongst the three randomized groups.

Eradication rates of H. pylori

The *H. pylori* eradication rates for each regimen are presented in Figure 2. In the ITT analysis, the *H. pylori* eradication rate was 80.2% [95% confidence interval (95%CI): 71.4%-86.8%] in the BQT group, 93.2% (95%CI: 86.6%-96.7%) in the VAT-10 group, and 92.2% (95%CI: 85.3%-96.0%) in the VAT-14 group. In the PP analysis, the *H. pylori* eradication rate was 80.9% (95%CI: 71.7%-87.5%) in the BQT group, 94.0% (95%CI: 87.5%-97.2%) in the VAT-10 group, and 93.9% (95%CI: 87.4%-97.2%) in the VAT-14 group. The results of ITT analysis proved that the *H. pylori* eradication rate in both the VAT-10 (93.2%) and VAT-14 (92.2%) groups was higher than that of the BQT group (80.2%), with rate differences of 13.9% between the BQT and VAT-10 groups, and 12.0% between the BQT and VAT-14 groups. All reported differences were statistically significant. PP analysis also showed significant differences, which were consistent with the results of ITT analysis (Figure 2). These results demonstrated that VAT-10 or VAT-14 was superior to BQT.

Compliances and adverse events

All participants received at least one dose of medication and the adverse events were assessed using the Adverse Event Analysis. The reported adverse events are displayed in Table 2. In total, the incidence of adverse events in the VAT-10 and VAT-14 groups was lower than in the BQT group (25.27% and 13.73% *vs* 37.62%, respectively; $P < 0.001$). Additionally, reported adverse events were more severe in the BQT group than in the VAT-10 and VAT-14 groups. Bitter taste was the most commonly reported adverse event in the BQT group. This adverse effect was more frequently reported in the BQT compared to the VAT-10 group (18.81% *vs* 3.88%; $P = 0.005$), and in the BQT compared to VAT-14 group (18.81% *vs* 5.88%; $P = 0.019$). Nausea was the second most commonly reported adverse event: BQT *vs* VAT-10 (14.85% *vs* 3.88%; $P = 0.042$), and BQT *vs* VAT-14 (14.85% *vs* 3.92%; $P = 0.042$). The other adverse events included dizziness: BQT *vs* VAT-10 (14.85% *vs* 3.88%; $P = 0.028$), and BQT *vs* VAT-14 (14.85% *vs* 1.96%; $P = 0.007$); abdominal discomfort: BQT *vs* VAT-10 (10.89% *vs* 0.97%; $P = 0.02$), and BQT *vs* VAT-14 (10.89% *vs* 3.92%; $P = 0.206$); and anorexia: BQT *vs* VAT-10 (9.90% *vs* 2.91%; $P = 0.158$), and BQT *vs* VAT-14 (9.90% *vs* 1.96%; $P = 0.107$). Patient compliance in the VAT-10 and VAT-14 groups was better than in the BQT group (97.09% and 97.06% *vs* 93.07%, respectively), although no statistical differences were found in these three groups ($P = 0.263$).

There are 4 patients in total who failed to take at least 80% of the study drugs due to adverse events. Among these 4 patients, 1 patient in the VAT-10 group ceased treatment due to dizziness, 1 patient in the VAT-14 group ceased treatment due to skin rash, and 2 patients in the BQT group ceased treatment due to diarrhea and nausea.

Remission of clinical symptoms for each regimen

The clinical symptom relief for the VAT-14 regimen (complete remission rate 58.82%, partial remission rate 33.33%, and no remission rate 7.84%), was significantly better than that of the BQT regimen (complete remission rate 40.59%, partial remission rate 41.58%, and no remission rate 17.82%) ($P = 0.014$). However, the clinical symptom relief for the VAT-10 regimen (complete remission rate 44.66%, partial remission rate 29.12%, and no remission rate 26.21%) was not better than that of the BQT regimen ($P = 1.000$) (Table 3).

Cost-effectiveness analysis of each regimen

Cost-effectiveness analysis showed that the cost-effectiveness ratio (CER) of VAT-10 and VAT-14 were both less than that of BQT (Table 4). Considering that the reported effectiveness for VAT-10 and VAT-14 were equal (Table 4), the VAT-14 regimen, which demonstrated the lowest CER, was the most cost-effective therapy.

DISCUSSION

Research on Vonoprazan has been conducted firstly in Japan. Recently, there have been some studies of Vonoprazan in other countries. Differences in populations may lead to different results from those observed in studies conducted in Japan. In the current study, we conducted a multicenter trial in Fujian, China to assess the efficacy and safety of VAT with different duration and dosages in the first-line treatment of *H. pylori* infection. Vonoprazan plus AMX was approved by the FDA and has been packaged in the Voquezna Dual Pak. However, the product has not been released on the market due to the detection of trace levels of a nitrosamine impurity[14].

Table 1 Baseline characteristics of patients, *n* (%)

	Total	BQT	VAT-10	VAT-14	*P* value
Age (years, mean ± SD)	45.15 ± 12.53	43.84 ± 14.26	45.52 ± 11.26	45.69 ± 12.39	0.428
Range	12.00-80.00	15.00-72.00	27.00-80.00	12.00-77.00	
Gender					0.027
Male	164 (53.59)	48 (51.06)	64 (64.00)	45 (45.45)	
Female	142 (46.41)	46 (48.94)	36 (36.00)	54 (54.55)	
Diagnosis					0.754
Peptic ulcer	42 (13.73)	15 (14.85)	12 (11.65)	15 (14.71)	
Non-ulcer dyspepsia	264 (86.72)	86 (85.15)	91 (88.35)	87 (85.29)	
Cigarette smoking	33 (10.78)	12 (12.77)	9 (9.00)	9 (9.09)	0.618
Alcohol drinking	14 (4.58)	6 (6.38)	4 (4.00)	3 (3.03)	0.524
Tea drinking	41 (13.40)	15 (15.96)	14 (14.00)	10 (10.10)	0.473
Coffee drinking	18 (5.88)	6 (6.38)	2 (2.00)	9 (9.09)	0.097
Family history of gastric cancer	17 (5.56)	4 (4.26)	4 (4.00)	5 (5.05)	0.940
H. pylori family gathering	51 (16.67)	15 (15.96)	15 (15.00)	19 (19.19)	0.581

H. pylori: *Helicobacter pylori*; VAT: Vonoprazan-amoxicillin dual therapy; BQT: Bismuth quadruple therapy.

Table 2 Adverse events for each regimen, *n* (%)

Adverse events	BQT	VAT-10	VAT-14	*P* value		
				Total	BQT *vs* VAT-10	BQT *vs* VAT-14
Total	38 (37.62)	25 (25.27)	14 (13.73)	< 0.001	0.112	0.001
Bitter taste	19 (18.81)	4 (3.88)	6 (5.88)	< 0.001	0.005	0.019
Nausea	15 (14.85)	4 (3.88)	4 (3.92)	0.003	0.042	0.042
Dizziness	15 (14.85)	4 (3.88)	2 (1.96)	< 0.001	0.028	0.007
Diarrhea	11 (10.89)	4 (3.88)	10 (9.80)	0.144	-	-
Abdominal discomfort	11 (10.89)	1 (0.97)	4 (3.92)	0.005	0.020	0.206
Abdominal pain	10 (9.90)	4 (3.88)	7 (6.86)	0.236	-	-
Anorexia	10 (9.90)	3 (2.91)	2 (1.96)	0.022	0.158	0.107
Constipation	9 (8.91)	6 (5.83)	5 (4.90)	0.481	-	-
Belching	9 (8.91)	2 (1.94)	4 (3.92)	0.062	-	-
Vomiting	8 (7.92)	1 (0.97)	4 (3.92)	0.040	0.114	0.719
Skin rash	7 (6.93)	1 (0.97)	2 (1.96)	0.054	-	-
Bloating	7 (6.93)	6 (5.83)	4 (3.92)	0.638	-	-
Heartburn	7 (6.93)	1 (0.97)	3 (2.94)	0.060	-	-
Insomnia	6 (5.94)	1 (0.97)	3 (2.94)	0.107	-	-

VAT: Vonoprazan-amoxicillin dual therapy; BQT: Bismuth quadruple therapy.

The physiological basis for an effective dual therapy is thought to be associated with the ability of the anti-secretory component of the therapy to maintain an intragastric pH above 6. Theoretically, raising the gastric pH above 6 causes bacteria to begin replicating and increases bacterial sensitivity to amoxicillin[15]. A high-dose, high-frequency dual therapy of AMX (≥ 2.0 g/day) and a PPI (at least twice daily) for 14 days has been reported to be effective in eradicating *H. pylori* as a first-line therapy[16,17]. However, the high medication doses and frequent medication administration

Table 3 Remission of clinical symptoms for each regimen, *n* (%)

Remission	BQT	VAT-10	VAT-14	*P* value		
				Total	BQT *vs* VAT-10	BQT *vs* VAT-14
Complete remission	41 (40.59)	46 (44.66)	60 (58.82)	< 0.001	1.000	0.014
Partial remission	42 (41.58)	30 (29.12)	34 (33.33)			
No remission	18 (17.82)	27 (26.21)	8 (7.84)			

VAT: Vonoprazan-amoxicillin dual therapy; BQT: Bismuth quadruple therapy.

Table 4 Cost-effectiveness analysis of each regimen

	BQT	VAT-10	VAT-14
Cost (CNY per percent)	334.18	216.80	163.52
Effectiveness (%)	80.90	94.00	93.90
CER (CNY per percent)	4.13	2.31	1.74
ICER (CNY per percent)		-1.82	-2.39

Effectiveness, the eradication rate in the per-protocol analysis (%). VAT: Vonoprazan-amoxicillin dual therapy; BQT: Bismuth quadruple therapy; CER: Cost-effectiveness ratio; Cost: Direct drug costs per patient; ICER: Incremental cost-effectiveness ratio.

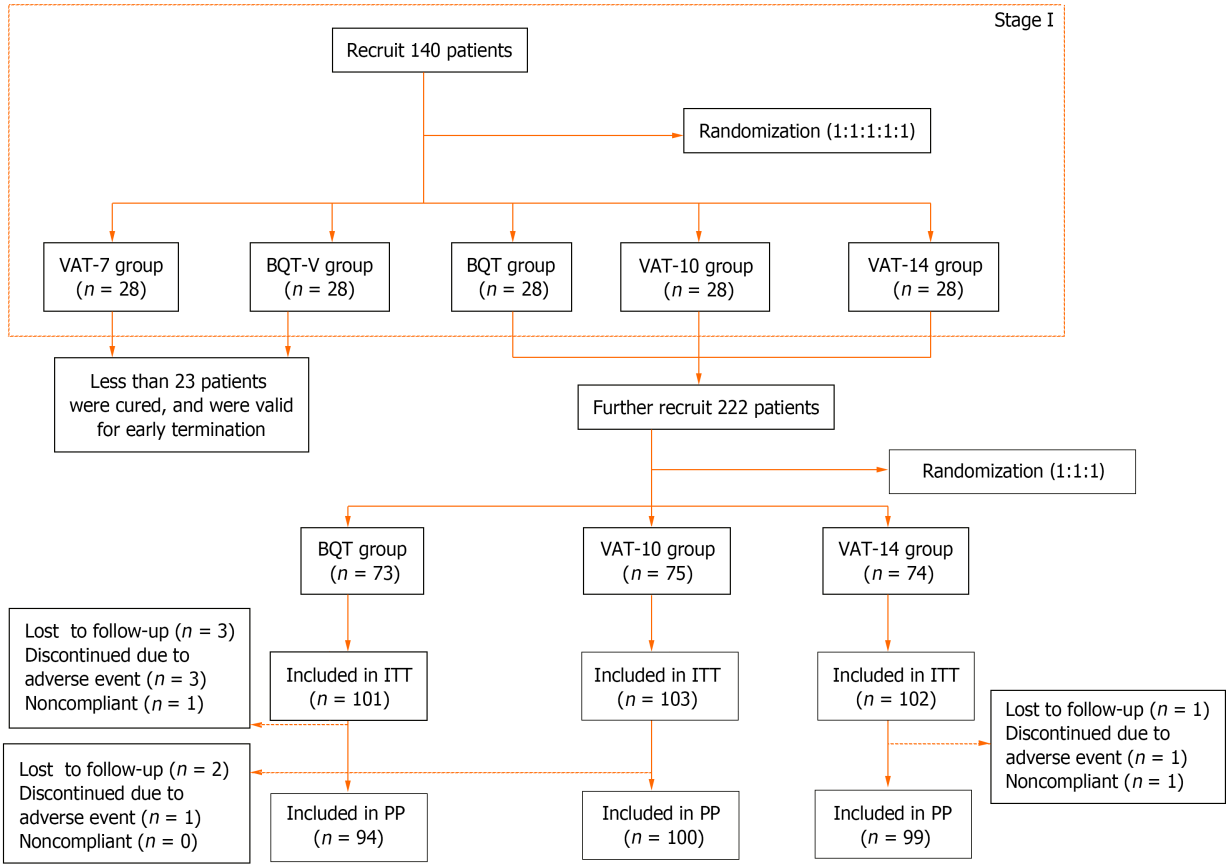

Figure 1 Flowchart of the clinical trial. VAT: Vonoprazan-amoxicillin dual therapy; BQT: Bismuth quadruple therapy; ITT: Intention-to-treat; PP: Per-protocol.

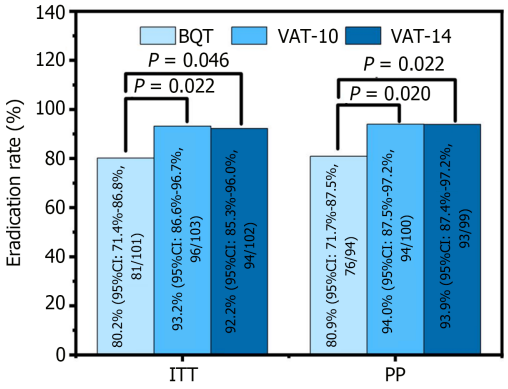

Figure 2 Intention-to-treat and per-protocol analyses of *Helicobacter pylori* eradication rates attained by bismuth quadruple therapy, Vonoprazan-amoxicillin dual therapy-10 and Vonoprazan-amoxicillin dual therapy-14. There were statistically significant differences in the eradication rates among the three regimens. VAT: Vonoprazan-amoxicillin dual therapy; BQT: Bismuth quadruple therapy; ITT: Intention-to-treat; PP: Per-protocol; 95%CI: 95% confidence interval.

required in such dual therapy regimens may induce some adverse events, resulting in poor patient compliance.

A dual therapy using Vonoprazan and AMX was first studied in Japan[18]. The therapy consisted of Vonoprazan (20 mg twice/day) and AMX (750 mg twice/day) for 7 days. This dual therapy regimen achieved an eradication rate of 92.9%, and was noninferior to Vonoprazan-amoxicillin-clarithromycin triple therapy in the ITT analysis. However, VAT-7 regimen in our study achieved a low eradication rate, which is similar to the results of two clinical studies in China[19,20]. As the course of VAT was extended to 10 or 14 days, the eradication rate of *H. pylori* increased to more than 90%. However, the eradication rate of 14 days VAT in Europe and the United States[12] was much lower than those of our study and other studies in China[21,22]. The underlying reason is unknown. It may be related to different species of *H. pylori* endemic in different areas around the world, leading to varied treatment outcomes. As for the dosage of Vonoprazan used in VAT, most studies proposed 20 mg twice a day. While we tried to use the dose of 20 mg a day in the VAT-14 group, we also achieved a satisfactory eradication rate. We showed that the dose of 20 mg a day for Vonoprazan was enough to maintain a sufficient intragastric pH for *H. pylori* eradication. A 10 or 14 days regimen is better than 7 days regimens, as previous numerous regimens.

The plasma concentration of amoxicillin decreases significantly after 6 to 8 hours. The effective blood concentration of amoxicillin can be maintained by increasing the frequency of administration. Compared with the frequency of twice a day, amoxicillin administered 3 to 4 times a day can improve the eradication rate of *H. pylori*[23]. As for the dosage of amoxicillin, a multicenter randomized trial in Japan showed low-dose amoxicillin (1.5 g/day) dual therapy provided acceptable and similar *H. pylori* eradication rates compared to Vonoprazan-based triple therapy in regions with high clarithromycin resistance[10]. However, the data from China indicated such regimen didn't provide the satisfactory eradication rate[20]. Thus, our study used a high dose of amoxicillin (1000 mg three times a day) to increase the eradication rate of *H. pylori* in VAT.

Due to its cytochrome P4502C19 (CYP2C19) polymorphism, Vonoprazan is less likely to interact with other drugs, such as warfarin and clopidogrel, than are PPIs, such as lansoprazole and omeprazole. CYP2C19 polymorphisms are not significant independent factors of *H. pylori* eradication using VAT. Furthermore, VAT regimens contain only two drugs and induce fewer adverse events than traditional BQT regimen. VAT regimens were also found to be more economical than BQT regimen. The eradication rate for BQT regimen was only 80.90% in our study. Our previous study showed that the rates of *H. pylori* antimicrobial resistance to amoxicillin, metronidazole, clarithromycin, and levofloxacin in Fujian were 14.90%, 50.96%, 38.94%, and 35.10%, respectively[24]. Hence, the treatment failure of BQT and BQT-V regimen may be due to the high resistance rate of CLA in Fujian. Therefore, under these circumstances, empiric treatment with a VAT regimen will have the potential to achieve higher eradication rates, and suppress the emergence of multidrug-resistant *H. pylori* strains, especially in areas where antibiotic resistance is growing or antimicrobial sensitivity tests are not readily available.

VAT regimen is also suitable for areas where bismuth cannot be obtained. If the patient is forbidden to use bismuth or cannot tolerate its adverse reactions, VAT regimen should be considered. VAT regimen is especially useful for elderly patients or patients with underlying diseases, such as liver and kidney dysfunctions. However, the VAT regimen is not suitable for patients who are allergic to penicillin antibiotics, such as amoxicillin. New regimens including other antibiotics combined with Vonoprazan warrants further investigation.

It should be noted that antibiotic resistance is not the sole reason for the failure of the effectiveness of *H. pylori* treatments in clinical practice. Poor patient compliance may also lead to treatment failure. In this study, medication side effects were the main reason for poor patient compliance. Failure of patients to take the full course of prescribed antibiotics increases the risk of bacteria developing antibiotic resistance. In the current study, the adverse reaction rate was higher in the BQT group than that in the VAT-10 group or VAT-14 group. However, overall patient compliance was excellent in all three groups. In our experience, patient education, including explanation of possible medication side effects and emphasis on the importance of treatment compliance is helpful in increasing patient compliance.

There are several limitations in our study. Firstly, the number of subjects in this study was small. The continuous involvement of more patients will be implemented in future studies since the *H. pylori* eradication rates observed were highly encouraging. Secondly, since most of the dual therapy studies published were performed in Asia, there might be bias caused by population characteristics. Therefore, a randomized, multicenter trial involving Europe, Africa, and other regions of the world should be designed to observe the effectiveness of VAT regimens in these regions in the future.

CONCLUSION

In summary, VAT with a duration of 10 days or 14 days, but not 7 days, achieved higher eradication rates than BQT with a more tolerable and manageable safety profile in patients in Fujian, China with *H. pylori* infection.

FOOTNOTES

Author contributions: Lin ZH and Wei JQ should be considered co-corresponding authors because of their significant contributions throughout the research; Lin ZH and Wei JQ conceived, designed, and supervised the study; Huang XP and Liu YJ contributed equally to the study; Huang XP and Liu YJ designed and performed the experiments, analyzed the data, prepared figures and tables, and authored and reviewed drafts of the article; Lin SW, Shao YF, Qiu F, Qiu QW, Xu ZK, Chen JX, Chen LH, Lin ZQ, Dai WH, Zhang MQ, Jiang Q, Xiao ZQ, Cheng XX, Zhang XF, You WB, Chen W, Li LQ, Lin WX, Wang YF, Lai FJ, Chen LQ, Huang ZH, and Zheng WQ collected and analyzed the data; and all authors have read and approved the final manuscript.

Supported by the Natural Science Foundation of Fujian Province, No. 2020J011087 and No. 2022J011025; Medical Innovation Project of Fujian Provincial Health Commission, No. 2020CXA006; and Zhuhai Science and Technology Project, No. 20181117E030040.

Institutional review board statement: The study was reviewed and approved by the Medical Ethics Committee of Fujian Provincial Hospital Institutional Review Board (Approval No. K2021-006-02).

Clinical trial registration statement: This study is registered at https://www.chictr.org.cn/showprojEN.html?proj=121897. The registration identification number is ChiCTR2100045778.

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

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

Data sharing statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONSORT 2010 statement: The authors have read the CONSORT 2010 statement, and the manuscript was prepared and revised according to the CONSORT 2010 statement.

Country of origin: China

ORCID number: Xue-Ping Huang 0000-0003-2803-2277; Wei-Xing Lin 0000-0002-3274-996X; Jin-Qi Wei 0000-0001-5308-4920; Zhi-Hui Lin 0000-0002-2165-9280.

S-Editor: Chen YL
L-Editor: A
P-Editor: Cai YX

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2024 July 21; 30(27): 3314-3325

DOI: 10.3748/wjg.v30.i27.3314

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Nomogram based on liver stiffness and spleen area with ultrasound for posthepatectomy liver failure: A multicenter study

Guang-Wen Cheng, Yan Fang, Li-Yun Xue, Yan Zhang, Xiao-Yan Xie, Xiao-Hui Qiao, Xue-Qi Li, Jia Guo, Hong Ding

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B, Grade C

Novelty: Grade B, Grade B, Grade C

Creativity or Innovation: Grade B, Grade B, Grade C

Scientific Significance: Grade B, Grade B, Grade C

P-Reviewer: Hernandez-Munoz R; Lampri E; Sivapatham S

Received: February 28, 2024

Revised: May 24, 2024

Accepted: June 17, 2024

Published online: July 21, 2024

Processing time: 133 Days and 16.2 Hours

Guang-Wen Cheng, Yan Fang, Li-Yun Xue, Xiao-Hui Qiao, Xue-Qi Li, Department of Ultrasound, Huashan Hospital, Fudan University, Shanghai 200040, China

Yan Zhang, Jia Guo, Department of Ultrasound, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

Xiao-Yan Xie, Department of Medical Ultrasonics, Institute of Diagnostic and Interventional Ultrasound, Sun Yat-sen University First Affiliated Hospital, Guangzhou 510080, Guangdong Province, China

Xue-Qi Li, Institute of Ultrasound in Medicine and Engineering, Shanghai Cancer Center, Shanghai 200040, China

Jia Guo, Department of Ultrasound, Eastern Hepatobiliary Surgical Hospital, Second Military Medical University, Shanghai 200433, China

Hong Ding, Department of Ultrasound, National Clinical Research Center for Aging and Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China

Co-first authors: Guang-Wen Cheng and Yan Fang.

Co-corresponding authors: Jia Guo and Hong Ding.

Corresponding author: Hong Ding, MD, PhD, Professor, Department of Ultrasound, Huashan Hospital, Fudan University, No. 12 Urumqi Middle Road, Shanghai 200040, China.
ding_hong@fudan.edu.cn

Abstract

BACKGROUND

Liver stiffness (LS) measurement with two-dimensional shear wave elastography (2D-SWE) correlates with the degree of liver fibrosis and thus indirectly reflects liver function reserve. The size of the spleen increases due to tissue proliferation, fibrosis, and portal vein congestion, which can indirectly reflect the situation of liver fibrosis/cirrhosis. It was reported that the size of the spleen was related to posthepatectomy liver failure (PHLF). So far, there has been no study combining 2D-SWE measurements of LS with spleen size to predict PHLF. This prospective study aimed to investigate the utility of 2D-SWE assessing LS and spleen area

(SPA) for the prediction of PHLF in hepatocellular carcinoma (HCC) patients and to develop a risk prediction model.

AIM

To investigate the utility of 2D-SWE assessing LS and SPA for the prediction of PHLF in HCC patients and to develop a risk prediction model.

METHODS

This was a multicenter observational study prospectively analyzing patients who underwent hepatectomy from October 2020 to March 2022. Within 1 wk before partial hepatectomy, ultrasound examination was performed to measure LS and SPA, and blood was drawn to evaluate the patient's liver function and other conditions. Least absolute shrinkage and selection operator logistic regression and multivariate logistic regression analysis was applied to identify independent predictors of PHLF and develop a nomogram. Nomogram performance was validated further. The diagnostic performance of the nomogram was evaluated with receiver operating characteristic curve compared with the conventional models, including the model for end-stage liver disease (MELD) score and the albumin-bilirubin (ALBI) score.

RESULTS

A total of 562 HCC patients undergoing hepatectomy (500 in the training cohort and 62 in the validation cohort) were enrolled in this study. The independent predictors of PHLF were LS, SPA, range of resection, blood loss, international normalized ratio, and total bilirubin. Better diagnostic performance of the nomogram was obtained in the training [area under receiver operating characteristic curve (AUC): 0.833; 95% confidence interval (95%CI): 0.792-0.873; sensitivity: 83.1%; specificity: 73.5%] and validation (AUC: 0.802; 95%CI: 0.684-0.920; sensitivity: 95.5%; specificity: 52.5%) cohorts compared with the MELD score and the ALBI score.

CONCLUSION

This PHLF nomogram, mainly based on LS by 2D-SWE and SPA, was useful in predicting PHLF in HCC patients and presented better than MELD score and ALBI score.

Key Words: Shear-wave elastography; Spleen; Hepatectomy; Posthepatectomy liver failure; Hepatocellular carcinoma

Core Tip: Posthepatectomy liver failure (PHLF) is a major complication after hepatectomy. Liver stiffness (LS) measured by ultrasound elastography can reflect liver reserve function, while splenic enlargement can also reflect liver reserve function. Ultrasound measurement of splenic size is simple, but there were few studies that used splenic size to predict PHLF. Our study used ultrasound elastography combined with spleen size and serological indicators to establish a predictive model for PHLF. It had the potential to predict PHLF, indicating that LS, and spleen size could be used for risk stratification in patients.

Citation: Cheng GW, Fang Y, Xue LY, Zhang Y, Xie XY, Qiao XH, Li XQ, Guo J, Ding H. Nomogram based on liver stiffness and spleen area with ultrasound for posthepatectomy liver failure: A multicenter study. *World J Gastroenterol* 2024; 30(27): 3314-3325
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3314.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3314

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common malignant liver tumor and the third-leading cause of cancer deaths worldwide[1]. Currently, surgical resection remains the preferred effective treatment for HCC. However, posthepatectomy liver failure (PHLF) is a major complication after hepatectomy, with a reported incidence ranging from 0.7% to 39.6%[2,3]. PHLF is a major cause of death in patients after hepatectomy with an approximate 50% mortality rate[4]. Therefore, an accurate risk prediction of PHLF is essential for improving clinical treatment strategies for HCC patients. The occurrence of PHLF is not only related to the scope of liver resection but also closely related to the liver reserve function of the residual liver. The presence of liver fibrosis or cirrhosis in over 70%-90% of HCC patients[5] has a significant impact on liver reserve function. Therefore, a comprehensive and effective preoperative evaluation of liver reserve function is crucial for developing a reasonable surgical plan to reduce the occurrence of PHLF.

The indocyanine green clearance test is widely used in Asia to evaluate liver reserve function. However, the accuracy of the results of this method may be influenced by multiple factors, so its effectiveness in predicting PHLF has been unsatisfactory in multiple studies[6-8]. In addition, some clinical models for assessing liver function reserve, such as the laboratory index-based model for end-stage liver disease (MELD) score and albumin-bilirubin (ALBI) score, have proven

to be of certain value in predicting the PHLF risk, but the predictive accuracy of these models remains inadequate with a ceiling effect[9,10]. Therefore, these methods have not been included in the current international HCC management guidelines and are not routinely used worldwide.

Computed tomography has been used to measure residual liver volume to predict PHLF in patients scheduled for major liver resection. However, residual liver volume cannot fully represent liver reserve function, especially for patients with liver cirrhosis[6]. Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid directly measures liver reserve function. However, this method is expensive and time-consuming, and previous reports have shown that its application requires complex calculations[11,12].

Liver stiffness (LS) measurement with two-dimensional shear wave elastography (2D-SWE) correlates with the degree of liver fibrosis and thus indirectly reflects liver function reserve[13-15]. Several previous studies showed a good predictive value of 2D-SWE for PHLF[6,16-18]. However, these studies investigated a small number of cases and lacked external validation. In addition, there was a deviation in LS measurements by ultrasound elastography during liver inflammation[19]. Splenomegaly is common in patients with liver fibrosis, especially cirrhosis. Due to the close correlation between liver fibrosis/cirrhosis and portal hypertension, as portal hypertension progresses, spleen size increases due to tissue proliferation, fibrosis, and portal vein congestion, which can indirectly reflect the situation of liver fibrosis/cirrhosis. Spleen size has been reported to be associated with PHLF[20,21]. So far, there have been no studies to predict PHLF by combining 2D-SWE measurement of LS with spleen size.

Therefore, the aim of the present study was to develop and validate a comprehensive PHLF prediction model based on LS measurement by 2D-SWE, spleen size, surgical factors, and laboratory indexes for providing better risk stratification of HCC patients before hepatectomy.

MATERIALS AND METHODS

Study design and population

This was a multicenter observational study consisting of two cohorts, a training cohort and a validation cohort. Between October 2019 and March 2022, consecutive patients undergoing hepatectomy were prospectively enrolled from centers A (Huashan Hospital), B (Eastern Hepatobiliary Surgical Hospital), and C (Shanghai Cancer Center) as the training cohort. Patients from centers D (Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine) and E (Sun Yat-sen University First Affiliated Hospital) were enrolled as the validation cohort. The inclusion criteria were as follows: (1) Age between 18 years and 85 years; (2) Patients with liver tumors prepared for partial hepatectomy; (3) Liver function classification of Child-Pugh A, B, or C; (4) Eastern Cooperative Oncology Group performance score 0-2[22]; and (5) LS measurement by 2D-SWE and spleen examination by ultrasound within 1 wk prior to surgery. The exclusion criteria were as follows: (1) Postoperative pathology indicating non-HCC; (2) Patients receiving preoperative anticancer treatment such as transhepatic arterial chemotherapy and embolization; (3) Patients receiving intraoperative ablation; (4) History of previous liver resection; (5) Failure in LS; and (6) Missing data. The detailed flowchart of patient selection is shown in Figure 1.

Data collection

The following patient data were collected: Demographic data (age and sex); preoperative laboratory data, including total bilirubin (TB), albumin, alanine transaminase, prothrombin time, international normalized ratio (INR), platelet (PLT) count, γ-glutamyl transpeptidase, white blood cell count, hemoglobin, alpha-fetoprotein, hepatitis B virus (HBV) status, and HBV-DNA level; tumor-related data (tumor size and number); surgical data [hepatic portal clamping time, blood loss (BL)]; liver resection range (RR) (major hepatectomy defined as liver resection of ≥ 3 Couinaud segments, minor hepatectomy defined as liver resection of < 3 Couinaud segments)[23]; and information about ultrasound imaging examination (LS measurement and spleen measurement).

Examination and interpretation of LS measurement by 2D-SWE

Liver 2D-SWE examination was performed on all patients using the Aixplorer ultrasound imaging system (Supersonic Imagine, Aix-en-Provence, France) equipped with a convex array probe SC6-1. In accordance with the European Federation of Societies for Ultrasound in Medicine and Biology guidelines, the procedures for liver 2D-SWE examination were as follows. The patient was asked to lie in a supine position with the right arm above the head after at least 4 h of fasting. An appropriate right intercostal or subcostal space was located for observing the right liver parenchyma using gray-scale ultrasound imaging; subsequently, the SWE model was switched on for elastography. The patient was then instructed to hold breath for at least 5 s to obtain a stabilized SWE image, and meanwhile the sampling frame (approximately 4 cm × 3 cm) was placed vertically on the liver parenchyma 1-2 cm below the liver capsule and at least 2 cm from the margins of liver masses, avoiding the intrahepatic vessels and bile duct. The color-coded elasticity map was required more than 80% filled. A region of interest (2 cm in diameter) was placed at the sampling frame for stiffness measurement in kPa. Five independent measurements were performed, and the measurements were considered successful when the interquartile range/median value was below 30%. Ultimately, the median of the five measurements was used as LS measurement.

Examination and interpretation of spleen area by ultrasound.

The longitudinal view of the spleen with the hilus was observed through the intercostal space near the tenth rib from the

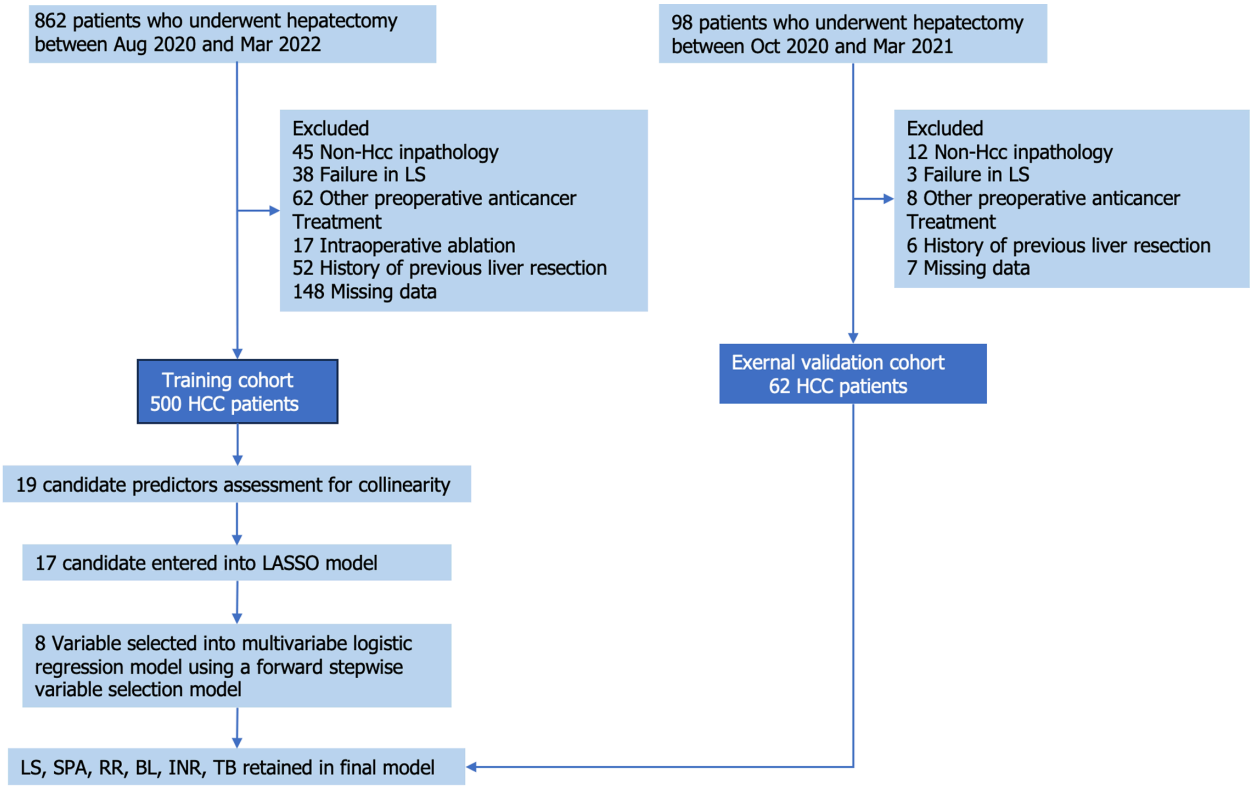

Figure 1 Flow chart of the cohorts in the study. BL: Blood loss; HCC: Hepatocellular carcinoma; INR: International normalized ratio; LASSO: Least absolute shrinkage and selection operator; LS: Liver stiffness RR: Resection range; SPA: Spleen area; TB: Total bilirubin.

posterior axillary line when the patient was placed in the right lateral position. In this location, the length and width of the spleen were measured. The spleen area (SPA, cm^2) was defined as the length (cm) × width (cm).

Diagnosis and definition

PHLF was diagnosed according to the criteria of the International Study Group on Liver Surgery[24]: According to the upper limit of normal values of the local laboratory on postoperative day 5, an increase in the INR (> 1.2) and hyperbilirubinemia (> 22 μmol/L or above preoperative value). The severity of PHLF was divided into three categories based on clinical management: Grade A, which does not require further clinical management; grade B, which requires active therapeutic intervention without invasive approaches; and grade C, which requires an invasive approach. We defined grade B and C PHLF as symptomatic PHLF (SPHLF), grade A or no PHLF were defined as non-SPHLF[25].

Statistical analysis

According to the sample size estimation of the area under receiver operating characteristic curve (AUC) of the diagnostic test and the incidence of PHLF in the literature, when the sensitivity = 0.90, the sample size was calculated for the diagnostic efficiency. AUC = 0.95, significance level = 0.05, power = 0.90, and the required sample size was calculated as 167 cases. A total sample size of 334 cases was required for the two subgroups.

Continuous variables in normal distribution were displayed as mean ± SD and analyzed by Student's *t* test, while continuous variables in non-normal distribution were presented as median (interquartile range) and analyzed by Mann-Whitney *U* test. In addition, categorical variables expressed as frequency (percentage) were compared by Pearson's χ^2 test or Fisher's exact test.

In the training cohort, the least absolute shrinkage and selection operator regression method was used to reduce the candidate predictor variables. We used logistic regression to further screen independent predictors and establish a multivariate prediction model. In this process, we used the stepwise forward method in SPSS to screen variables in the logistic regression model and used the default $P = 0.1$ in SPSS to determine the independent variables included in the model. A nomogram based on the predictive model was constructed and further validated in the validation cohort.

The AUC was used to assess the diagnostic performance of the predictive model compared with other traditional models (MELD score and ALBI score), and the AUC values were compared by DeLong's tests. Bootstrap with 2000 resampling was generated for the calibration curve in the training and validation cohorts as internal and external validation. The decision curve analysis (DCA) was used to evaluate the clinical effectiveness of the prediction model. $P < 0.05$ indicated a statistically significant difference. All the above statistical analyses were performed in R software (v.4.1.0; http: //www.r-project.org/) and SPSS (version 20.0; IBM Corp., Armonk, NY, United States).

RESULTS

Clinical features

The study included 500 eligible participants in the training cohort and 62 in the validation cohort. There were 142 cases and 22 cases of PHLF in the training cohort and the validation cohort, respectively. Among them, the number of PHLF A, PHLF B and PHLF C cases were 106, 32, and 4 cases in the training cohort, and 15, 6, and 1 cases in the validation cohort, respectively. One patient in the training cohort died within 90 d after surgery, with a mortality rate of 0.2%. The baseline characteristics of the patients are listed in Table 1. The baseline clinicopathologic data, including sex, age, laboratory indexes such as TB, INR, PLT, alpha-fetoprotein, HBV status, and HBV-DNA, tumor-related data, and surgical data such as BL, RR, LS, and SPA did not show significant differences between the training and validation cohorts ($P > 0.05$).

Selection of predictors and construction of nomogram model

Least absolute shrinkage and selection operator regression of the training cohort showed the right clinical and ultrasound features with non-zero coefficients with a minimum lambda value of 0.06. These features included the following eight variables: LS; SPA; RR; BL; alanine transaminase; prothrombin time; INR; and TB. Based on the above-screened variables, logistic regression was used to construct a multivariate prediction PHLF model (PM), which ultimately included six variables shown in Figure 2. Based on the multivariate prediction model, we developed a PM nomogram (Figure 3) to predict the risk of PHLF to provide a quantitative method for the clinicians. The score and predicted probability of PHLF can be calculated using the following formulas: PM = -8.343 + 0.176 × LS + 0.082 × SPA + 0.001 × BL - 1.086 × RR (major = 1; minor = 0) + 0.049TB + 0.148 × INR (multiplied by 10). The predicted probability of PHLF = 1/[1 + exp (-PM + 8.298)].

Diagnostic performance of the PM compared with previously reported models

In order to confirm the clinical utility of PM, we analyzed the correlation between the PM model and the previous commonly used ALBI and MELD models, and the spearman correlation coefficients between PM and ALBI and MELD were 0.62 and 0.59, respectively (both $P < 0.05$). The receiver operating characteristic curve and AUC values of the PM and the previously reported models (ALBI score and MELD score) for estimating PHLF risk were calculated and compared in the training and validation cohorts (Figure 4, Table 2). In both the training and validation cohorts, the predictive performance of PM on PHLF were significantly higher than those of ALBI and MELD ($P < 0.05$).

Calibration and DCA

The calibration curves (2000 bootstrap resamples) are graphically shown for the validation of the PM in both cohorts (Figure 5). The Hosmer-Lemeshow tests exhibited $P = 0.752$ in the validation cohort, which suggested that the predicted probability of the PM was well consistent with the actual outcome. The DCA curve also indicated that the PM had good clinical utility.

Subgroup analysis of SPHLF and non-SPHLF

The median LS of the SPHLF group was significantly higher than that of the non-SPHLF group (14.50 kPa *vs* 13.34 kPa, $P = 0.048$). Multivariate logistic regression analysis showed that LS ($P < 0.05$) and major liver resection ($P < 0.001$) were the independent predictors of SPHLF. Namely, patients with LS ≥ 12.52 kPa have an increased risk of SPHLF (odds ratio: 1.28), at which point the AUC of LS diagnosis of SPHLF is 0.80. Among all patients with PHLF, the incidence of SPHLF was significantly higher in patients with major liver resection than in those with minor liver resection (51.2% *vs* 14.1%, $P < 0.001$).

Subgroup analysis of the major liver resection group and the minor liver resection group using dual cutoff diagnosis based on LS and SPA

In patients with PHLF, the LS value and SPA in the major liver resection group were significantly lower than those in the minor liver resection group (LS: 13.00 kPa *vs* 14.24 kPa; $P = 0.046$; SPA: 45.3 cm^2 *vs* 53.8 cm^2; $P = 0.0013$). The diagnostic cutoff values of LS and SPA in 2D-SWE for diagnosing PHLF in the major liver resection and minor liver resection groups were evaluated using the dual cutoff diagnosis: For LS, 10.34 kPa in the major liver resection group (AUC = 0.74) and 13.48 kPa in the minor liver resection group (AUC = 0.78); and for SPA: 33.7 cm^2 in the major liver resection group (AUC = 0.78) and 43.2 cm^2 in the minor liver resection group (AUC = 0.84).

DISCUSSION

It is clinically important to assess preoperative liver function reserve to predict the development of PHLF. Our model comprehensively considered the effects of preoperative liver status and intraoperative factors. Multiple variable screening methods were used, combined with ultrasound indicators, serological indicators, and surgical-related indicators, to comprehensively evaluate the impact of relevant factors on the occurrence of PHLF. Through the nomogram, the contribution of various predictive indicators in the PM was visually displayed.

INR, TB, RR, and BL are all independent risk factors for PHLF. This is reasonable because INR and TB are the recognized indicators that reflect PHLF and are used to develop PHLF prediction models[9]. As for the RR, a high volume of hepatectomy is related to increased risks of PHLF[26]. BL is also an independent risk factor for PHLF, which is consistent with the study by Fang *et al*[27] Considering that the liver is a blood-rich organ, excessive bleeding may

Table 1 Descriptive characteristics of the study population, *n* (%)

Characteristics	Training cohort	Validation cohort	*P* value
Patients	500	62	
PHLF	142 (28.40)	22 (35.50)	0.250
Sex			0.080
Male	413 (89.01)	45 (72.58)	
Female	87 (10.99)	17 (27.42)	
Age in yr, mean ± SD	55.70 ± 10.70	53.05 ± 10.62	0.067
TB in mg/dL, median; IQR	12.8; 9.9-17.0	13.4; 9.2-17.2	0.740
ALB in g/L, median; IQR	43; 40.0-46.0	41; 38.8-45.0	0.130
ALT in U/L, median; IQR	27; 19.0-38.0	32; 21.0-39.0	0.250
PT in s, median; IQR	12.4; 11.7-13.2	12.0; 11.5-13.0	0.080
INR, median; IQR	10.5; 9.9-11.1	10.2; 9.7-10.9	0.110
PLT as × 10^9/L, median; IQR	148.5; 111.0-197.0	167.5; 139.8-192.0	0.060
GGT in U/L, median; IQR	43.0; 11.0-1019.0	44.5; 16.0-543.0	0.190
WBC as × 10^9, median; IQR	5.55; 1.84-14.07	5.88; 2.01-14.30	0.250
HB in g/L, median; IQR	142; 66-203	145; 105-267	0.200
AFP			0.680
≤ 20	239 (47.8)	27 (43.5)	
> 20	261 (52.2)	35 (56.5)	
LS in kPa, median; IQR	10.8; 7.9-14.0	9.6; 8.0-12.3	0.150
SPA in cm^2, median; IQR	38.70; 38.50-41.10	39.16; 37.90-44.80	0.370
Tumor size in cm, median; IQR	3.1; 0.5-25.0	3.8; 0.7-13.0	0.230
Tumor number, median; IQR	1; 1.0-15.0	1; 1.0-2.0	0.090
RR			0.070
Minor	400 (80.0)	43 (69.4)	
Major	100 (20.0)	19 (30.6)	
BL in mL, median; IQR	100; 50-200	175; 50-300	0.390
Clamping time in min, median; IQR	15.0; 0-69	13.5; 0-60	0.150
HBV			0.570
Positive	468 (93.6)	60 (96.7)	
Negative	32 (6.4)	2 (3.3)	
HBV-DNA level			0.680
≥ 10^3 IU/mL	286 (57.2)	32 (51.6)	
< 10^3 IU/mL	32 (42.8)	30 (48.4)	
PHLF			0.250
Absent	358 (71.6)	40 (64.5)	
Present	142 (28.4)	22 (35.5)	
PHLF ISGLS grade			0.310
0-A	464 (92.8)	55 (88.7)	
B-C	36 (7.2)	7 (11.3)	

Data in parentheses were used to calculate percentages. AFP: Alpha-fetoprotein; ALB: Albumin; ALT: Alanine transaminase; BL: Blood loss; GGT: γ-

glutamyl transpeptidase; HB: Hemoglobin; HBV: Hepatitis B virus; INR: International normalized ratio, multiplied by 10; IQR: Interquartile range; ISGLS: International Study Group on Liver Surgery; LS: Liver stiffness; PHLF: Posthepatectomy liver failure; PLT: Platelet; PT: Prothrombin time; RR: Range of resection; SD: Standard deviation; SPA: Spleen area; TB: Total bilirubin; WBC: White blood cell.

Table 2 Comparison of model discrimination

Variables	Training cohort, *n* = 500			Validation cohort, *n* = 62		
Model	**PM**	**ALBI**	**MELD**	**PM**	**ALBI**	**MELD**
AUC (95%CI)	0.833 (0.792-0.873)	0.651 (0.598-0.703)	0.508 (0.436-0.548)	0.802 (0.684-0.920)	0.658 (0.536-0.774)	0.631 (0.499-0.750)
Sensitivity, %	83.100 (118/142)	43.700 (62/142)	62.000 (88/142)	95.500 (21/22)	72.300 (13/22)	59.100 (13/22)
Specificity, %	73.500 (263/358)	80.200 (287/358)	53.100 (190/358)	52.500 (21/40)	57.500 (29/40)	72.500 (29/40)
P value	-	< 0.001[1]	< 0.001[2]	-	0.040[3]	0.048[4]

[1]Area under receiver operating characteristic curve (AUC) values of the albumin-bilirubin (ALBI) score compared to that of the posthepatectomy liver failure model (PM) in the training cohort.
[2]AUC values of the model for end-stage liver disease (MELD) score compared to that of the PM in the training cohort.
[3]AUC values of the ALBI score compared to that of the PM in the validation cohort.
[4]AUC values of the model for end-stage liver disease score compared to that of the PM in the validation cohort.
CI: Confidence interval.

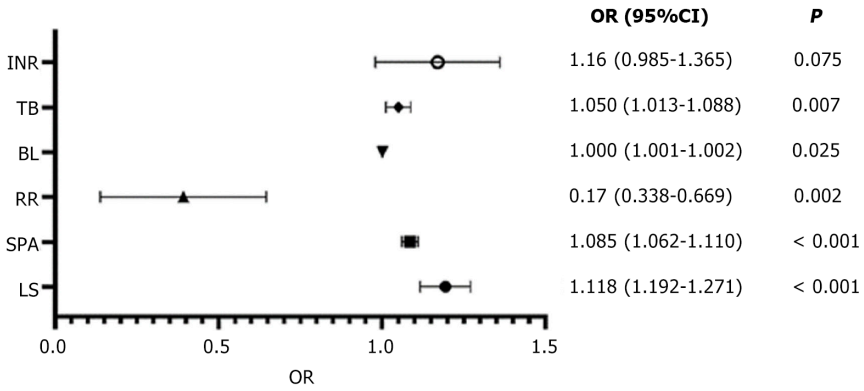

Figure 2 Forest plot of odds ratio for the multiple variables in logistic regression analysis. 95%CI: 95% confidence interval; BL: Blood loss; INR: International normalized ratio; LS: Liver stiffness OR: Odds ratio; RR: Resection range; SPA: Spleen area; TB: Total bilirubin.

inevitably lead to liver cell damage and decreased liver function. However, with the continuous refinement and standardization of surgical procedures, effective control of BL is not a complex and difficult task. By contrast, only a more accurate assessment of liver fibrosis/cirrhosis can predict liver reserve function more accurately, thereby improving the accuracy of predicting the occurrence of PHLF.

Ultrasound SWE has been confirmed and recommended by multiple guidelines for measuring LS to evaluate the degree of liver fibrosis[28-30], providing a theoretical basis for predicting PHLF by SWE-based LS measurement. Splenomegaly is associated with portal hypertension caused by cirrhosis and with poor prognosis[31,32]. Ultrasound is a convenient and useful tool for measuring spleen size.

In the prediction model we established, we found that LS and SPA measured by ultrasound were the independent risk factors for PHLF. Although many studies have established predictive models for PHLF based on LS measured by SWE in the past, the LS measured by SWE can be affected by inflammation. Indeed, there is often inflammation in HCC patients with liver fibrosis or even cirrhosis[33,34]. Therefore, considering the insufficient use of SWE alone to evaluate liver reserve function, a comprehensive evaluation of spleen size reflecting liver conditions was added. Bae *et al*[20] used specific software to measure spleen volume in three-dimensional computed tomography, and the results showed that spleen volume was an independent risk factor for predicting PHLF. However, their study required the use of unique software (Liver analysis, IntelliSpace Portal, Philips Health Systems), and the operation was time-consuming, which is not conducive to routine clinical use. The ultrasound measurement of spleen size in our study was simple and convenient, especially for patients with an enlarged spleen, making it more practical.

Previous studies have shown that PLT count was one of the risk factors for predicting PHLF[33], but our study did not show that PLT count was useful for predicting PHLF, which might be related to the criteria used when we included patients. For these thrombocytopenic patients, they were considered not eligible for surgery at our center. Therefore,

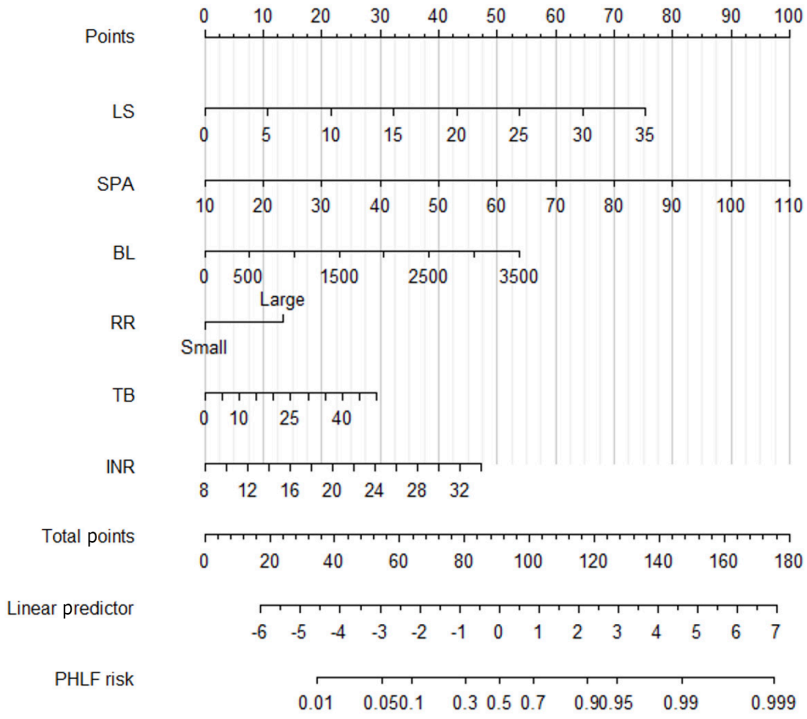

Figure 3 Nomogram of posthepatectomy liver failure model. BL: Blood loss; INR: International normalized ratio; LS: Liver stiffness; PHLF: Post-hepatectomy liver failure; RR: Resection range; SPA: Spleen area; TB: Total bilirubin.

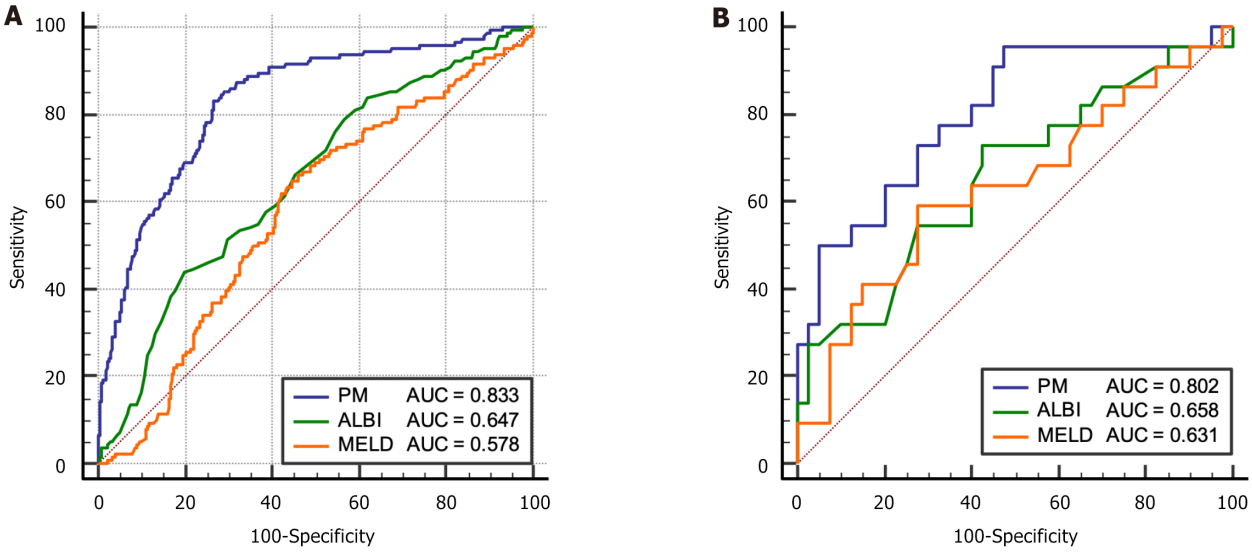

Figure 4 Receiver operating characteristic of models in training cohort and validation cohort. A: Training cohort; B: Validation cohort. ALBI: Albumin-bilirubin; MELD: Model of end-stage liver disease; PM: Posthepatectomy liver failure model.

many patients with severe thrombocytopenia were not included in this study.

We compared the established PM model with previous serological models ALBI and MELD in predicting liver failure, and the results showed that the PM model had a significantly higher AUC in predicting PHLF compared to ALBI and MELD. The sensitivity was always higher than the serological model, and the specificity was not always higher than the serological model. Since we hoped to effectively identify patients who might experience liver failure, we paid more attention to the sensitivity of the model in identifying liver failure. This model has achieved satisfactory sensitivity in both the training and validation cohort, and the AUC that reflected the diagnostic performance of the entire model was significantly better than the serological model.

We conducted a subgroup analysis of SPHLF and non-SPHLF. In the subgroup analysis, it was found that LS and RR were the independent risk factors of SPHLF, which seems understandable. Both RR and LS determine the number of effective liver cells in the residual liver after hepatectomy, thereby reflecting the liver reserve function of the residual liver after hepatectomy, which has been confirmed in previous studies[9,18,35]. We have determined that LS ≥ 12.52 kPa is the

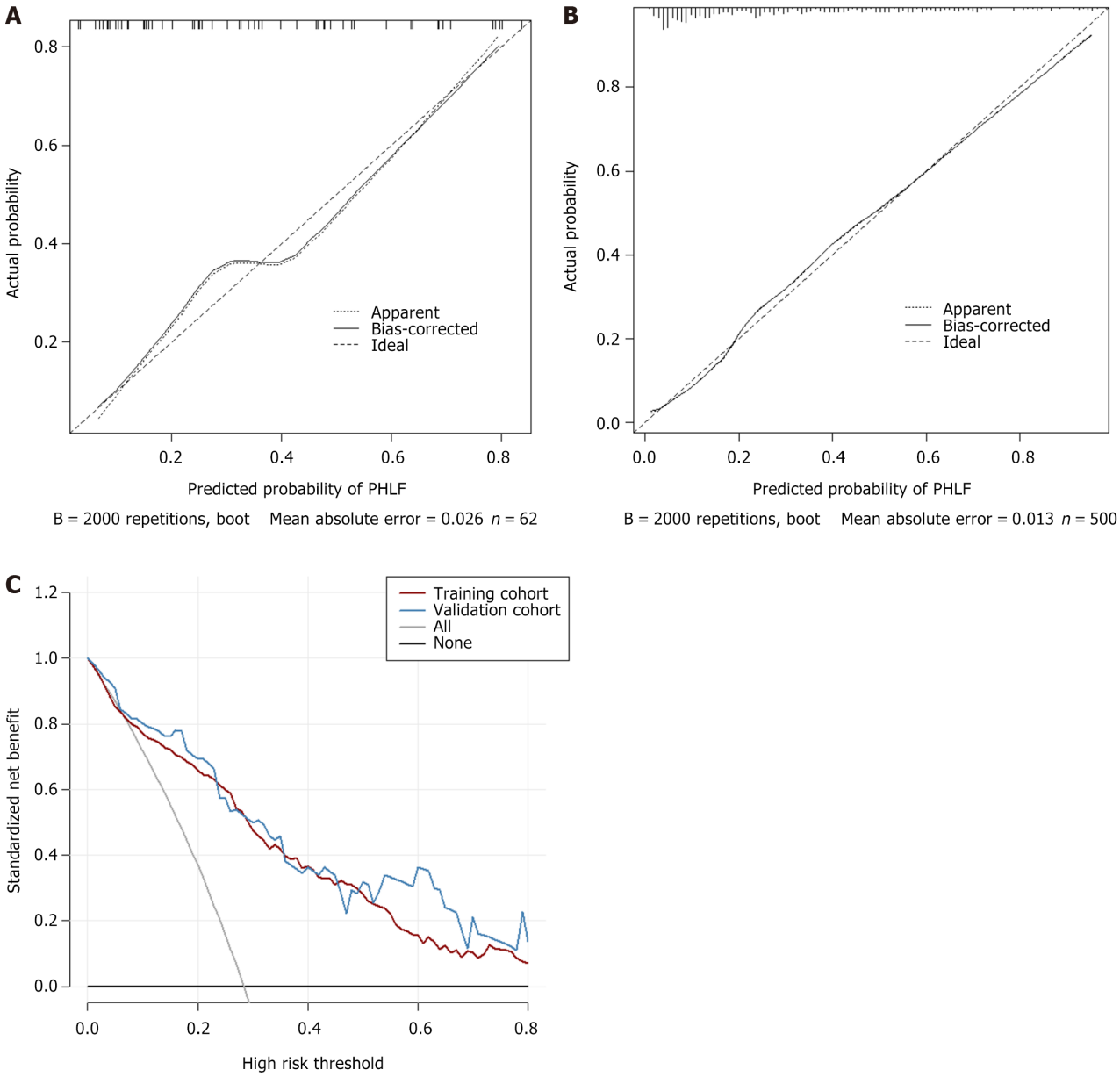

Figure 5 Figure calibration curves in the training cohort and the validation cohort and decision curve analysis of the prediction model. A: Training cohort; B: Validation cohort; C: Decision curve analysis of the prediction model. PHLF: Posthepatectomy liver failure.

cutoff value for diagnosing SPHLF. This is similar to the 11.90 kPa result obtained by Shen *et al*[34]. However, in the study of Long *et al*[18], the cutoff value for diagnosing SPHLF was 9.50 kPa and was quite different from our study, which might be related to the different number of cases and incidence rate of SPHLF between these studies. Namely, in the study by Long *et al*[18], 38 of 119 patients had SPHLF (an incidence rate of 31.9%), while in our study, 36 out of 500 patients had SPLF (an incidence rate of 7.2%). According to the new diagnostic criteria and literature, the incidence rate of PHLF is 9.0%-18.6%[36]. From the perspective of data, our incidence rate is closer to the literature reports, and our study was a multicenter study with a large sample size, better reflecting reality.

In addition, we conducted a subgroup analysis of the range of liver resection in the major liver resection group and the minor liver resection group. The results showed that the LS and SPA of PHLF patients in the major liver resection group were significantly lower than those in the minor liver resection group. In the case of a liver tumor with a large size that requires major liver resection, the LS greater than 10.34 kPa is recommended to prevent the occurrence of PHLF. However, when the tumor has a small range, the liver RR is also small, and the LS value reaches 13.48 kPa, we need to be alert to the occurrence of PHLF. Similarly, when the SPA is greater than 33.7 cm^2 and large liver resection is required, there may be a risk of PHLF. If minor liver resection is performed and the SPA reaches 43.2 cm^2 or more, there is a risk of PHLF.

The study had some limitations. First, almost the entire target population for this study included patients with HBV-related HCC, so this predictive nomogram needs further validation in patients with HCC of other etiologies, such as hepatitis C virus and alcohol abuse. Second, LS measurement by 2D-SWE reflects the stiffness of the focal liver tissue rather than that of the whole liver, which is an inherent limitation of ultrasound elastography. Third, the sample size in

the external validation cohort was not very large, so it is indispensable to increase the sample size for further external validation of the predictive nomogram.

CONCLUSION

In summary, our study established a nomogram for predicting the risk of developing PHLF by using data of patients from different centers. The nomogram showed better predictive performance than traditional models in both training and validation cohorts. In addition, the corresponding subgroup analysis for different situations provided surgeons with diagnostic cutoff values in different clinical scenarios, which can more effectively guide preoperative assessment of PHLF risk and effectively screen patients suitable for surgery.

ACKNOWLEDGEMENTS

We thank other centers involved in this multicenter study for their support in data collection.

FOOTNOTES

Author contributions: Cheng GW and Fang Y were responsible for designing the study, collecting data, analyzing data, and writing the paper. They contributed equally to this study. Xue LY, Zhang Y, Xie XY, Qiao XH, and Li XQ were responsible for the collection of cases and the collation of data. Ding H, Guo J and Xie XY were responsible for the authenticity and completeness of data from the center A and C, center B and D, and center E, respectively. All authors approved the final version of the manuscript for submission for publication. Ding H and Guo J contributed equally to this work as co-corresponding authors in responsibility for the design of the study, ethics, and overall research process, as well as for the writing and revision of the article.

Supported by the National Natural Science Foundations of China, No. 81873897 and No. 82102050; Shanghai Science and Technology Development Foundation, No. 22Y11911500; and Shanghai Municipal Health Commission of Science and Research Fund, No. 202140378.

Institutional review board statement: The study was reviewed and approved by the Medical Ethics Committee of Huashan Hospital of Fudan University (Approval No. 2020-1204).

Informed consent statement: All patients signed a presurgical informed consent form.

Conflict-of-interest statement: The authors declare that they have no competing interests.

Data sharing statement: The data underlying this article are available in HIMedc data manage system based on REDCap, at http://www.himedc.cn and requires authors' consent to be shared.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Country of origin: China

ORCID number: Guang-Wen Cheng 0000-0002-8831-9213; Hong Ding 0000-0002-9998-0904.

S-Editor: Chen YL
L-Editor: Filipodia
P-Editor: Zhang XD

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World Journal of Gastroenterology

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World J Gastroenterol 2024 July 21; 30(27): 3326-3335

DOI: 10.3748/wjg.v30.i27.3326

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Randomized Controlled Trial

Endoscopic polidocanol foam sclerobanding for the treatment of grade II-III internal hemorrhoids: A prospective, multi-center, randomized study

Chun-Ying Qu, Fei-Yu Zhang, Wen Wang, Feng-Yu Gao, Wu-Lian Lin, Hao Zhang, Guang-Yu Chen, Yi Zhang, Ming-Ming Li, Zheng-Hong Li, Mei-Hong Cai, Lei-Ming Xu, Feng Shen

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C

Novelty: Grade B, Grade C

Creativity or Innovation: Grade A, Grade B

Scientific Significance: Grade B, Grade B

P-Reviewer: Kumar R; Seow-Choen F

Received: March 11, 2024

Revised: May 28, 2024

Accepted: June 14, 2024

Published online: July 21, 2024

Processing time: 122 Days and 6.8 Hours

Chun-Ying Qu, Fei-Yu Zhang, Yi Zhang, Ming-Ming Li, Zheng-Hong Li, Mei-Hong Cai, Lei-Ming Xu, Feng Shen, Department of Gastroenterology & Endoscopy, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China

Wen Wang, Wu-Lian Lin, Department of Gastroenterology, The 900th Hospital of the People's Liberation Army Joint Service Support Force, Fuzhou 350025, Fujian Province, China

Feng-Yu Gao, Department of Endoscopy, Shandong Provincial Maternal and Child Health Care Hospital Affiliated to Qingdao University, Jinan 250014, Shandong Province, China

Hao Zhang, Department of Endoscopy, Baoshan People's Hospital of Yunnan Province, Baoshan 678000, Yunnan Province, China

Guang-Yu Chen, Department of Gastroenterology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China

Co-first authors: Chun-Ying Qu and Fei-Yu Zhang.

Co-corresponding authors: Lei-Ming Xu and Feng Shen.

Corresponding author: Feng Shen, MD, PhD, Chief Doctor, Department of Gastroenterology & Endoscopy, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, No. 1665 Kongjiang Road, Shanghai 200092, China. shenfeng01@xinhuamed.com.cn

Abstract

BACKGROUND

Endoscopic rubber band ligation (ERBL) is a nonsurgical technique for the treatment of symptomatic internal hemorrhoids but is limited by recurrence and post-procedural pain.

AIM

To evaluate satisfaction, long-term recurrence, and post-procedural pain in managing internal hemorrhoids using a combination of polidocanol foam sclerotherapy and ERBL.

METHODS
This was a prospective, multicenter, randomized study. A total of 195 consecutive patients diagnosed with grade II-III internal hemorrhoids were enrolled from four tertiary hospitals and randomly divided into a cap-assisted endoscopic polidocanol foam sclerobanding (EFSB) or an ERBL group. All patients were followed-up for 12 months. Symptom-based severity and post-procedural pain were assessed using a hemorrhoid severity score (HSS) and a visual analog scale (VAS). Continuous variables were reported as medians and interquartile range.

RESULTS
One hundred and ninety-five patients were enrolled, with 98 in the EFSB group. HSS was lower in the EFSB group than in the ERBL group at 8 weeks [4.0 (3.0-5.0) *vs* 5.0 (4.0-6.0), $P = 0.003$] and 12-month [2.0 (1.0-3.0) *vs* 3.0 (2.0-3.0), $P < 0.001$] of follow-up. The prolapse recurrence rate was lower in the EFSB group at 12 months (11.2% *vs* 21.6%, $P = 0.038$). Multiple linear regression analysis demonstrated that EFSB treatment [B = -0.915, 95% confidence interval (CI): −1.301 to −0.530, $P = 0.001$] and rubber band number (B = 0.843, 95%CI: 0.595-1.092, $P < 0.001$) were negatively and independently associated with the VAS score 24 hours post-procedure. The median VAS was lower in the EFSB group than in the ERBL [2.0 (1.0-3.0) *vs* 3.0 (2.0-4.0), $P < 0.001$].

CONCLUSION
Cap-assisted EFSB provided long-term satisfaction and effective relief from the recurrence of prolapse and pain 24 hours post-procedure.

Key Words: Internal hemorrhoids; Endoscopic therapy; Polidocanol foam; Sclerotherapy; Rubber band ligation; Sclerobanding

Core Tip: Endoscopic rubber band ligation (RBL) is a nonsurgical technique for the management of symptomatic internal hemorrhoids. However, it is constrained by recurrence and post-procedural pain. Endoscopic polidocanol foam sclerobanding (EFSB) is a novel approach known that has been proposed to address these challenges. In this study, we integrated endoscopic polidocanol foam sclerotherapy with RBL in patients presenting with grade II-III internal hemorrhoids and conducted a prospective, multicenter, randomized study to assess the long-term symptomatic and endoscopic efficacy of EFSB. Our findings demonstrated that EFSB offered long-term satisfaction and effective relief from prolapse recurrence and post-procedural pain.

Citation: Qu CY, Zhang FY, Wang W, Gao FY, Lin WL, Zhang H, Chen GY, Zhang Y, Li MM, Li ZH, Cai MH, Xu LM, Shen F. Endoscopic polidocanol foam sclerobanding for the treatment of grade II-III internal hemorrhoids: A prospective, multi-center, randomized study. *World J Gastroenterol* 2024; 30(27): 3326-3335
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3326.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3326

INTRODUCTION

Internal hemorrhoids are the most common benign condition of the anorectum. They are characterized by bleeding and prolapse during defecation, resulting in pain. Rubber band ligation (RBL) is a simple, cost-effective, and long-term treatment for symptomatic internal hemorrhoids. Injection sclerotherapy (IS) and RBL are the most commonly employed nonsurgical techniques for hemorrhoids[1]. Considering both efficacy and post-operative complications, Tutino *et al*[2] recommended IS as a first-line treatment option, and RBL for treating persistent symptomatic grade II-III prolapse. More recently, Pata *et al*[3] reported that sclerobanding, which combines RBL with polidocanol foam sclerotherapy using an anoscope, was a safe technique and had a low rate of post-operative complications.

The development of flexible endoscopy allowed use of retroflex endoscopic RBL (ERBL) to provide improved maneuverability, photographic documentation, as well as better performance of hemorrhoidal ligation[4]. However, RBL is associated with a higher long-term recurrence rate than that observed with surgical treatment[5]. The recurrence rate of RBL has been reported to range from 15.5% to 31.1% and varies depending on the hemorrhoid grade, ligation method, follow-up time, and retreatment sessions[5-7]. Post-procedural pain was identified as the most common complication[8, 9].

Polidocanol is a nonionic surfactant that mainly targets endothelial cells, causes vasospasm and inflammation, and is a local anesthetic[10]. Polidocanol induces an inflammatory reaction with sclerosis of the submucosal tissue and consequent contraction of the hemorrhoidal tissue, thus improving bleeding and prolapse. Use of this foam formulation is associated with reduced rates of post-procedural pain and bleeding recurrence when used to manage grade I and II internal hemorrhoids[11]. However, no significant series of retroflex endoscopy sclerobanding have been reported.

In addition, there is a lack of clinical trials comparing the endoscopic-based improvement of sclerobanding and ERBL for the long-term management of patients with grade II and III internal hemorrhoids. We hypothesized that the submucosal injection of polidocanol foam before RBL would lift the mucosa for easy ligation and increase fibrosis in the submucosal tissue, thus helping to improve long-term efficacy. Therefore, in this study, we evaluated the long-term symptomatic and endoscopic efficacy of endoscopic polidocanol foam sclerobanding (EFSB) for the treatment of grade II and III internal hemorrhoids.

MATERIALS AND METHODS

This multicenter, single-blind, randomized study was conducted in four Chinese Gastroenterology and Endoscopy centers (Xinhua Hospital, Shanghai; Shangdong Provincial Maternal and Child Health Care Hospital, Shangdong; Baoshan People's Hospital of Yunnan Province, Yunnan; The 900th Hospital of the People's Liberation Army Joint Service Support Force, Fuzhou). The ethics committee of the Xinhua Hospital approved the study (XHEC-C-2020-003-1), and all patients provided written informed consent before enrolling in the study.

Inclusion and exclusion criteria

Consecutive adult patients who were 18-60 years of age and diagnosed with grade II or III internal hemorrhoids according to the traditional Goligher classification between May 2020 and March 2021 were prospectively enrolled. The inclusion criteria were obvious symptoms of prolapse with or without bleeding, no previous history of endoscopic treatment, failure of conservative treatment (such as adequate fluid or fiber intake, medication, and lifestyle changes), and a willingness to undergo simultaneous colonoscopy. The exclusion criteria were: (1) Over 60 years of age; (2) Severe cardiopulmonary insufficiency; (3) Diagnosis of malignant tumors; (4) Colon polyps > 1 cm in diameter or at > 3 sites; (5) Diagnosis of inflammatory bowel disease or other perianal diseases (*e.g.*, anal fistulas and fissures); (6) Autoimmune disease; (7) History of internal hemorrhoid surgery; (8) Allergy to polidocanol; and (9) Lost to follow-up.

Sample size and randomization

Our primary aim was a superiority comparison of prolapse recurrence at 12 months post-procedure between the EFSB and ERBL groups. In previous studies, the average long-term recurrence rate was approximately 15%[5,6]. However, EFSB is known to reduce the recurrence rate to 10%. First, we performed a power calculation. With a statistical power of 90%, a type I error of 5%, and a superiority margin of 10%, the analysis indicated that we required 166 participants (83 in each group). Assuming a dropout rate of 15%, a final sample of 195 patients was required.

Randomization was performed after the colonoscopy had been completed and indicated that endoscopic treatment of internal hemorrhoids was necessary. Random numbers were generated by a computer with SPSS version 22.0 (IBM Corp., Armonk, NY, United States), written on cards, and placed in envelopes. The participants were randomized to two groups according to the corresponding random numbers to an EFSB group that received 10 mg/mL of polidocanol foam sclerotherapy combined with RBL, and an ERBL group that received endoscopic RBL only.

Clinic and laboratory variables

We calculated the symptom-based hemorrhoid severity score (HSS)[12] at baseline (T0) to assess its potential effect on the quality of the life of patients. The HSS was used to record five self-assessed symptoms (pain on defecation, itching, bleeding, soiling, and prolapse requiring manual repositioning) according to their frequency (0, never; 1, monthly; 2, weekly; 3, daily) during the previous month, with a total score ranging from 0 to 15. Low scores indicated elevated levels of patient satisfaction[13]. The anthropometric measurements obtained during the initial interview included height, body mass, and body mass index (BMI, kg/m^2). Fasting venous blood samples were collected to determine preprocedural hemoglobin level, prothrombin time, and alanine aminotransferase (ALT) level 1 d before the procedure.

Preprocedural preparation

Anticoagulant drugs were stopped 5-7 d before the procedure. A polyethylene glycol solution (with a split dose) was utilized for bowel preparation. A 1% polidocanol foam (20 mg/2 mL, Hameln Pharmaceuticals GmbH, Germany) was prepared as described by Moser *et al*[14], with a temporary preparation before injection. The endoscopy equipment included an Olympus GIF-H290 gastroscope, a CF H290I colonoscope, a transparent cap (Olympus, Japan), an Interject injection therapy needle catheter (23 g, Boston Scientific, United States), and a Speedband Superview Super 7 Multiple Band Ligator (Boston Scientific, United States).

Treatment and endoscopic classification

All patients were required to undergo a complete colonoscopic examination under propofol intravenous anesthesia prior to internal hemorrhoid therapy. Intestinal polyps were removed by cold snare polypectomy before hemorrhoid treatment. We changed to a gastroscope, and the anal canal was opened with a transparent cap, fully inflated, and rinsed with a water jet. Subsequently, the patient was examined by gastroscopy. The evaluation items were assessed in both forward and retroflexed positions and then classified by the degree of range, form, and the presence of red color signs [15]. The range was determined by the circumferential distribution of the internal hemorrhoids and was classified into five grades (0, no hemorrhoids; 1, one-quarter the circumference; 2, half the circumference; 3, three-quarters the circumference; 4, the whole circumference). The form was determined by the diameter of the largest hemorrhoid and was

classified into three grades (0, no hemorrhoids; 1, < 12 mm in diameter; 2, ≥ 12 mm or more in diameter). In the EFSB group, polidocanol foam was primarily injected into the submucosa under the pedicle of the hemorrhoids or around the prominent red signs above the dentate line (Figure 1A). The injection was stopped when the mucosa was fully raised, and the amount of foam at each point was < 4 mL (Figure 1B). A transparent cap was used to suppress extensive bleeding while pulling out the needle. A multiple rubber band ligator was attached to the top of the gastroscope in a retroflexed position to remove negative pressure and ligate the upper mucosa of the hemorrhoid. A single band was released in each hemorrhoid while avoiding repeated ligation of the same plane (Figure 1C). The complete procedure is shown in Video, attached. Patients in the ERBL group underwent endoscopic RBL without injection. All patients were treated by gentle manual massage and assisted during their recovery from prolapse to prevent acute thrombosis following the procedure [16]. At each center, all procedures were performed by the same physician.

Follow-up and outcomes

Patients were kept under observation in a day ward for 24 hours post-operatively to exclude serious adverse events or complications, and were discharged only after completing the post-procedural pain assessment. All participants were instructed to consume a low-fiber diet for 3 d to ensure a soft stool consistency and were advised to maintain external cleanliness. Participants were followed-up for 12 months and completed questionnaires in the outpatient department, at 24 hours (T1), 1 week (T2), 4 weeks (T3), 8 weeks (T4), and 12 months (T5) after the procedure. Participants in both groups were followed-up as outpatients at 3 months intervals thereafter until 12 months had elapsed or recurrence occurred. Symptom-based HSS questionnaires, post-procedural pain, treatment-induced complications, and recurrence of prolapse and/or bleeding were recorded.

The primary study endpoint was prolapse and/or bleeding recurrence after long-term evaluation. Recurrence was defined as an improved but persistent prolapse with or without bleeding in both grade II and III hemorrhoids. All cases were confirmed by the same endoscopic physician who performed the treatment (Figure 2). Overall satisfaction was assessed at T4 and T5 with the HSS. Safety was evaluated as an additional primary outcome of patient-reported adverse reactions. The secondary endpoint was post-procedural pain and bleeding in terms of short-term and long-term satisfaction, and patient-reported adverse reactions.

Statistical analysis

Statistical analysis was performed with SPSS version 22.0 for Windows (IBM Corp. Armonk, NY, United States). Continuous variables were reported as medians and interquartile range (IQR). The Mann-Whitney *U* test was used to compare between-group differences of continuous variables. Count datas were reported as numbers and proportions. The χ^2 test was used to compare differences of categorical variables. Correlations of the VAS score and other variables were assessed by Spearman's correlation coefficient (ρ). Stepwise multiple linear regression was used for multivariate analysis, and 95% confidence intervals (CIs) were calculated. All statistical tests were two sided. Statistical significance was set at $P < 0.05$. The statistical methods were reviewed by Guang-Yu Chen of Clinical Research Unit, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine.

RESULTS

Patient characteristics

During the study period, 217 patients with grade II or III internal hemorrhoids with prolapse and/or bleeding underwent endoscopy. A total of 22 patients were excluded, 2 patients with ulcerative colitis (remission), 4 with malignant tumors, 1 with sicca syndrome, 3 over 60 years of age, 7 with colon polyps > 1 cm in diameter or > 3 sites, and 5 who were lost to follow-up (Figure 3). The remaining 195 patients with symptomatic internal hemorrhoids were prospectively enrolled. The majority were men (n = 108, 55.4%) and the median age (IQR) was 46.0 (40.0-50.0) years. Of the 97 patients in the ERBL group, 73.2% (n = 71) were grade II, of the 98 in the EFSB group, 67.3% (n = 66) were grade II. No statistically significant differences of sex, age, BMI, hemoglobin level, prothrombin time, or ALT level in the two groups in terms were observed (Table 1).

Endoscopy treatment

Of the total participants (n = 195), 5.0 (4.0-5.0) rubber bands were used for grade III hemorrhoids, which was significantly higher than those used for grade II [3.0 (3.0-3.5), $P < 0.001$]. The number of rubber bands used in the ERBL and EFSB groups were 3.0 (3.0-4.5) and 3.0 (3.0-4.3), respectively, and the between-group differences were not significant (P = 0.821). In the EFSB group, 13.0 (12.0-15.0) mL polidocanol foam was injected at 4.6 (3.8-5.0) sites; no polidocanol was injected in the ERBL group. In the ERBL group, the rubber band fell off during the operation in 3.1% of participants (n = 3) because of insufficient suction or premature release. Exhausted rubber bands were identified in 1.0% of the patients (n = 1) because of incomplete absorption of intestinal gas following the completion of treatment. All patients underwent colonic endoscopy. A total of 35.9% (n = 70) of the patients had intestinal polyps and underwent cold snare polypectomy before ERBL or EFSB (39.2% *vs* 32.7%, n = 38 *vs* n = 32, P = 0.212).

Long-term recurrence and satisfaction

The rate of prolapse recurrence in the EFSB group was 11.2% (n = 11) and was significantly lower than that in the ERBL group (21.6%, n = 21) at T5 (P = 0.038). In general, the HSS decreased with follow-up time in both the ERBL and EFSB

Table 1 Clinical and laboratory characteristics of patients in the endoscopic rubber band ligation and endoscopic foam sclerobanding groups

Characteristic	Total patients, *n* = 195	ERBL, *n* = 97	EFSB, *n* = 98	*P* value
Male sex	108 (55.4)	58 (59.8)	50 (51.0)	0.250
Age in years	46.0 (40.0-50.0)	47.0 (40.5-50.0)	45.0 (39.5-50.0)	0.948
Goligher classification				
Grade II	137 (70.3)	71 (73.2)	66 (67.3)	0.231
Grade III	58 (29.7)	26 (26.8)	32 (32.7)	
Haemorrhoid severity score	8.0 (6.0-9.0)	9.0 (7.5-10.0)	8.00 (6.00-9.25)	0.564
Body mass index in kg/m^2	21.7 (20.0-23.0)	20.7 (19.0-22.1)	21.8 (20.0-23.2)	0.896
Haemoglobin in g/L	130.0 (125.0-138.0)	129.0 (123.0-139.0)	134.5 (125.0-138.0)	0.319
Prothrombin time in sec	12.0 (11.0-12.0)	12.0 (11.0-12.0)	11.0 (10.1-12.8)	0.752
Alanine aminotransferase in U/L	25.0 (16.9-33.0)	25.0 (15.0-33.0)	24.0 (19.0-32.3)	0.316

Continuous variables are medians (interquartile ranges) and compared using the Mann-Whitney *U* test. Count data are percentages and analysed using the χ^2 test. EFSB: Endoscopic foam sclerobanding; ERBL: Endoscopic rubber band ligation.

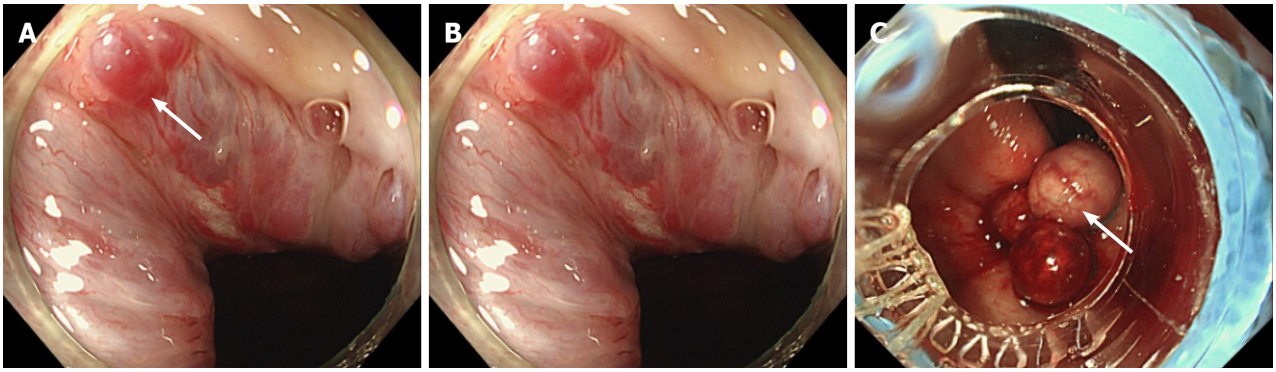

Figure 1 Endoscopic transparent cap-assisted endoscopic foam sclerobanding for the treatment of internal hemorrhoids. A: An obvious red color sign of internal hemorrhoids was observed with the transparent cap (indicated by arrows); B: Submucosal foam polidocanol injection, the white foam, was prominently raised; C: Negative pressure remove and ligation of the hemorrhoids with a retroflexed position. The white ball (arrow) is the ligated mucosa after foam sclerotherapy injection.

groups. The HSS was significantly lower in the EFSB group than in the ERBL group at both T4 [4.0 (3.0-5.0) *vs* 5.0 (4.0-6.0), $P = 0.003$], and T5 [2.0 (1.0-3.0) *vs* 3.0 (2.0-3.0), $P < 0.001$], see Figure 4A.

Post-procedural pain

At T1, the VAS in the EFSB group was 2.0 (1.0-3.0), which was significantly lower than that in the ERBL group [3.0 (2.0-4.0), $P < 0.001$]. However, the difference was not significant at T2 (Figure 4B). At T1, 65.3% ($n = 64$) of patients in the EFSB group had non-mild pain and the percentage was significantly higher than that in the ERBL group (40.2%, $n = 39$, $P = 0.001$). No statistically significant differences were observed at T2 (Table 2).

Next, we focused on T1 and discovered that the VAS values were strongly associated with the EFSB group ($\rho = 0.264$, $P < 0.001$), grade ($\rho = 0.251$, $P < 0.001$), and the number of rubber bands ($\rho = 0.391$, $P < 0.001$). Multiple linear regression analysis revealed that the EFSB group (B = -0.915, 95%CI: −1.301 to −0.530, $P = 0.001$) and the number of rubber bands (B = 0.843, 95%CI: 0.59-1.092, $P < 0.001$) were independently associated with VAS. In the ERBL group, one female patient experienced severe persistent pain for more than 4 hours following the procedure. An endoscopic examination revealed that the anal papilla and internal hemorrhoids were bandaged together. Her pain gradually decreased with the use of an anti-inflammatory analgesic suppository.

Post-procedural complications

Post-procedural bleeding was another frequent complication, and was recorded in 52.8% ($n = 103$) of the patients at T2. The rates of no, mild, moderate, and severe bleeding in the ERBL and EFSB groups were 49.5% and 44.9% ($n = 48$ *vs* $n = 44$), 40.2% and 41.8% ($n = 39$ *vs* $n = 41$), 8.2% and 9.2% ($n = 8$ *vs* $n = 9$), and 2.1% and 4.1% ($n = 2$ *vs* $n = 4$), respectively. No statistically significant differences were observed between the groups ($P = 0.815$). A 42-year-old male patient with severe

Table 2 Comparison of post-procedural pain of patients in the endoscopic rubber band ligation and endoscopic foam sclerobanding groups

Visual analogue scale	Total patients, *n* = 195	ERBL, *n* = 97	EFSB, *n* = 98	*P* value
T1[1]				
None-mild, ≤ 2	103 (52.8)	39 (40.2)	64 (65.3)	0.001
Moderate, 3-6	90 (46.2)	56 (57.7)	34 (34.7)	
Severe, ≥ 7	2 (1.0)	2 (2.1)	0 (0)	
T2[2]				
None-mild, ≤ 2	175 (89.7)	85 (87.6)	90 (91.8)	0.232
Moderate, 3-6	20 (10.3)	12 (12.4)	8 (8.2)	
Severe, ≥ 7	0 (0)	0 (0)	0 (0)	

[1]T1: 24 hours post-procedure.
[2]T2: 1 week post-procedure.
Count data are percentages and analysed using the χ^2 test.

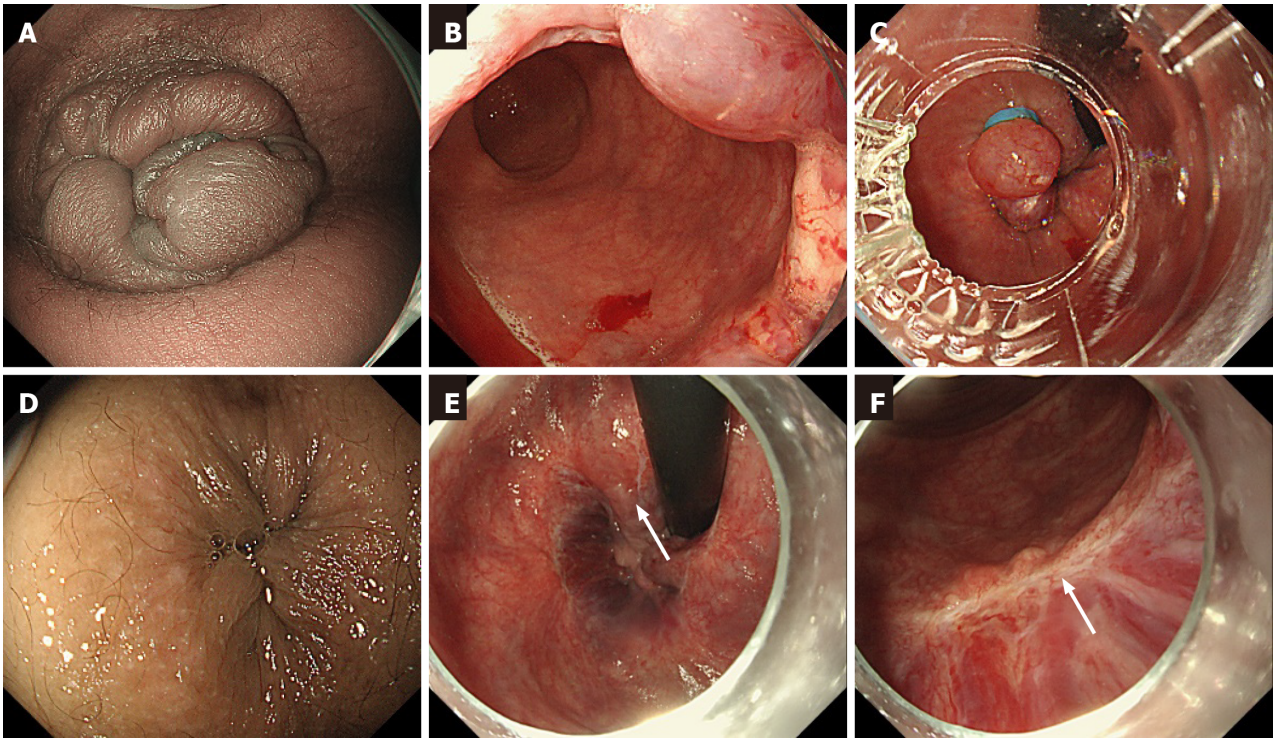

Figure 2 Treatment and follow-up of a patient with grade III internal hemorrhoids. A: Internal hemorrhoids with prolapse; B and C: Endoscopic foam sclerobanding (EFSB); D: 12 months after EFSB without apparently prolapse; E: Formation of scars (arrow) in retroflexed; F: Formation of scars (arrow) in normal position.

bleeding in the EFSB group was diagnosed with an anal ulcer that resolved with conservative treatment using a hemorrhoid suppository. At T3, the rates of no and mild bleeding in the ERBL and EFSB groups were 55.7% and 59.2% (n = 54 *vs* n = 58), and 44.3% and 40.8% (n = 43 *vs* n = 40), respectively (P = 0.363). None of the patients experienced moderate or severe bleeding at T3. A 58-year-old male patient developed urination difficulties that improved after the application of heat without special treatment. No pelvic abscesses, fever, thrombosis, anal fistulas, or fissures were recorded in either group.

DISCUSSION

To our knowledge, this is the first study to use an endoscope combined with polidocanol foam sclerotherapy and RBL to

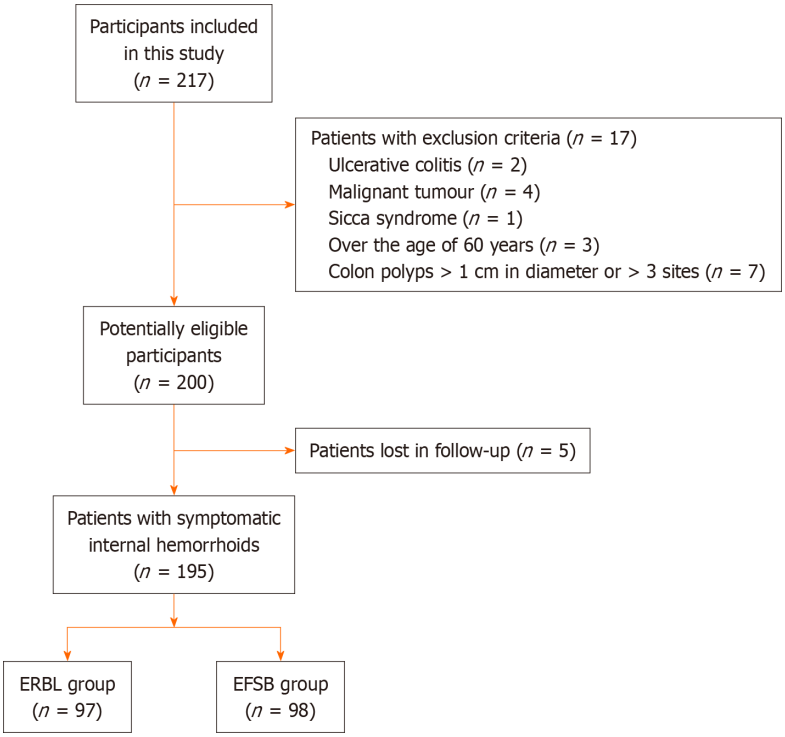

Figure 3 Flowchart of patient inclusion, exclusion and intervention. During the study period, 217 patients with grade II or III internal hemorrhoids were enrolled. A total of 22 patients were excluded, 2 with ulcerative colitis (remission), 4 with malignant tumors, 1 with sicca syndrome, 3 over 60 years of age, 7 with colon polyps > 1 cm in diameter or at > 3 sites, and 5 who were lost to follow-up. The remaining patients were randomly assigned to endoscopic rubber band ligation (*n* = 97) or endoscopic foam sclerobanding (*n* = 98). EFSB: Endoscopic polidocanol foam sclerobanding; ERBL: Endoscopic rubber band ligation.

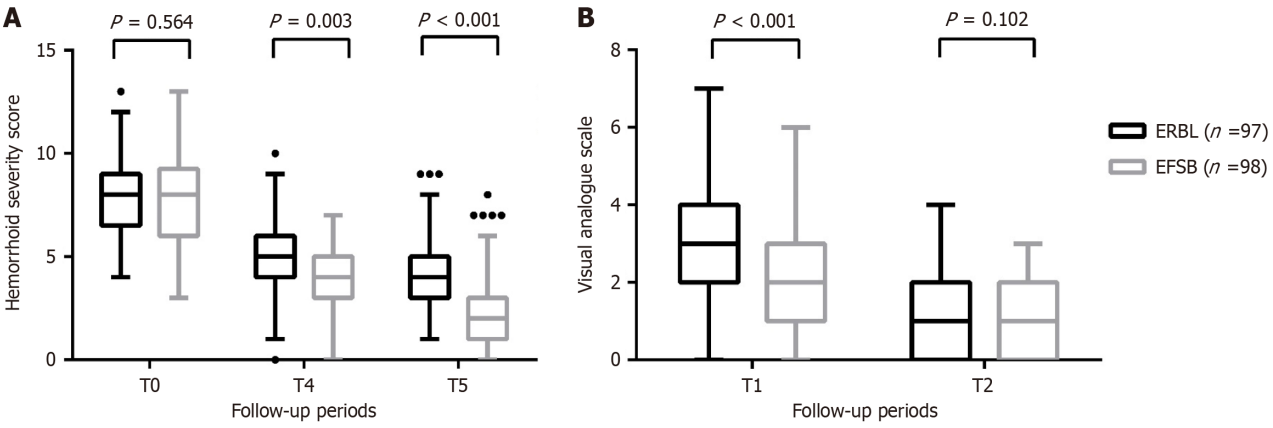

Figure 4 Box plots. A: Hemorrhoid severity score in the endoscopic rubber band ligation (ERBL) group and the endoscopic foam sclerobanding (EFSB) group according to follow-up time. Before procedure (T0), at 8 weeks (T4), and at 12 months (T5) post-procedure. Upper and lower whiskers indicate the 75th percentile plus 1.5 interquartile range (IQR) and the 25th percentile indicates minus 1.5 IQR. Outlier: A value greater than the 75th percentile plus 1.5 IQR; B: Pain visual analogue scale score in the ERBL group and the EFSB group on follow-up at 24 hours (T1) and 1 week (T2) post-procedure. Upper and lower whiskers indicate the 75th percentile plus 1.5 IQR and the 25th percentile minus 1.5 IQR.

treat patients with grade II and III internal hemorrhoids. We demonstrated that the submucosal injection of polidocanol foam before RBL reduced long-term prolapse recurrence and relieved short-term post-procedural pain. Compared to the ERBL group, the EFSB group experienced a lower recurrence rate (11.2% *vs* 21.6%, *n* = 11 *vs* *n* = 21) and a significant reduction in symptomatic HSS [2.0 (1.0-3.0) *vs* 3.0 (2.0-3.0)] at the end of follow-up. Multiple linear regression analysis confirmed that EFSB treatment was independently and negatively associated with the VAS at 24 hours post-procedure. After 1 week, pain was relieved in both groups. This multi-center study found that EFSB was associated with a high level of satisfaction, low recurrence at 12 months follow-up, and effective relief of pain at 24 hours post-procedure.

Currently, nonoperative management of internal hemorrhoids is considered a valid alternative to surgical therapy[7]. One theory suggests that internal hemorrhoids are caused by abnormal expansion and distortion of blood vessels and destructive changes in the anal cushion that supports connective tissue [17]. The primary principle of RBL is to remove abnormal anal cushion tissue, promote fibrosis, and attach the anal cushion to the mucosa, thereby alleviating the main

symptoms of prolapse. Compared to traditional proctoscopy, RBL use of a flexible endoscope is considered to provide better maneuverability, better vision, more rubber band release, and a lower bleeding recurrence rate[18]. However, use of RBL or ERBL to treat internal hemorrhoids with severe prolapse is associated with a high recurrence rate. A systematic review by Dekker *et al*[5] reported a recurrence rate of 31.1% (n = 50) with RBL treatment and was higher than that observed after hemorrhoidectomy. Compared to hemorrhoidal artery ligation, the recurrence rate of grade II and III internal hemorrhoids 1 year after RBL treatment was 49%[19]. To date, various evaluation criteria such as hemorrhoid grades, session times, and different follow-up periods have been used to determine the recurrence rate, and the results have been variable [7]. The primary goal of treating grade II and III internal hemorrhoids with EBL is to alleviate prolapse. Thus, the primary aim of our study was to investigate the recurrence of prolapse but not bleeding. This multicenter study, which had a 12-month follow-up period, indicated that sclerotherapy decreased the recurrence of prolapse by promoting submucosal fibrosis. The submucosal injection of foam helps lift the mucosa for easy ligation and prevents aspiration of the muscularis propria, thereby reducing post-operative pain caused by visceral innervation.

The treatment of internal hemorrhoids is now believed to involve the improvement of symptoms instead of cushion removal. Therefore, in the present study, we used the HSS to assess overall satisfaction, and symptom-based questionnaires are now required to guide the management of internal hemorrhoidal disease[20]. In this study, we observed that post-procedural pain was frequently experienced following treatment with RBL or ERBL. Approximately, 25% to 50% of the patients experienced post-procedural pain, especially in the first 48 hours following the procedure[21]. Komporozos *et al*[7] reported that multiple banding, a young age, male sex, and external hemorrhoids were all risk factors for increased pain. In this study, post-procedural pain mainly occurred after 24 hours and was independently associated with the number of rubber bands. The findings agree with those reported by Komporozos *et al*[7]. Thus, a clear need exists to improve the post-procedural pain caused by band ligation. The infiltration of local anesthesia, the administration of analgesia, warm salt baths, and the removal of the elastic bands, have all been used to reduce pain[7,9]. In this study, the submucosal injection of a foam sclerosing agent effectively improved acute perianal pain caused by the rubber band. We hypothesize that complete separation of the mucosa and muscularis propria after submucosal injection can avoid incorrect ligation or excessive tension of the muscular layer and results in relieving splanchnic-nerve pain.

Polidocanol is a detergent-type sclerosant that is widely used for the management of varicose veins[22]. Polidocanol is also a local anesthetic and the foam formulation leads to a homogeneous distribution of drug microbubbles[10]. Previous studies confirmed that 30 mg/mL (3%) of polidocanol foam is a safe, cost-effective, and repeatable conservative treatment for grade I, II, and III hemorrhoids[10,14]. Additionally, polidocanol foam leads to marked vasospasm, damage of the hemorrhoidal endothelium, an inflammatory reaction within 2 min, and induces a fibrotic reaction 30 min after administration. Anoscope-assisted sclerobanding (*i.e.* combined RBL with polidocanol foam sclerotherapy) has recently been deemed a safe technique with a low rate of minor post-operative complications[3]. Our findings were similar, but we used 10 mg/mL (1%) polidocanol and performed one treatment procedure to avoid potential side effects and maintain participant safety.

Post-operative bleeding is a common complication of this procedure. In this study, we discovered that the multi-site injection of a foam sclerosing agent in the EFSB group increased the incidence of post-operative bleeding that may have been related to mucosal injury with oozing at the needle insertion site. Most patients experienced mild-to-no bleeding without special medical therapy. In a previous study, Caetano *et al*[23] reported that a micronized purified flavonoid fraction, an oral drug used to treat capillary fragility, reduced the amount of bleeding during the first month. A recent study reported that clopidogrel bisulfate did not increase bleeding complications in patients undergoing RBL[24]. However, considering the risk of massive bleeding caused by antiplatelet or anticoagulant drugs, these patients were instructed to discontinue these drugs 5–7 d before the procedure.

Rare complications of RBL including perianal abscesses, vascular-vagal symptoms, priapism, dysuria, anal fistula, and longitudinal mucosal ulcers have been reported[25-27]. The complications of RBL with negative-pressure suction are believed to be less likely than those of forceps-assisted band ligation, which often results in unclear vision while performing the procedure and is more likely to damage the mucosa[28]. In this study, 1 patient had dysuria and another had a mucosal ulcer, confirming that EFSB was safe, with minimal adverse events. We believe that transparent cap assistance and rectal rinsing with a water jet provides clear vision and avoids injection-induced infections.

Our study had some limitations to consider. First, patients with severe disease may require additional treatment sessions to improve their symptoms and success rate. However, EFSB was performed for the first time, and only one session was conducted to avoid unexpected side effects. Second, the study followed patients for only 12 months. Therefore, long-term follow-up studies should be conducted. Third, recurrence is known to be associated with other factors that require further exploration. Finally, no established criteria are available for the endoscopic diagnosis of internal hemorrhoids. Inserting a scope could reduce the prolapsing cushions to their normal position, thus leading to misdiagnosis[29].

CONCLUSION

Prospective analysis of grade II and III internal hemorrhoids revealed that the use of the EFSB technique effectively reduced the 12-m prolapse recurrence rate and relieved 24-h post-procedure pain. However, whether repeated treatment is effective and safe remains unclear. Therefore, further long-term follow-up and treatment sessions should be conducted to further reduce the recurrence of prolapse.

ACKNOWLEDGEMENTS

The authors thank the patients for their consent to share the whole treatment and follow-up procedures. All authors agree to be accountable for the content of the work.

FOOTNOTES

Author contributions: Qu CY and Zhang FY contributed equally to this work as co-first authors; Xu LM and Shen F contributed equally to this work as co-corresponding authors; Qu CY and Zhang FY drafted the manuscript; Xu LM and Shen F designed the study and supervised its implementation; Chen GY analyzed the data; Qu CY, Wang W, Gao FY, Lin WL, Zhang H, Zhang Y, Li MM, Xu LM, and Shen F completed the endoscopic manipulations; Zhang FY, Li ZH and Cai MH participated in the experiments; and all authors made critical revisions and approved the final version to be published. The reasons for designating Xu LM and Shen F as co-corresponding authors are that Xu LM is responsible for multicenter coordination and quality control; Shen F is responsible for multi-center coordination and design of the project.

Supported by the Hospital Funded Clinical Research of Xinhua Hospital, No. 19XHCR16D.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Boards of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine and Ethics Committees of the Xinhua Hospital (Approval No. XHEC-C-2020-003-1).

Clinical trial registration statement: This study is registered at https://classic.clinicaltrials.gov/ct2/show/NCT04398823?term=NCT04398823&draw=2&rank=1. The registration identification number is NCT04398823.

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

Conflict-of-interest statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data sharing statement: Consent was not obtained but the presented data are anonymized and risk of identification is low.

CONSORT 2010 statement: The authors have read the CONSORT 2010 Statement, and the manuscript was prepared and revised according to the CONSORT 2010 Statement.

Country of origin: China

ORCID number: Fei-Yu Zhang 0009-0005-7203-6322; Wen Wang 0000-0003-0001-3672; Feng-Yu Gao 0000-0002-7226-7842; Guang-Yu Chen 0000-0002-9564-8971; Yi Zhang 0000-0002-1363-3291; Ming-Ming Li 0000-0003-1753-6533; Lei-Ming Xu 0000-0002-6735-4853; Feng Shen 0000-0001-7782-2211.

S-Editor: Chen YL
L-Editor: Filipodia
P-Editor: Zheng XM

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2024 July 21; 30(27): 3336-3355

DOI: 10.3748/wjg.v30.i27.3336

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Distinct gut microbiomes in Thai patients with colorectal polyps

Thoranin Intarajak, Wandee Udomchaiprasertkul, Ahmad Nuruddin Khoiri, Sawannee Sutheeworapong, Kanthida Kusonmano, Weerayuth Kittichotirat, Chinae Thammarongtham, Supapon Cheevadhanarak

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade B

Scientific Significance: Grade B, Grade B

P-Reviewer: Dziegielewska-Gesiak S, Poland

Received: February 21, 2024

Revised: April 30, 2024

Accepted: May 31, 2024

Published online: July 21, 2024

Processing time: 140 Days and 23.4 Hours

Thoranin Intarajak, Bioinformatics Unit, Chulabhorn Royal Academy, Lak Si 10210, Bangkok, Thailand

Thoranin Intarajak, Ahmad Nuruddin Khoiri, Kanthida Kusonmano, Weerayuth Kittichotirat, Bioinformatics and Systems Biology Program, School of Bioresources and Technology, and School of Information Technology, King Mongkut's University of Technology Thonburi, Bang Khun Thian 10150, Bangkok, Thailand

Thoranin Intarajak, Sawannee Sutheeworapong, Kanthida Kusonmano, Weerayuth Kittichotirat, Supapon Cheevadhanarak, Systems Biology and Bioinformatics Unit, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bang Khun Thian 10150, Bangkok, Thailand

Wandee Udomchaiprasertkul, Molecular Genomic Research Laboratory, Chulabhorn Royal Academy, Lak Si 10210, Bangkok, Thailand

Chinae Thammarongtham, National Center for Genetic Engineering and Biotechnology, King Mongkut's University of Technology Thonburi, Bang Khun Thian 10150, Bangkok, Thailand

Supapon Cheevadhanarak, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bank Khun Thian 10150, Bangkok, Thailand

Supapon Cheevadhanarak, Fungal Biotechnology Unit, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bang Khun Thian 10150, Bangkok, Thailand

Corresponding author: Supapon Cheevadhanarak, PhD, Associate Professor, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, 49 Soi Thian Thale 25, Bang Khun Thian Chai Thale Road, Bank Khun Thian 10150, Bangkok, Thailand. supapon.che@mail.kmutt.ac.th

Abstract

BACKGROUND

Colorectal polyps that develop *via* the conventional adenoma-carcinoma sequence [*e.g.*, tubular adenoma (TA)] often progress to malignancy and are closely associated with changes in the composition of the gut microbiome. There is limited research concerning the microbial functions and gut microbiomes associated with colorectal polyps that arise through the serrated polyp pathway, such as hyperplastic polyps (HP). Exploration of microbiome alterations asso-

ciated with HP and TA would improve the understanding of mechanisms by which specific microbes and their metabolic pathways contribute to colorectal carcinogenesis.

AIM

To investigate gut microbiome signatures, microbial associations, and microbial functions in HP and TA patients.

METHODS

Full-length 16S rRNA sequencing was used to characterize the gut microbiome in stool samples from control participants without polyps [control group (CT), n = 40], patients with HP (n = 52), and patients with TA (n = 60). Significant differences in gut microbiome composition and functional mechanisms were identified between the CT group and patients with HP or TA. Analytical techniques in this study included differential abundance analysis, co-occurrence network analysis, and differential pathway analysis.

RESULTS

Colorectal cancer (CRC)-associated bacteria, including *Streptococcus gallolyticus* (*S. gallolyticus*), *Bacteroides fragilis*, and *Clostridium symbiosum*, were identified as characteristic microbial species in TA patients. *Mediterraneibacter gnavus*, associated with dysbiosis and gastrointestinal diseases, was significantly differentially abundant in the HP and TA groups. Functional pathway analysis revealed that HP patients exhibited enrichment in the sulfur oxidation pathway exclusively, whereas TA patients showed dominance in pathways related to secondary metabolite biosynthesis (*e.g.*, mevalonate); *S. gallolyticus* was a major contributor. Co-occurrence network and dynamic network analyses revealed co-occurrence of dysbiosis-associated bacteria in HP patients, whereas TA patients exhibited co-occurrence of CRC-associated bacteria. Furthermore, the co-occurrence of SCFA-producing bacteria was lower in TA patients than HP patients.

CONCLUSION

This study revealed distinct gut microbiome signatures associated with pathways of colorectal polyp development, providing insights concerning the roles of microbial species, functional pathways, and microbial interactions in colorectal carcinogenesis.

Key Words: Gut microbiome; Colorectal adenoma; Hyperplastic polyp; Full-length 16s rRNA; Microbial correlation networks; Predicted functional mechanisms

Core Tip: This study identified gut microbiome signatures and metabolic pathways associated with two types of colorectal polyps. It is the first report of enrichment in the sulfur oxidation pathway among patients with hyperplastic polyps (HP) and the involvement of *Streptococcus gallolyticus* in the secondary metabolite biosynthesis pathway among patients with tubular adenoma (TA). Additionally, analysis of microbial associations in the gut microbiomes of HP and TA patients revealed a decrease in the co-occurrence of short chain fatty acid-producing bacteria. Conversely, there was an increase in the co-occurrence of dysbiosis and colorectal cancer-associated bacteria.

Citation: Intarajak T, Udomchaiprasertkul W, Khoiri AN, Sutheeworapong S, Kusonmano K, Kittichotirat W, Thammarongtham C, Cheevadhanarak S. Distinct gut microbiomes in Thai patients with colorectal polyps. *World J Gastroenterol* 2024; 30(27): 3336-3355
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3336.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3336

INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent cancer worldwide and the second leading cause of cancer-related mortality[1]. In Thailand, it is the fourth highest in terms of incidence and mortality rates; more than 20000 new cases have been reported in the 2020s[2]. The distinct molecular pathways leading to CRC are associated with various colorectal polyps. The most common pathway is the conventional adenoma-carcinoma sequence, which involves progression from tubular, tubulovillous, and villous adenomas. Another key pathway, the serrated polyp pathway, is characterized by the presence of colorectal polyps such as hyperplastic polyps (HP), sessile serrated adenoma/polyps (SSA/Ps), and traditional serrated adenoma. All the abovementioned colorectal polyps are regarded as neoplastic polyps with the potential to become malignant; they represent key phases in CRC progression[3].

The gut microbiome plays a pivotal role in connecting environmental factors to colorectal polyps because these factors are correlated with both compositional and functional changes within the collective microbial community residing in the colon. Alterations in the gut microbiome have been linked to colorectal polyps, and specific microbial species have been identified as potential drivers of oncogenesis. Microbial species commonly identified in precancerous lesions and

implicated in colorectal carcinogenesis include *Escherichia coli* (*E. coli*)[4,5], *Bacteroides fragilis* (*B. fragilis*)[6], and *Streptococcus gallolyticus*[7]. Additionally, shifts in *Fusobacterium mortiferum* and pro-inflammatory bacteria (*e.g.*, *Bilophilia* and *Desulfovibrio*), along with decreases in short chain fatty acid (SCFA)-producing bacteria such as *Faecalibacterium prausnitzii* and *Bifidobacterium pseudocatenulatum*, have been observed in adenoma patients[8,9]. Moreover, functional studies have elucidated the roles of specific microbes in colorectal polyp formation through processes such as co-metabolic dysfunction, inflammation, epigenetic alterations, and DNA damage[10,11]. Because the gut microbiome modulates the host metabolic environment, it can directly or indirectly influence mutagenesis rates, thereby influencing carcinogenesis. Importantly, gut microbiome differences have been discovered between healthy individuals and patients with serrated polyps[12,13]. It has been hypothesized that the unique microbiome alterations associated with early adenomas and premalignant colorectal polyps can serve as biomarkers for early cancer detection or the identification of individuals with a risk of colorectal polyps. Furthermore, an exploration of the microbiome alterations specific to premalignant or benign polyps would provide insights regarding the mechanisms by which specific microbes and their metabolic pathways contribute to colorectal carcinogenesis. However, there has been limited research concerning microbial alterations in colorectal polyps; substantial discrepancies in microbial markers across studies may be attributed to diverse biological factors that impact gut microbiome composition, as well as inconsistencies in microbial sequencing data processing[14,15].

In this study, we compared gut microbiome signatures between two types of colorectal polyps: Tubular adenoma (TA, high potential for malignancy) and HP (low potential for malignancy). Our results revealed distinct gut microbiome signatures associated with each pathway of colorectal polyp development. Additionally, we identified microbes and microbial functions significantly associated with TA and HP. These findings suggest that gut microbiome signatures can serve as early biomarkers of CRC risk and help to identify potential targets for cancer prevention strategies.

MATERIALS AND METHODS

Participant recruitment and criteria

Participants in this study were volunteers who took part in the CRC Screening Development with Multiple Technologies Project 2020s, established by Chulabhorn Royal Academy in Thailand. The inclusion criteria were as follows: (1) Individuals aged 50-80 years who underwent CRC screening by colonoscopy between February 1, 2021, and June 30, 2022; (2) individuals who had not taken antibiotics within the preceding 3 mo; (3) individuals who did not use proton pump inhibitors or enemas, reported no history of constipation, or had undergone colonoscopy within the previous 1 mo; and (4) individuals who cooperated with the screening program and were willing to provide written informed consent. The exclusion criteria were as follows: (1) Incomplete clinical data; (2) loss of follow-up; (3) presence of inflammatory polyps; or (4) no stool sample collection. Each participant was recruited to take part in the study at the initial screening visit. They were interviewed by a physician, who recorded their clinical data and health history. After endoscopic examination of the large intestine, any detected polyps were removed and examined by a pathologist. In total, 152 participants were categorized into three groups based on the histopathology findings of the detected polyps: Control (CT), HP, and TA group. The CT group consisted of participants who exhibited no polyps during colonoscopy, the HP group comprised participants who had HP, and the TA group encompassed participants with at least one tubular or tubulovillous adenoma. The study protocol was approved by the Ethics Committee of Chulabhorn Research Institute and the Institutional Review Boards of Chulabhorn Royal Academy (Project Code 045/2563). Informed consent was obtained from all participants involved in the study.

Sample collection and DNA extraction

Stool samples were collected using DNA/RNA Shield Fecal Collection Tubes (Zymo Research, Irvine, CA, United States) prior to routine bowel preparation and colonoscopy. Participants were given a fecal collection kit and instructions for home stool sampling. Collected stool samples were stored at -20 °C until analysis. Microbial DNA was extracted from stool samples using the DNeasy PowerSoil Pro Kit (Qiagen, Germantown, MD, United States) in accordance with the manufacturer's protocol. The extracted DNA was quantified using a NanoDrop and the Qubit dsDNA HS Assay Kit (both from Thermo Fisher Scientific, Waltham, MA, United States). The ZymoBIOMICS Gut Microbiome Standard (Zymo Research) was utilized as the positive control for DNA extraction, full-length 16S rRNA amplification, and sequencing.

Full-length 16S library preparation and sequencing

Full-length 16S rRNA (V1-V9) was amplified using a set of barcoded primers: (27F) 5'-GCATC/barcode/AGRGTTYGATYMTGGCTCAG-3' and (1492R) 5'-GCATC/barcode/RGYTACCTTGTTACGACTT-3'. The barcoded primer sequences were provided by PacBio. Each polymerase chain reaction (PCR) mixture consisted of 0.5 U Q5 High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA, United States), 200 μmol/L dNTPs, 0.5 μmol/L each forward and reverse primers, and 1 ng extracted DNA. PCR was performed using a thermocycler with the following protocol: 98 °C for 30 seconds; 22 cycles of 98 °C for 10 seconds, 55 °C for 30 seconds, and 72 °C for 1 minute; and 72 °C for 2 minutes. Library constructs for full-length 16S rRNA analysis were prepared using the SMRTbell Express Template Prep Kit 2.0 (PacBio, Menlo Park, CA, United States) and Sequel Binding Kit 3.0 in accordance with the manufacturer's protocol. Sequencing was performed using the PacBio Sequel I platform.

Microbiome data analysis

Gut microbiome analysis was conducted after a series of quality control steps using the FastQC[16] and MultiQC[17] pipelines. SMRT link v11.0 (PacBio) was utilized for demultiplexing and primer removal. Next, the QIIME2 (version 2023.2) pipeline[18] was used for microbiome profiling. Specifically, the q2-dada2 plugin (version 2023.2.0)[19] within QIIME2 was implemented for read denoising with the following criteria: pooling method, pseudo; minimum and maximum sequence lengths, 1000 and 1800 bases, respectively. Amplicon sequence variants (ASVs) were subjected to filtering, chimerism screening, and base correction. Taxonomic assignment was conducted using the Greengenes2 2022.10 database[20], which was trained with a naïve Bayes classifier. Common contaminants, unclassified ASVs, spike-ins, mitochondrial sequences, and chloroplast sequences were removed. Microbiome diversity and abundance were analyzed using the PhyloSeq (version 1.42.0)[21] and microbiome (version 1.20.0)[22] packages in R software[23]. Statistical analysis was conducted by the Wilcoxon and Kruskal-Wallis rank sum tests. Microbial richness and evenness were assessed *via* metrics such as the Shannon index[24], Simpson index[25], phylogenetic diversity (PD)[26], and Pielou index[27]. β-diversity was determined by Bray-Curtis distance-based principal coordinate analysis (PCoA)[28,29].

Gut microbiome signature discovery and co-occurrence network analysis

Gut microbiome signatures were identified using the linear discriminant analysis effect size (LEfSe) discovery tool[30,31]. Before data entry into LEfSe, normalization was performed by rarefaction of 13500 reads. Class comparisons were conducted using the Kruskal-Wallis test, whereas subclass comparisons were conducted with the Wilcoxon test. Both tests used a significance threshold of $P < 0.05$. Differential abundance analyses based on the negative binomial distribution were performed with the DESeq2 package (version 1.38.3)[32]. The abundances were imported into the DESeq2 package, and the Wald test was used to determine statistical significance[33]. Co-occurrence correlation analyses were carried out using FastSpar (version 0.0.10) software[34]. Correlation coefficients were averaged across five inference iterations, and *P* values were determined by 1000 bootstrap correlations. Correlation coefficients with *P* values less than 0.05 and absolute correlation values greater than 0.4 were selected for visualization in Cytoscape (version 3.10.0) software [35]. Comparisons among three networks (one network per group) were performed using the DyNet application[36] in Cytoscape. Microbial species with a rewiring metric or D_n-score of ≥ 2.0, as well as an edge count of ≥ 4, were regarded as rewired nodes for each dataset in comparisons between the CT and HP groups and between the CT and TA groups.

Functional pathway and enzyme commission enrichment analyses

To explore the metabolic functions and pathways of the gut microbiome in each group, PICRUSt2 software[37] was utilized to make predictions about microbial functions within metabolic pathways. The compositions of the identified microbes were aligned with the MetaCyc database[38] to obtain estimates of their metabolic functions. Statistical Analysis for Metagenomic Profiles (STAMP; version 2.1.3) software[39] was employed to detect variations in metabolic function abundance among groups using Welch's *t*-test[40] with a confidence interval of 0.95 and a significance threshold of $P < 0.05$. Gut microbiome contributions to specific pathways were determined by collecting pathway-associated enzyme commission (EC) numbers from MetaCyc and sorting on the basis of relative abundance.

RESULTS

Participant characteristics

In total, 440 participants were invited to participate in the CRC Screening Development with Multiple Technologies Project 2020s between February 1, 2021, and June 30, 2022; of these, 152 participants were enrolled in the experiment (Figure 1). Participants were categorized into three groups: 40 in the CT group, 52 in the HP group, and 60 in the TA group. Table 1 presents the demographic and clinical characteristics of the study participants. Participants in the HP and TA groups tended to be older than participants in the CT group. The number of women did not significantly differ among the three groups, but the CT group had the lowest number of men. Most participants in the HP group had polyps in the distal colon, whereas participants in the TA group had polyps in the distal and proximal colon.

Microbial community investigation

Data preprocessing with the QIIME2 pipeline revealed an average read length of 1452.95. The minimum and maximum sequence lengths were 1363 and 1788 bases, respectively. Supplementary Figure 1 shows the sequence length statistics and alpha rarefaction curve. Source Data Supplementary Table 1 lists the numbers of reads during the denoising process.

Microbiome diversities were compared among study groups using the Wilcoxon rank sum test and Kruskal-Wallis rank sum test. In terms of α-diversity, there were no significant differences among the CT, HP, and TA groups according to the Wilcoxon rank sum test. Analyses using the Shannon index ($P = 0.47$), PD ($P = 0.85$), Simpson index ($P = 0.42$), and Pielou index ($P = 0.72$) indicated that α-diversity did not significantly differ between the HP and CT groups (Figure 2A). Furthermore, α-diversity did not significantly differ between the CT and TA groups [Shannon index ($P = 0.38$), PD ($P = 0.75$), Simpson index ($P = 0.29$), and Pielou index ($P = 0.48$); Figure 2B].

Bray-Curtis distance-based PCoA was performed to assess β-diversity among participants in the CT, HP, and TA groups. As shown in Figure 2C and D, there were no significant differences in gut microbiome composition between the CT and HP groups or the CT and TA groups. Thus, the CT, HP, and TA groups did not demonstrate significant differences in richness and evenness at the species level.

Table 1 Participant demographic and clinical characteristics

Group	CT, *n* = 40	HP, *n* = 52	TA, *n* = 60
Age in years, median (range)	56 (52-59)	60 (51-71)	61 (52-71)
Sex			
Female	31 (77.5)	32 (61.5)	31 (51.7)
Male	9 (22.5)	20 (38.5)	29 (48.3)
BMI in kg/m^2, median (range)	21.7 (18-26.56)	23.4 (18.6-38.3)	24 (19-32)
Polyp location			
Proximal		6 (11.5)	21 (35)
Distal		39 (75)	22 (36.7)
Both		7 (13.5)	17 (28.3)

Data are *n* (%). BMI: Body mass index; CT: Control; HP: Hyperplastic polyps; TA: Tubular adenoma.

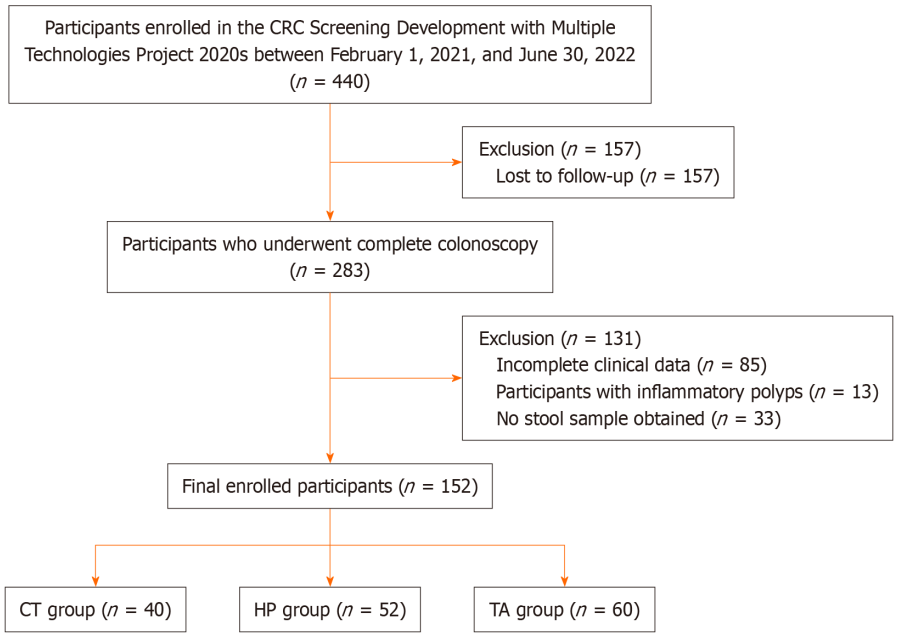


Figure 1 Flowchart of participant enrollment. CRC: Colorectal cancer; CT: Control group; HP: Hyperplastic polyps; TA: Tubular adenoma.

Groupwise comparative analyses of gut microbial species: CT vs HP and CT vs TA

To identify gut microbiome signatures that could distinguish the CT group from the HP and TA groups, we compared microbial species between the CT and HP groups, and between the CT and TA groups, using the LEfSe method and DESeq2. LEfSe, a powerful tool for the discovery of high-potential signatures, combines non-parametric standard tests for statistical significance with linear discriminant analysis (LDA). The LDA model within LEfSe identifies microbial species that are differentially abundant between groups, then estimates the effect size of each significantly different microbial species[31]. In contrast, DESeq2 utilizes a negative binomial generalized linear model to estimate log fold changes between two groups, then evaluates the significance of those changes using the Wald test[33].

LEfSe revealed that ten microbial species significantly differed between the CT and HP groups (Figure 3A, Source Data Supplementary Table 2). Among these, four microbial species were enriched in the HP group: *Blautia* A 141780 *hansenii* (*B. hansenii*), *Ruminococcus* C 58660 sp000433635, UBA9414 sp003458885, and *Veillonella* A *atypica* (*V. atypica*). Additionally, LEfSe revealed that 20 microbial species significantly differed between the CT and TA groups (Figure 3B, Source Data Supplementary Table 2). Seven microbial species were significantly enriched in the TA group, including two CRC-associated bacteria (*Bacteroides* H *fragilis* and *Clostridium* Q 134516 *symbiosum*), as well as *Bacteroides nordii* and *Clostridium* Q *fessum* (Figure 3B, Source Data Supplementary Table 2).

According to DESeq2, 22 microbial species exhibited significantly different abundances in the HP group; these included dysbiosis and gastrointestinal diseases-associated bacteria, such as *Mediterraneibacter gnavus* (*M. gnavus*) and *Fusobacterium varium* (*F. varium*), as well as commensal and SCFA-producing bacteria (*e.g.*, *B. hansenii, Butyribacter*

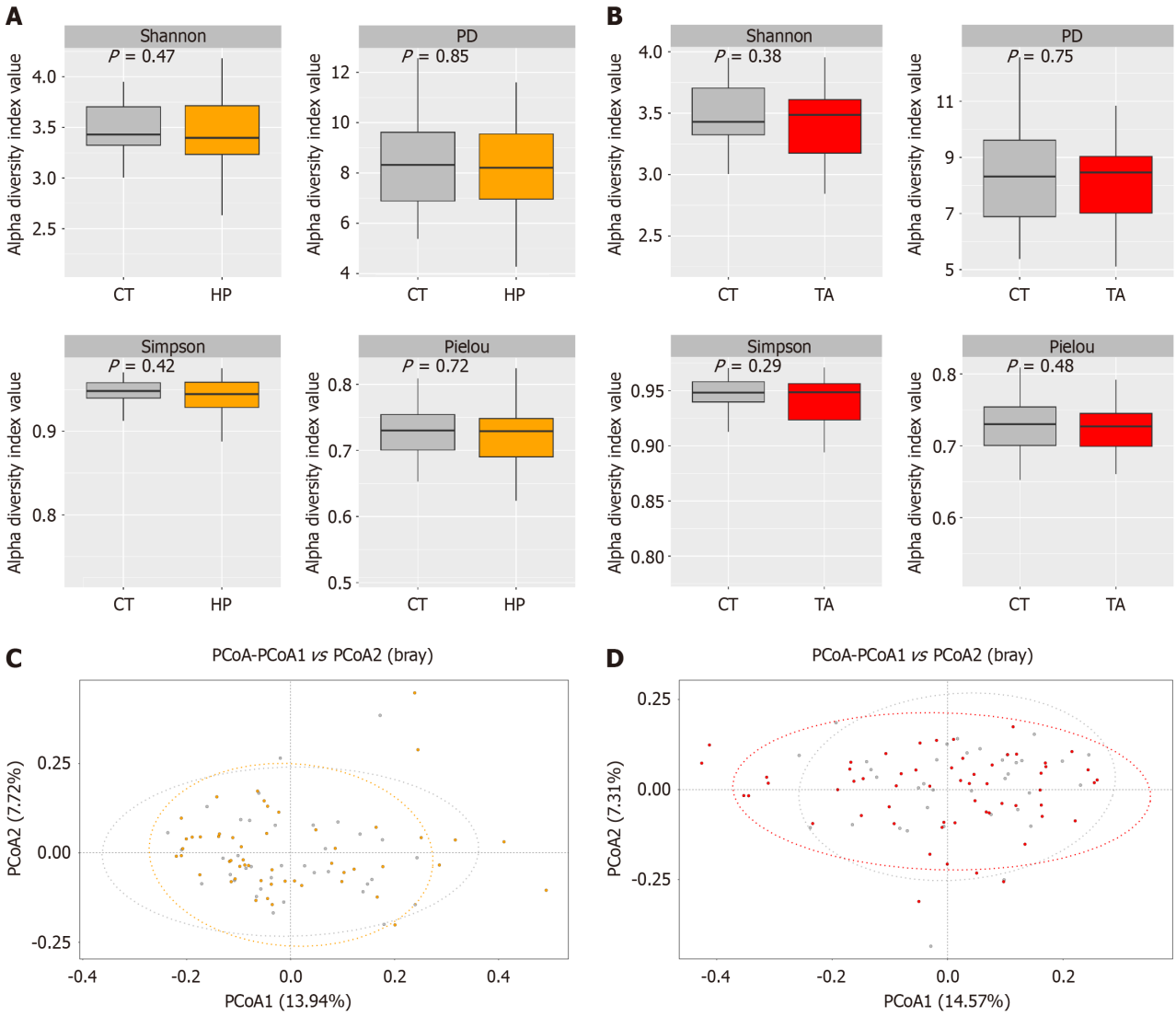
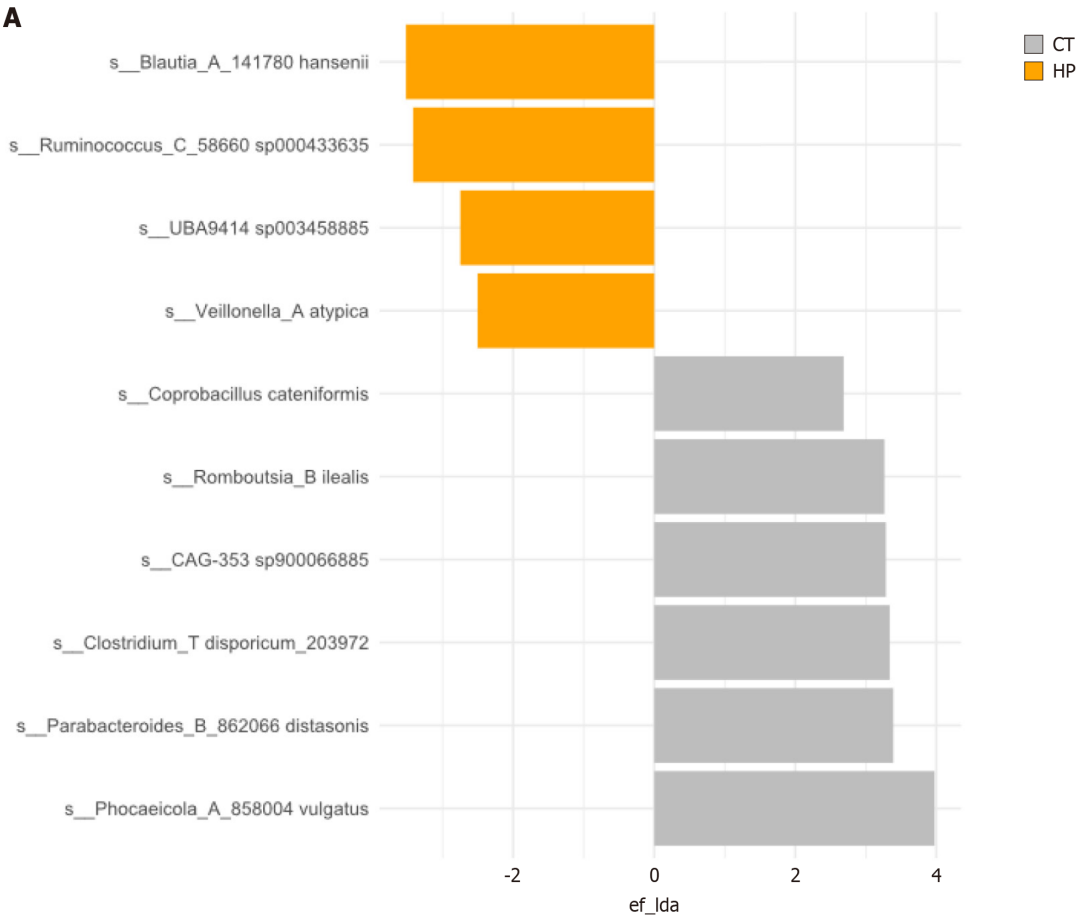


Figure 2 Microbial diversity of the gut microbiome in control, hyperplastic polyps, and tubular adenoma groups. A: Box plots show α-diversity between the control (CT) and hyperplastic polyps (HP) groups; B: Box plots show α-diversity between the CT and tubular adenoma (TA) groups; C: Principal coordinate analysis (PCoA) plots show β-diversity between the CT and HP groups; D: PCoA plots show β-diversity the CT and TA groups. PD: Phylogenetic diversity.

sp001916135, *Bifidobacterium catenulatum*, and *Faecalimonas umbilicata*) (Figure 3C, Source Data Supplementary Table 2). Additionally, 20 microbial species showed significantly different abundances in the TA group (Figure 3D), including *S. gallolyticus* (a well-known CRC-associated species), *M. gnavus*, and *F. varium*. Additionally, *V. atypica* demonstrated significantly different abundances in the HP and TA groups. *V. atypica* is commonly localized in the oral cavity and has been identified in fecal samples from older patients with CRC[41,42]. Notably, the TA group had increased abundances of well-known CRC-associated bacteria. These findings suggest that increases in *S. gallolyticus* and *B. fragilis* contribute to TA development. The findings also support the notion that patients with colorectal polyps exhibit a dysregulated microbiome characterized by high abundances of potentially pathogenic bacteria. In summary, our results imply that the identified microbial species could be used as signatures for HP and TA. We also assessed the presence of pathogenic bacteria commonly associated with CRC, such as *F. nucleatum* and *E. coli*. In this study, *F. nucleatum* and *E. coli* were not detected in the taxonomic annotation (Source Data Supplementary Table 2).

Predicted functional signatures in HP and TA groups

To identify the mechanisms by which the gut microbiome influences CRC carcinogenesis and detect biologically significant differences, we examined changes in functional composition using the MetaCyc pathway database. Compared with the CT group, the HP and TA groups had 20 and eight enriched pathways, respectively (Figure 4). Enriched pathways in the HP group included cell structure biosynthesis [*e.g.*, peptidoglycan biosynthesis I (meso-diaminopimelate containing)]; inorganic nutrient metabolism [super pathway of sulfur oxidation (*Acidianus ambivalens*)]; fatty acid and lipid biosynthesis (*e.g.*, CDP-diacylglycerol biosynthesis I); cofactor, carrier, and vitamin biosynthesis; carboxylic acid biosynthesis; secondary metabolite biosynthesis; tetrapyrrole biosynthesis; and amino acid biosynthesis. Secondary metabolite biosynthesis; aromatic compound degradation; cofactor, carrier, and vitamin biosynthesis; and cell structure biosynthesis were enriched in the TA group. The main differential pathways in the TA group were related to secondary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s__UBA9414 sp003458885
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s__Coprobacillus cateniformis
s__Romboutsia_B ilealis
s__CAG-353 sp900066885
s__Clostridium_T disporicum_203972
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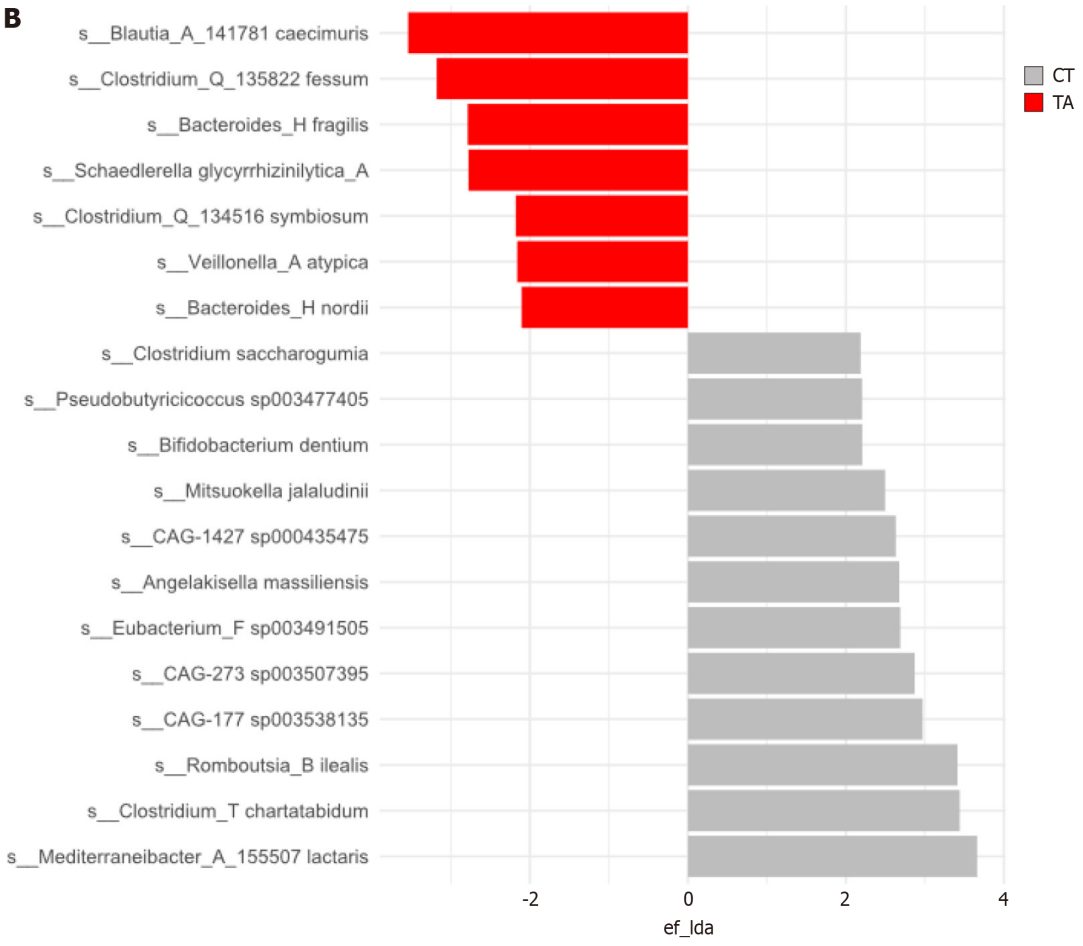
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s__Blautia_A_141781 caecimuris
s__Clostridium_Q_135822 fessum
s__Bacteroides_H fragilis
s__Schaedlerella glycyrrhizinilytica_A
s__Clostridium_Q_134516 symbiosum
s__Veillonella_A atypica
s__Bacteroides_H nordii
s__Clostridium saccharogumia
s__Pseudobutyricicoccus sp003477405
s__Bifidobacterium dentium
s__Mitsuokella jalaludinii
s__CAG-1427 sp000435475
s__Angelakisella massiliensis
s__Eubacterium_F sp003491505
s__CAG-273 sp003507395
s__CAG-177 sp003538135
s__Romboutsia_B ilealis
s__Clostridium_T chartatabidum
s__Mediterraneibacter_A_155507 lactaris
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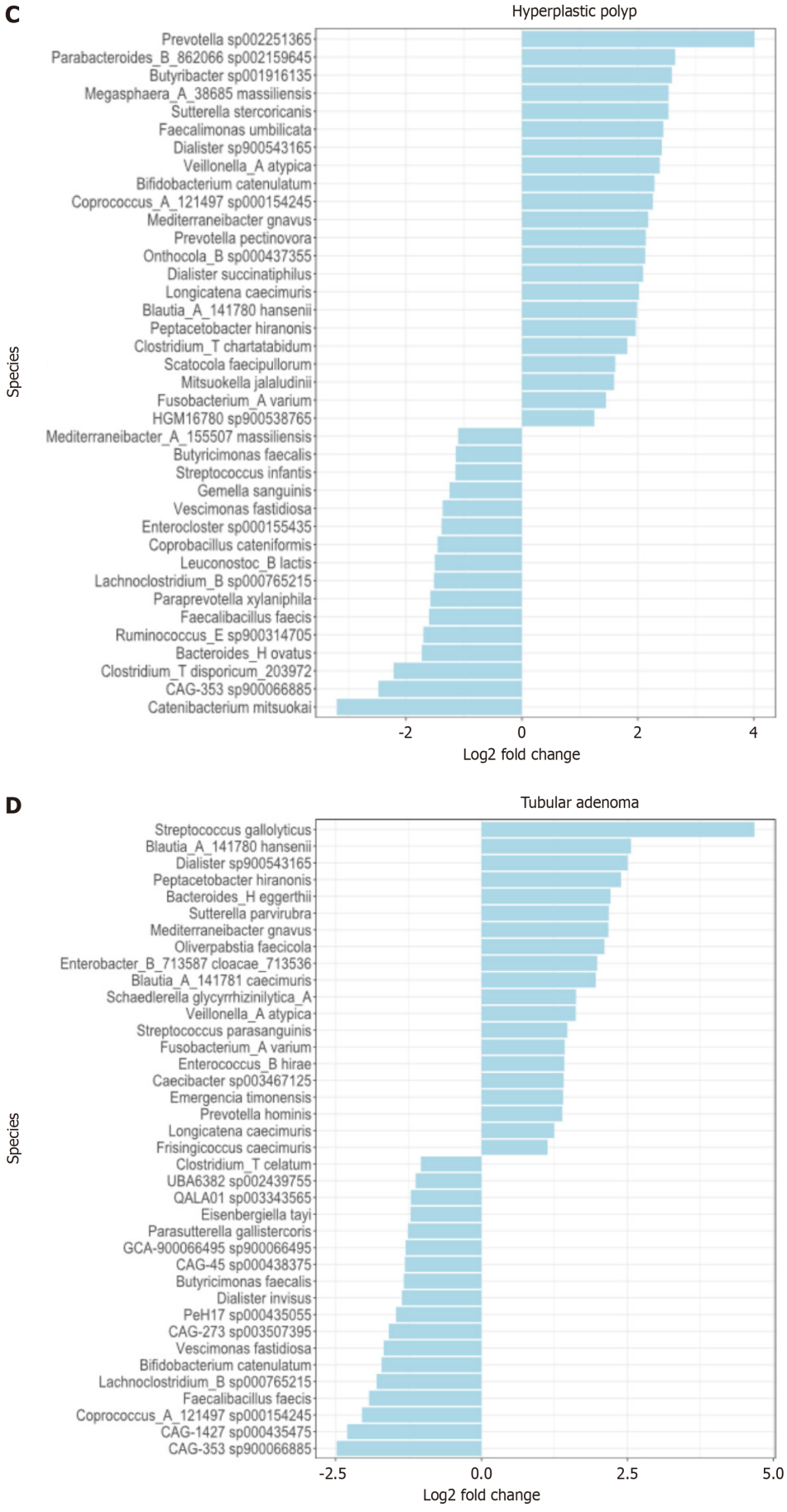


Figure 3 Linear discriminant analysis effect size and DESeq2 identified the most enriched microbial species in the control, hyperplastic

polyps, and tubular adenoma groups. A: Linear discriminant analysis (LDA) effect size (LEfSe) showed significant microbial differences between the control (CT) and hyperplastic polyps (HP) groups; B: LEfSe showed significant microbial differences the CT and tubular adenoma (TA) groups (LDA score > 2); C: DESeq2 analysis graphs illustrate the $\log_2$ fold differential abundances of microbial species between the CT and HP groups; D: DESeq2 analysis graphs illustrate the $\log_2$ fold differential abundances of microbial species between the CT and TA groups. In the graphs, $\log_2$ fold changes > 0 indicate an increase in the corresponding microbial species, whereas $\log_2$ fold changes < 0 indicate a decrease. Microbial species positioned above the zero threshold demonstrated higher relative abundance in either the HP or TA group compared to the CT group.

metabolite biosynthesis: Taxadiene biosynthesis (engineered), mevalonate pathway I (eukaryotes and bacteria), and the super pathway of geranylgeranyl diphosphate biosynthesis I (*via* mevalonate). Notably, mevalonate pathway I (eukaryotes and bacteria) overlapped between the HP and TA groups.

Next, we investigated the contributions of microbial species to the enriched pathways in the HP and TA groups. The dominant reactions in the sulfur oxidation (*Acidianus ambivalens*) pathway are adenylyl-sulfate reductase (APS reductase (EC:1.8.99.2)) and the thiosulfate dehydrogenase (quinone) pathway (EC:1.8.5.2). In the HP group, 17 and 22 microbial species contributed to the APS reductase pathway (EC:1.8.99.2) and the thiosulfate dehydrogenase (quinone) pathway (EC:1.8.5.2), respectively. *Parabacteroides* B 862066 *distasonis* (*P. distasonis*), *Bacteroides* H *thetaiotaomicron* (*B. thetaiotaomicron*), and *Bacteroides* H *cellulosilyticus* were the main contributors to the thiosulfate dehydrogenase (quinone) pathway. Furthermore, the APS reductase pathway was associated with *Bilophila wadsworthia* (*B. wadsworthia*) and *Desulfovibrio piger* (*D. piger*), both sulfate-reducing bacteria (SRB) (Source Data Supplementary Table 3).

In the TA group, several enzymes in enriched pathways had large contributions from *S. gallolyticus*; these enzymes included hydroxymethylglutaryl-CoA synthase (EC:2.3.3.10), mevalonate kinase (EC:2.7.1.36), phosphomevalonate kinase (EC:2.7.4.2), diphosphomevalonate decarboxylase (EC:4.1.1.33), and isopentenyl-diphosphate delta-isomerase (EC:5.3.3.2) (Source Data Supplementary Table 4). These findings suggested that the development of TA could be influenced by functional pathways associated with distinct gut microbiome signatures.

Analysis of microbial correlation networks in CT, HP, and TA groups

Microbial correlation networks and hub species were explored *via* co-occurrence network analyses that examined patterns of microbial correlation in the two colorectal polyp types. Microbial association networks were constructed for CT, HP, and TA groups using FastSpar. Table 2 presents the network properties of the CT, HP, and TA groups. The CT network exhibited the largest size in terms of the numbers of nodes and edges, as well as network diameter. These findings indicated that the CT network was more extensive and complex compared with the HP and TA networks. Moreover, the CT network contained a higher number of microbial contributors compared with the HP and TA networks, suggesting that it plays a crucial role in maintaining the overall status of the system.

Nodes with a high degree (> 10) were regarded as hub species in the co-occurrence networks. In the CT network (Figure 5A), 29 hub species were identified, including *Coprococcus* A 187866 *catus* (*C. catus*), *Clostridium ramosum* (*C. ramosum*), ER4 sp000765235, *Lawsonibacter* sp000177015, *Sellimonas intestinalis, Blautia* A 141781 *caecimuris* (*B. caecimuris*), *Blautia* A 141781 *massiliensis* (*B. massiliensis*), and *Anaerobutyricum soehngenii* (*A. soehngenii*) (Supplementary Figure 2A). In the HP network (Figure 5B), 27 hub species were identified, including *M. gnavus, C. ramosum, Dysosmobacter* sp000403435, *Agathobacter rectalis* (*A. rectalis*), *B. hansenii, Clostridium* Q 135853 *saccharolyticum* A, *B. caecimuris, Enterocloster bolteae* (*E. bolteae*), *Dorea* A *formicigenerans* (*D. formicigenerans*), and *B. thetaiotaomicron* (Supplementary Figure 2B). In the TA network (Figure 5C), 19 hub species were identified. *Blautia* A 141781 *obeum* had the highest node degree, followed by *E. bolteae, Dorea* A *longicatena, Clostridium* AQ *innocuum, Dysosmobacter* sp000403435, *M. gnavus, D. formicigenerans*, *C. catus*, *C. ramosum*, and *B. caecimuris* (Supplementary Figure 2C). Furthermore, the CT, HP, and TA networks contained commensal bacteria within the *Blautia* genus: *B. massiliensis, Blautia* A 141781 *faecis, Blautia* A 141780 *argi, B. obeum, B. caecimuris,* and *B . wexlerae*. Notably, *B. hansenii* was exclusively present in the HP and TA networks. Many SCFA-producing bacteria were identified as hub species, including *C. catus, Faecalibacterium prausnitzii* C 71358, *A. rectalis, Anaerobutyricum hallii, A. soehngenii,* and *F. umbilicata.* Some hub species were opportunistic pathogens, dysbiosis-associated bacteria, and CRC-associated bacteria, such as *E. bolteae, M. gnavus, C. symbiosum*, and *C. ramosum.*

The microbial correlation networks indicated that although many microbial interactions were present in all groups, some interactions were exclusive to one or two groups, suggesting that altered microbial interactions partly contribute to the distinct gut microbiome signatures of the HP and TA groups; these findings also highlight the potential role of the gut microbiome in promoting colorectal polyp development. Numerous microbial interactions present in the CT network were absent from the HP and TA networks. Specifically, the CT network showed unique microbial associations, such as negative interactions of *C. catus* with *Lawsonibacter* sp000177015 and *C. ramosum*. There were also positive interactions among CT-associated bacteria, such as CAG-45 sp000438375 (*Lachnospiraceae*) with *Coprococcus* A 121497 *eutactus* and CAG-353 sp900066885 (*Ruminococcaceae*). Additionally, some positive interactions between SCFA-producing bacteria such as *A. hallii* and *A. soehngenii*, and between *C. eutactus* and *Butyribacter* sp003529475, were absent from the HP and TA networks. Furthermore, the HP and TA networks exhibited specific microbial associations that were absent from the CT network, especially the interactions between commensal bacteria and SCFA-producing bacteria, as well as dysbiosis and CRC-associated bacteria. For example, positive interactions of *M. gnavus* with *B. hansenii* and *F. umbilicata* were observed. Positive interactions of *E. bolteae* with *B. hansenii, Ruthenibacterium lactatiformans,* and *Clostridium* Q 135853 *saccharolyticum* A, as well as positive interactions of *M. gnavus* with *Mediterraneibacter* A 155507 *torques*, *B. thetaiotaomicron*, *Bacteroides caccae, Faecalimonas phoceensis,* and *Phocaeicola* A *858004 vulgatus*, were exclusively present in the HP network. In contrast, positive interactions of *E. bolteae* with *B. fragilis* and *P. distasonis*; *B. fragilis* with *C. innocuum* and *C. ramosum*; and *C. ramosum* with *B. thetaiotaomicron* were present in the TA network. These findings support the notion of dysbiosis

Table 2 Correlation network properties of the control, hyperplastic polyp, and tubular adenoma groups

Network properties	CT	HP	TA
Number of nodes	187	137	117
Number of edges	469	372	302
	(pos = 311, neg = 158)	(pos = 245, neg = 127)	(pos = 210, neg = 92)
Clustering coefficient	0.2	0.306	0.302
Network diameter	10	8	7
Average number of neighbors	5.613	6.698	5.650
Network density	0.035	0.064	0.055
Network centralization	0.130	0.255	0.15353

CT: Control; HP: Hyperplastic polyps; Neg: Negative; Pos: Positive; TA: Tubular adenoma.

involvement in colorectal polyp development.

To further characterize the microbial community structure and key microbial associations among patients with different types of polyps, dynamic changes in interactions between the CT and HP networks, and between the CT and TA networks, were explored *via* DyNet analyses that identified synchronized and rewired nodes across the two datasets. The rewired node score was determined by the D_n-score, which reflects the altered interactions of microbial species across the synchronized networks in the two datasets. DyNet visualization of the synchronized CT and HP networks revealed 92 rewired nodes; 70 rewired nodes were present in both datasets (Figure 6A). Similarly, the synchronized CT and TA networks exhibited 108 rewired nodes; 83 rewired nodes were present in both datasets (Figure 6B). Additionally, the synchronized networks showed seven and two rewired nodes that were exclusive to the HP and TA datasets, respectively. DyNet visualization revealed that unique rewired nodes in the HP group consisted of SCFA-producing bacteria, commensal bacteria, and CRC-associated bacteria. For example, *A. rectalis*, *Ruminococcus* D *bicirculans*, *Blautia* A 141780 *stercoris*, *Eubacterium* I *ramulus*, and *S. gallolyticus* were identified as unique rewired nodes in the HP group. In contrast, *A. rectalis* and *Megamonas funiformis* were identified as unique rewired nodes in the TA group. Many interactions involving rewired nodes exclusive to the HP group were between SCFA-producing bacteria and commensal bacteria. Examples include interactions of *A. rectalis* with *F. prausnitzii*, *Lachnospira eligens*, and *A. soehngenii*; *E. ramulus* with *C. catus*; *R. bicirculans* with *A. hallii*; and *A. rectalis* with *D. formicigenerans*. However, rewired nodes exclusive to the TA group displayed fewer interactions between SCFA-producing bacteria and commensal bacteria compared with nodes in the HP group. Examples include interactions of *A. rectalis* with *C. catus*. Notably, *S. gallolyticus* was identified as a unique rewired node in the HP dataset, demonstrating interactions with SCFA-producing bacteria and commensal bacteria such as *F. prausnitzii*, *B. faecis*, *B. caecimuris*, *Dysosmobacter* sp000403435, *Anaerotignum lactatifermentans,* and *Amedibacillus dolichus*. These findings suggest that co-occurrence patterns and microbial interactions differ between the HP and TA groups, which could also describe the development of the two colorectal polyp types and their distinct malignancy landscapes.

DISCUSSION

The development of colorectal polyps is significantly influenced by alterations in gut microbiome community composition and ecology. It typically arises from an imbalance in the gut microbiome community and the colonization of microbial species that trigger chronic inflammation, eventually leading to the multistep process of polyp formation[43]. Here, we investigated differences in gut microbiome communities between individuals with and without colorectal polyps, which provided insights concerning the microbial species, functions, and mechanisms that impact colorectal polyp development through the conventional adenoma-carcinoma sequence and the serrated polyp pathway. Furthermore, we identified multiple differentially abundant species in HP patients and TA patients; many of these species have been associated with CRC, dysbiosis, and colorectal adenoma[44-50].

In the present study, the overall gut microbiome compositions did not significantly differ among the CT, HP, and TA groups. However, there were variations in the microbial species associated with each group. These findings are consistent with the results of previous cohort studies that did not demonstrate differences in overall gut microbiome composition between normal samples and samples from patients with adenoma[13,15]. However, previous studies regarding colorectal polyps have yielded inconsistent results regarding community diversity[51,52]. Some studies showed no differences in diversity, whereas others revealed increased diversity in patients with polyps. These discrepancies may be influenced by factors such as sample size, statistical power, or the presence of population-specific microbial drivers or pathogens. Another possible explanation is that the gut microbiome associated with colorectal polyps is similar to the gut microbiome of healthy individuals[52,53]. The discrepancies also may have arisen from the limited taxonomic coverage and reliance on reference genomes during whole metagenomic sequencing taxonomic profiling[54,55].

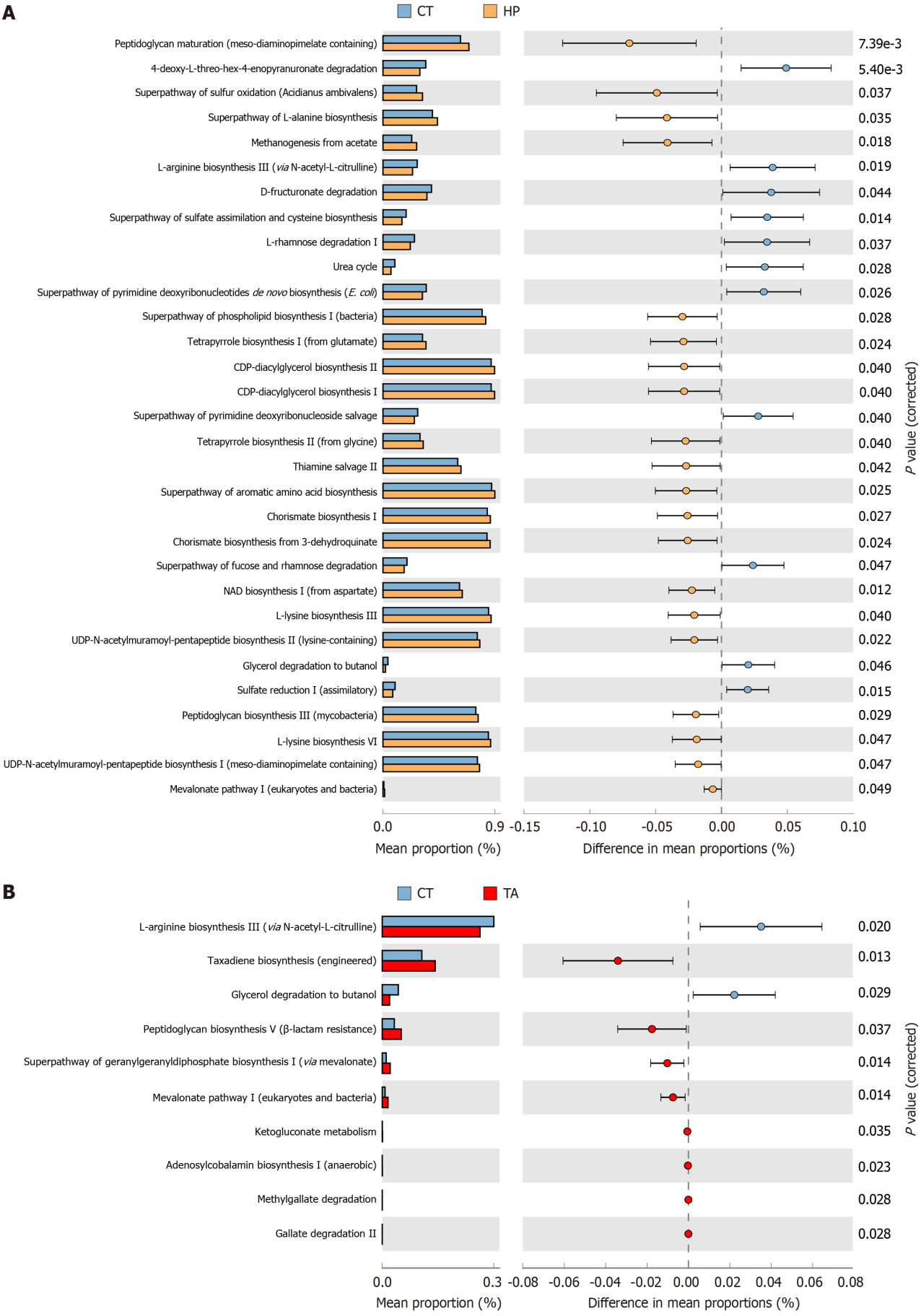


Figure 4 Functional differences in the gut microbiome among control, hyperplastic polyps, and tubular adenoma groups. A: PICRUSt2

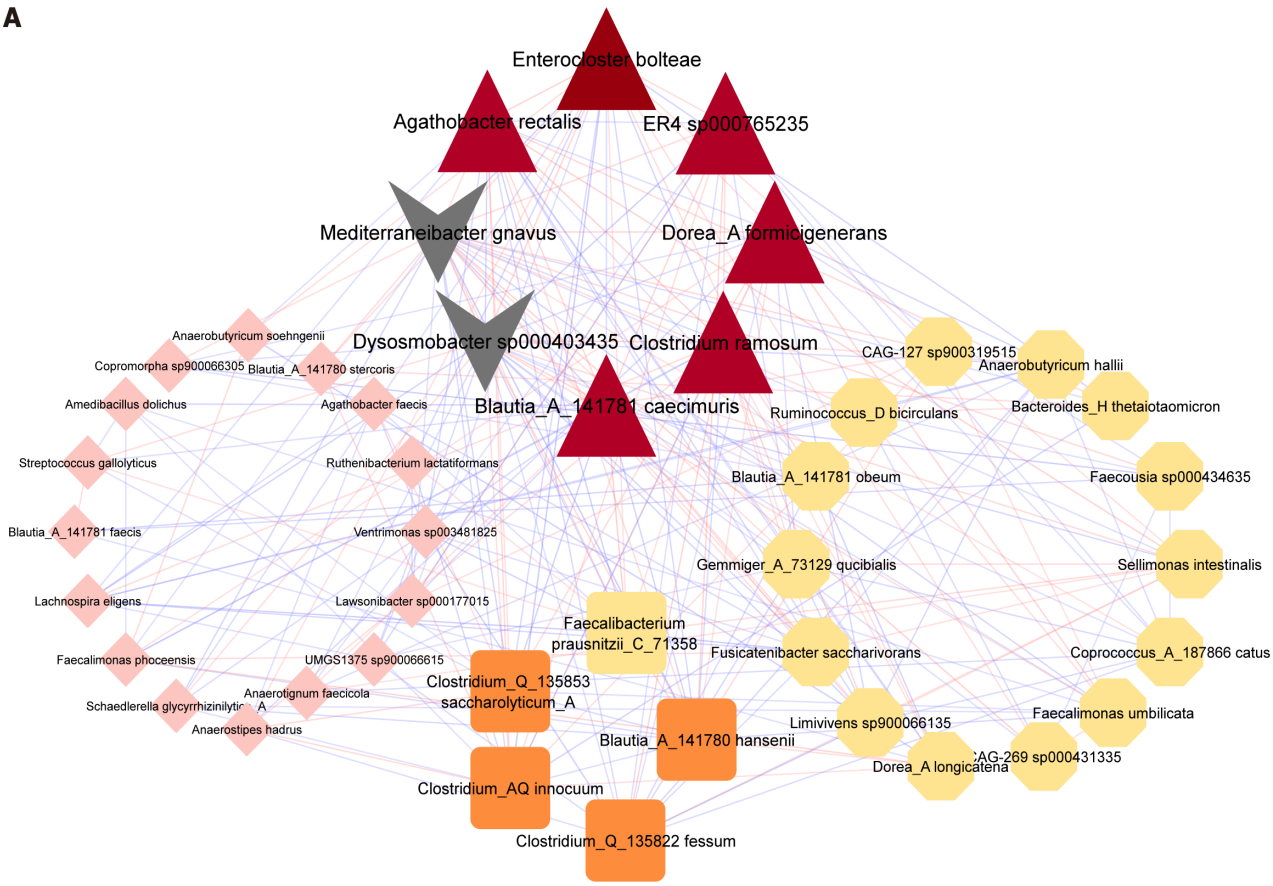
demonstrated significant differences in the gut microbiome within MetaCyc pathways between the control (CT) and hyperplastic polyps (HP) groups; B: PICRUSt2 demonstrated significant differences in the gut microbiome within MetaCyc pathways between the CT and tubular adenoma (TA) groups.

Multiple studies have shown that some pathogenic bacteria, such as *Fusobacterium*, *B. fragilis*, and *E. coli*, are highly abundant in patients with colorectal polyps[4,5,7-9,56]. For example, biofilm formation and virulence factors from *E. coli*, responding to environmental changes in the mucosa, can induce genotoxic effects as well as inflammatory and neoplastic processes. This occurs through the activation of DNA damage, oxidative stress, and the NF-κB and STAT3 signaling pathways[57]. We did not identify *E. coli* and *Fusobacterium* in the HP and TA groups. These results are consistent with previous findings concerning colorectal adenomas[44,47]. Furthermore, they underscore the importance of developing polyp-specific biomarkers that are specifically associated with colorectal adenomas.

Furthermore, we observed substantial variation in differential microbial species among the HP, TA, and CT groups. The present study revealed a significant increase in *M. gnavus* abundance among HP and TA patients, whereas the abundances of *B. fragilis* and *S. gallolyticus* were only significantly increased in TA patients. *M. gnavus* abundance is elevated in various gastrointestinal diseases, including inflammatory bowel disease, irritable bowel syndrome, CRC, Crohn's disease, and ulcerative colitis[50,58]. This elevated abundance may be associated with inflammation and bowel neoplasia[58]. *M. gnavus* is capable of metabolizing primary bile acids, which are not absorbed by the small intestine, into secondary bile acids[59]. Elevated levels of secondary bile acids can induce oxidative and nitrosative stresses, DNA damage, apoptosis, and mutations in host cells. Furthermore, secondary bile acids interact with the farnesoid X receptor in an antagonist manner, leading to enhanced Wnt signaling in the conventional adenoma-carcinoma sequence[60]. A meta-analysis of case reports and case series from 1970 to 2010 indicated that approximately 60% of patients with *S. gallolyticus* infections also had concurrent colon adenomas or carcinomas, a rate significantly higher than the percentage observed in the general population[61]. Additional studies have shown associations of colorectal adenoma or carcinoma with *S. gallolyticus* infection[62].

The current study offers additional evidence to support the strong association between *S. gallolyticus* and colorectal adenomas. It has been observed that *S. gallolyticus* is closely linked to the transformation of colorectal mucosa into adenoma, potentially through mechanisms such as epithelial barrier invasion or virulence factor release. *S. gallolyticus* enhances inflammation and tumorigenesis by targeting NF-κB and Wnt/β-catenin signaling, upregulating β-catenin levels, and inducing inflammation *via* cytokines (*e.g.*, interleukin-1, interleukin-8, and cycloooxygenase-2)[63-65]. Therefore, *S. gallolyticus* is presumed to participate in neoplastic transformation. Our findings indicate that *S. gallolyticus* is a key contributor to the mevalonate pathway, which is substantially enriched in patients with TA. This pathway plays a crucial role in the biosynthesis of compounds such as isopentenyl pyrophosphate, farnesyl pyrophosphate, and geranyl-geranyl pyrophosphate, which serve as building blocks for various essential biomolecules, including lipoproteins, dolichol, ubiquinone, and cholesterol-derived products (*e.g.*, steroid hormones, oxysterols, vitamin D, and bile acids). These metabolites play important roles in the regulation of cellular metabolism[66]. However, isopentenyl pyrophosphate, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate can also contribute to inflammation-mediated tumor growth through oncogenic activation of Ras[66]. Previous studies have revealed that mevalonate pathway inhibition can impede the growth and proliferation of colon cancer cell lines[67]. The present study revealed a novel link between *S. gallolyticus* and the mevalonate pathway, which involved cell signaling in carcinogenesis. Notably, mevalonate pathway activity was predicted to substantially increase in the HP and TA groups compared with the CT group. This finding strongly implies that the mevalonate pathway plays a key role in colorectal polyp formation. Furthermore, the present study revealed significant increases in the abundances of *B. caecimuris*, *C. symbiosum*, and *B. fragilis* among patients with TA. *B. caecimuris* is a commensal bacterium in the human gut, and there is no evidence linking it to colorectal polyps[68]. However, *B. caecimuris* has been detected in fecal samples from CRC patients[69]. The abundance of *C. symbiosum* is increased in colorectal adenoma, making it a promising biomarker for the noninvasive detection of colorectal adenoma[70,71]. *B. fragilis*, a mucin-degrading bacterium[72], can adhere to intestinal mucus and utilize it as a nutrient source for growth[73]. *B. fragilis* produces a metalloprotease that alters signaling pathways and induces the production of reactive oxygen species, resulting in DNA damage and E-cadherin cleavage[74,75]. These results indicated that TA patients predominantly had microbial species associated with inflammation and the adenoma-carcinoma sequence. Our findings highlight the distinct contributions of the gut microbiome to the conventional adenoma-carcinoma sequence and the serrated polyp pathway. In the conventional adenoma-carcinoma sequence, the presence of pathogens and inflammation-enhancing microbial species in an inflammatory environment can promote the development of colorectal carcinogenesis. Our findings also suggest that the microbial species detected in this study may be useful for identifying patients with a high risk of colorectal adenoma.

Functional analysis provides valuable insights into the complex mechanisms underlying the development of serrated polyps. In the present study, we predicted enrichment of the APS reductase pathway in HP patients; this pathway contains a microbial enzyme that metabolizes sulfate to sulfite. Our findings suggest that the APS reductase pathway is associated with SRB, including *D. piger* and *B. wadsworthia*, which can increase H_2S levels. Previous studies concerning gut microbiome alterations among individuals with colorectal adenomas have revealed increased levels of SRB such as *Bilophila*, *Desulfovibrio*, and *B. wadsworthia* in patients with adenomatous polyps[76]. Endogenous production of H_2S primarily occurs through the metabolic activities of SRB and other bacteria, which metabolize inorganic sulfur compounds such as sulfate and sulfite (commonly found in processed food preservatives), as well as organic sulfur compounds (*e.g.*, cysteine or taurine, present in red meat)[77]. There is emerging evidence that the metabolism of organic sulfur by SRB in the human gut may link diets high in red and processed meat to increased risks of early-onset adenomas [78,79]. H_2S can damage the mucosal layer by disrupting disulfide bonds, which causes the mucus layer to become less


A
Enterocloster bolteae
Agathobacter rectalis
ER4 sp000765235
Mediterraneibacter gnavus
Dorea_A formicigenerans
Dysosmobacter sp000403435
Clostridium ramosum
Blautia_A_141781 caecimuris
Anaerobutyricum soehngenii
Copromorpha sp900066305
Blautia_A_141780 stercoris
Amedibacillus dolichus
Agathobacter faecis
Streptococcus gallolyticus
Ruthenibacterium lactatiformans
Blautia_A_141781 faecis
Ventrimonas sp003481825
Lachnospira eligens
Lawsonibacter sp000177015
Faecalimonas phoceensis
UMGS1375 sp900066615
Anaerotignum faecicola
Schaedlerella glycyrrhizinilytica_A
Anaerostipes hadrus
Clostridium_Q_135853 saccharolyticum_A
Clostridium_AQ innocuum
Clostridium_Q_135822 fessum
CAG-127 sp900319515
Anaerobutyricum hallii
Ruminococcus_D bicirculans
Bacteroides_H thetaiotaomicron
Blautia_A_141781 obeum
Faecousia sp000434635
Gemmiger_A_73129 qucibialis
Sellimonas intestinalis
Faecalibacterium prausnitzii_C_71358
Fusicatenibacter saccharivorans
Coprococcus_A_187866 catus
Limivivens sp900066135
Faecalimonas umbilicata
Blautia_A_141780 hansenii
Dorea_A longicatena
CAG-269 sp000431335
B
Blautia_A_141780 sp001304935
Enterocloster bolteae
Blautia_A_141781 massiliensis
ER4 sp000765235
Bacteroides_H caccae
Dorea_A formicigenerans
Bacteroides_H thetaiotaomicron
Parabacteroides_B_862066 distasonis
Blautia_A_141781 faecis
NSJ-61 sp003433845
Agathobaculum butyriciproducens
Escherichia marmotae
Eisenbergiella tayi
Clostridium ramosum
Agathobacter faecis
Blautia_A_141781 wexlerae
UBA1417 sp003531055
Fusicatenibacter saccharivorans
Lawsonibacter sp000177015
Dorea_A longicatena
Faecalimonas umbilicata
Schaedlerella glycyrrhizinilytica_A
Limivicinus sp002320035
Clostridium_AQ innocuum
Clostridium_Q_135853 saccharolyticum_A
UBA3402 sp003478355
Coprococcus_A_121497 eutactus
Blautia_A_141781 caecimuris
Dysosmobacter sp000403435
Enterocloster sp000155435
Faecousia sp000434635
Mediterraneibacter gnavus
Coprococcus_A_187866 catus
Blautia_A_141781 obeum
Enterocloster lavalensis
Limivivens sp900066135
Anaerobutyricum soehngenii
Dialister invisus
Lachnospira eligens
Faecalibacterium prausnitzii_C_71358
Intestinibacter bartlettii
Copromorpha sp900066305
Clostridium_Q_134516 symbiosum
Blautia_A_141780 coccoides
Lactonifactor longoviformis
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UMGS1375 sp900066615
Anaerobutyricum hallii
Sellimonas intestinalis
Clostridium_Q_135822 fessum

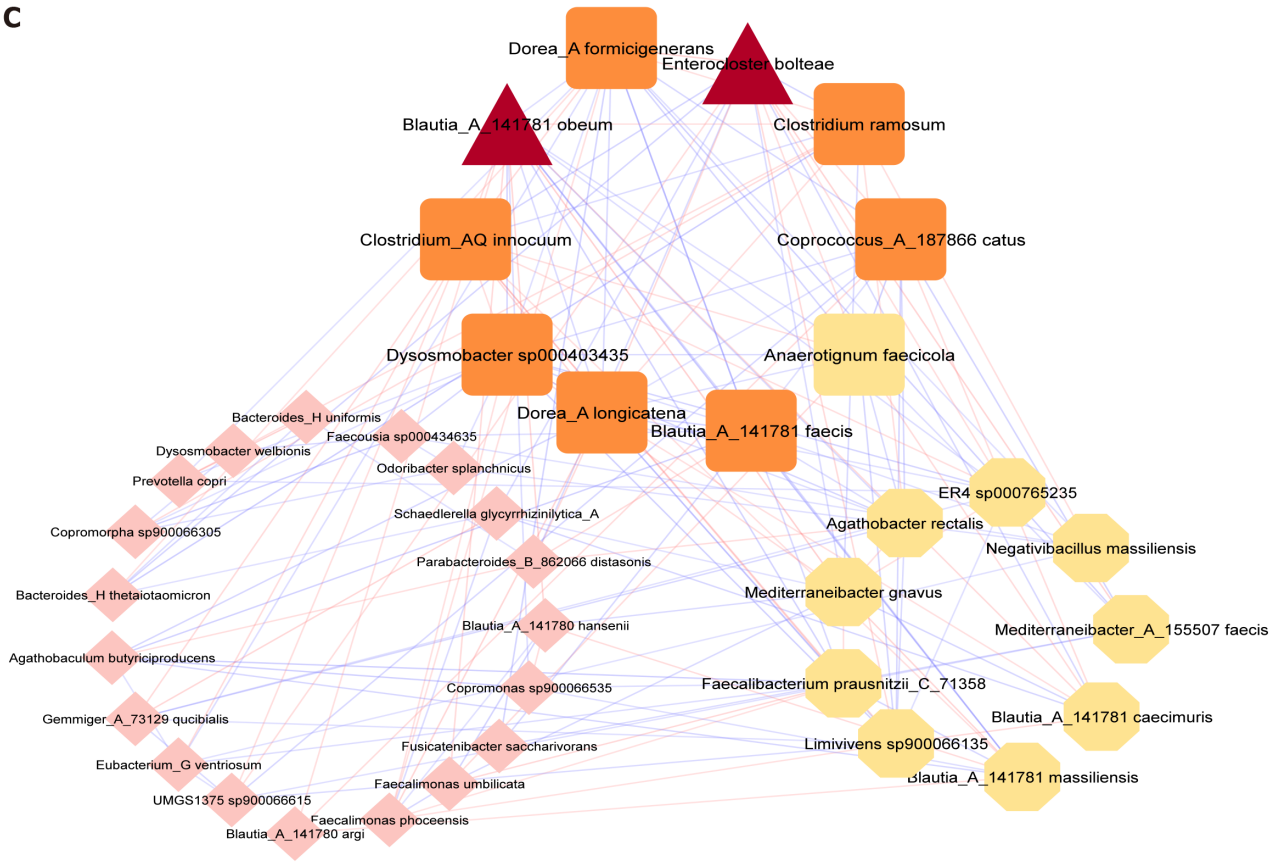

Figure 5 Microbial interaction network of the gut microbiome. A: Co-occurrence networks were constructed at the species level using abundance data from the control (CT) group; B: Co-occurrence networks were constructed at the species level using abundance data from hyperplastic polyps (HP); C: Co-occurrence networks were constructed at the species level using abundance data from tubular adenoma (TA) group. The figure shows that all nodes had more than five connections. Each node in the network represents a single microbial species. The color of each node corresponds to its degree (*i.e.* number of connections with other nodes). Nodes are represented as follows, according to their degree: > 20, red; 16-19, orange; 10-15, yellow; and 6-9, pink. Edges between nodes represent correlations between those nodes; blue indicates a positive correlation, whereas red indicates a negative correlation.

viscous and more permeable. These changes allow toxic compounds and microbial species from the gut lumen to directly interact with the epithelial cell surface, leading to cellular damage, triggering immune responses, and promoting inflammation[79,80]. Chronic inflammation is frequently associated with gastrointestinal cancers, and individuals with colitis have an increased risk of cancer[81]. Therefore, mucin restoration and mucosal barrier strengthening are therapeutic objectives during chronic inflammation, particularly in patients with extensive colitis. This approach is likely to reduce neoplastic processes in the intestinal epithelium and improve health outcomes.

A previous study involving a Thai population revealed decreased *B. thetaiotaomicron* abundance among individuals with adenoma, whereas the abundance was increased among individuals with CRC. Conversely, *P. distasonis* abundance was increased among patients with adenoma and patients with CRC[82]. The present study showed no significant difference in the relative abundances of *B. thetaiotaomicron* and *P. distasonis* between the polyp groups (HP and TA) and the CT group. Both microbial species contribute to thiosulfate oxidation within the sulfur oxidation pathways. Specifically, they are involved in the enzymatic process known as thiosulfate:quinone oxidoreductase, which facilitates thiosulfate oxidation and subsequent tetrathionate production. These intermediates in the sulfur cycle may serve as key sites for electron transfer and energy generation[83]. Nevertheless, it is important to note that the present study specifically focused on the contributions of *B. thetaiotaomicron* and *P. distasonis* to metabolic pathways that exhibited significant differences compared with the CT group, rather than conducting a comprehensive evaluation of all pathways.

Co-occurrence network analysis revealed fewer interactions between beneficial microbial species in the HP and TA groups, which may be associated with the occurrence of colorectal polyps. Notably, positive interactions among beneficial microbial species such as *C. catus*, *Dysosmobacter* sp000403435, and the butyrate-producing genus *Eubacterium* were diminished. Additionally, the HP and TA groups showed fewer negative associations between *C. catus* and opportunistic pathogens such as *E. bolteae*. These results suggest that decreased interactions among beneficial microbial species contribute to colorectal polyp formation. Furthermore, synchronized network analysis demonstrated differences in co-occurrence patterns between the HP and TA groups. For example, the TA group exhibited fewer occurrences of beneficial microbial species compared with the HP group; they also displayed higher levels of co-occurrence involving CRC-associated bacteria. These microbial species are associated with the inflammation that leads to the progression of HP and TA. SCFAs can reduce gut inflammation by promoting gut barrier integrity and permeability through various mechanisms that also help to maintain homeostasis[84-86]. Therefore, the co-occurrence of SCFA-producing bacteria in

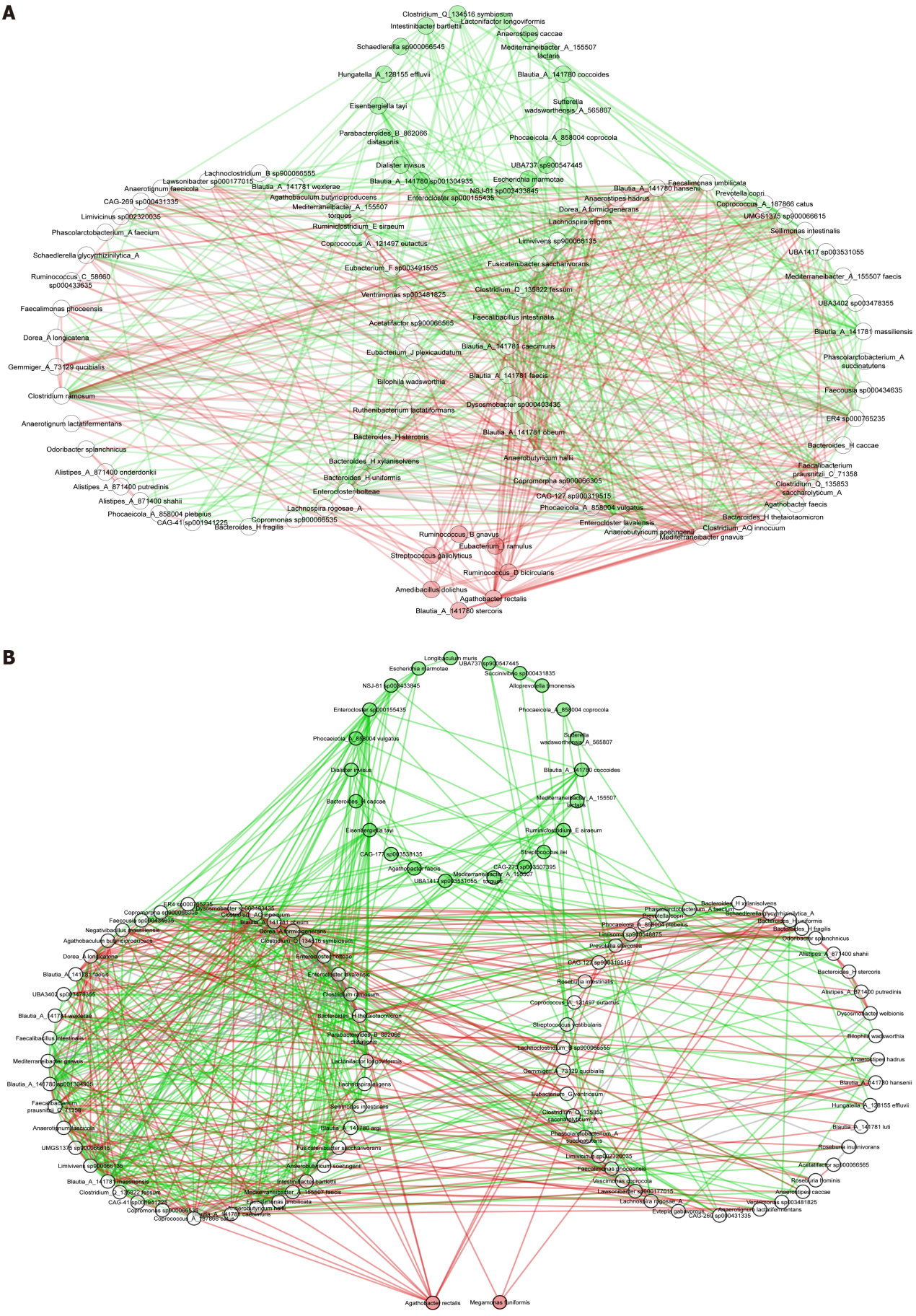

Figure 6 Co-occurrence networks and DyNet visualization of synchronized co-occurrence networks. A: The control (CT) and hyperplastic polyps

(HP) networks; B: The CT and tubular adenoma (TA) networks. Nodes represent microbial species, whereas edges represent correlation coefficients between microbial species. Green edges are only present in the CT network; red nodes and edges are exclusive to the HP and TA groups; and white nodes and gray edges are shared between the CT and HP groups, as well as the CT and TA groups.

patients with HP may enable the maintenance of a good health status[56]. In contrast, a decrease in the co-occurrence of SCFA-producing bacteria, accompanied by an increase in the co-occurrence of CRC-associated bacteria, may be involved in colorectal adenoma formation and colorectal carcinogenesis in TA patients. This hypothesis is supported by a previous report in which patients with cystic fibrosis, who carry adenomas and have a high risk of CRC, exhibited reductions in SCFA-producing bacteria and an increased relative abundance of *B. fragilis*[87].

Colorectal polyps can develop through two main genetic pathways. The conventional adenoma-carcinoma sequence is characterized by mutations in the *adenomatous polyposis coli* gene, chromosomal instability, or microsatellite instability, as well as the absence of CpG island methylator phenotype (CIMP) alterations; this pathway leads to TA. The alternative pathway, the serrated polyp pathway, is mainly characterized by BRAF mutations and high numbers of CIMP alterations; this pathway leads to HP, SSA/Ps, and traditional serrated adenoma. There is substantial histological overlap between SSA/Ps and HP; SSA/Ps constitute up to 30% of all colon cancers. SSA/Ps can develop either as primary tumors or evolve from HP[3], suggesting CRC susceptibility in HP patients. Our investigation of gut microbiome characteristics in different types of colorectal polyps revealed that HP and TA had distinct gut microbiome signatures. This comprehensive analysis of gut microbiome signatures has provided valuable insights concerning gut microbiome contributions to colorectal polyp development, particularly with respect to gut microbiome effects on carcinogenesis. In the serrated polyp pathway, dysbiosis and gastrointestinal disease-associated bacteria, along with the inflammation-inducing sulfur oxidation pathway, contribute to the establishment of a tumor microenvironment. However, this phenomenon is counterbalanced by increased abundances and co-occurrence of SCFA-producing bacteria in HP patients. Furthermore, we speculate that the increased abundance of CRC-associated bacteria mediating the mevalonate pathway involves inflammation and cell proliferation in TA patients, suggesting that such bacteria contribute to the conventional adenoma-carcinoma sequence.

The present study utilized long-read 16S rRNA sequencing, which offers higher resolution for assignments of microbial identity at the species or strain levels. However, it is important to acknowledge the limitations of this study. In particular, it had a small sample size, the results should be confirmed in another cohort, and experimental validation is needed. Furthermore, during bioinformatics analysis, ASVs were aggregated to the species level and then used as input for alpha and beta diversity analyses, as well as differential abundance and co-occurrence analyses.

CONCLUSION

This study utilized differential abundance, co-occurrence, and differential pathway analyses to characterize the gut microbiome signatures in colorectal polyps. The differential abundance analysis identified candidate microbial species that could serve as biomarkers for colorectal polyps. The co-occurrence analysis provided insights concerning the dynamic changes in microbial correlation networks among the CT, HP, and TA groups. The differential pathway analysis predicted functional pathways and determined the roles of microbial species in metabolic function during colorectal polyp development. The results highlight the importance of numerous pathways in colorectal polyp development, offering evidence to support interventions and treatment in the context of CRC carcinogenesis. Furthermore, analyses of the dynamic changes between the CT group and colorectal polyp groups (HP and TA) enhanced the understanding of gut microbiome interactions within the community. Specifically, our findings suggest that HP patients have an increased risk of CRC; more effective strategies are needed to identify and manage such patients.

ACKNOWLEDGEMENTS

In memory of Assistant Professor Dr. Bunchorn Siripongpreeda — his robust leadership and project management made this work possible. We sincerely thank Watsawan Sangkaew, Watcharapol Khoonin, Piangpit Kamolphan, Teeramade Thidseesang, Yanee Poomjaroen, Supanan Makkram, Suyada Sukho, Phaktheema Phoothong, Suchada Panaiem, Chanisara Suebwongdit, Napatsawan Opad, and Tulyawat Prasongmaneerut for assistance with participant recruitment. We especially thank Thanrada Popraros, Napong Leelasithorn, Kawin Chinpong, Tanabhorn Kookongkritsadakorn, Kamonwan Soonklang, Penpisut Homsuwan, and Kerati Jirawattanapalin for assistance with collecting patient information from questionnaires. We also thank Chirayu Auewarakul, Thaniya Sricharunrat, and Montri Gururatsakul for providing medical advice.

FOOTNOTES

Author contributions: Cheevadhanarak S, Sutheeworapong S, Thammarongtham C, Intarajak T and Udomchaiprasertkul W contributed to conceptualization; Cheevadhanarak S, Kusonmano K, Intarajak T and Udomchaiprasertkul W contributed to methodology;

Sutheeworapong S, Intarajak T and Khoiri AN contributed to software; Intarajak T, Kittichotirat W and Khoiri AN contributed to validation; Intarajak T and Khoiri AN contributed to formal analysis; Cheevadhanarak S, Intarajak T and Udomchaiprasertkul W contributed to investigation, resources, data curation and writing – original draft preparation; Cheevadhanarak S, Thammarongtham C, Kusonmano K, Khoiri AN, Intarajak T and Udomchaiprasertkul W contributed to writing – review and editing; Intarajak T, contributed to visualization; Cheevadhanarak S, Thammarongtham C, Sutheeworapong S, Kusonmano K and Kittichotirat W contributed to supervision; Intarajak T and Kittichotirat W contributed to project administration and funding acquisition; All authors gave final approval of the version of the article to be published.

Supported by Chulabhorn Royal Academy (Fundamental Fund: Fiscal year 2022 by National Science Research and Innovation Fund), No. FRB650039/0240 Project Code 165422.

Institutional review board statement: The study protocol was reviewed and approved by the Ethical Committee of Chulabhorn Research Institute and the Institutional Review Boards of Chulabhorn Royal Academy (Project Code 045/2563).

Informed consent statement: Informed consent was obtained from all participants involved in the study.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: Raw sequence data have been deposited in the SRA database under accession number PRJNA940282.

Country of origin: Thailand

ORCID number: Thoranin Intarajak 0000-0001-7756-2528; Wandee Udomchaiprasertkul 0009-0004-6112-4194; Ahmad Nuruddin Khoiri 0000-0003-2883-4149; Sawannee Sutheeworapong 0000-0002-1988-1624; Kanthida Kusonmano 0000-0002-6532-9144; Weerayuth Kittichotirat 0000-0001-8397-633X; Chinae Thammarongtham 0000-0001-5834-4795.

S-Editor: Li L
L-Editor: Filipodia
P-Editor: Zheng XM

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World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2024 July 21; 30(27): 3356-3360

DOI: 10.3748/wjg.v30.i27.3356

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LETTER TO THE EDITOR

More on the interplay between gut microbiota, autophagy, and inflammatory bowel disease is needed

Arunkumar Subramanian, Afrarahamed Jahabardeen, Tamilanban Thamaraikani, Chitra Vellapandian

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade D

Novelty: Grade B, Grade C

Creativity or Innovation: Grade C, Grade C

Scientific Significance: Grade B, Grade C

P-Reviewer: He L; Mu YP

Received: April 24, 2024

Revised: June 15, 2024

Accepted: June 27, 2024

Published online: July 21, 2024

Processing time: 72 Days and 14.2 Hours

Arunkumar Subramanian, Afrarahamed Jahabardeen, Tamilanban Thamaraikani, Chitra Vellapandian, Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Chennai 603203, India

Corresponding author: Tamilanban Thamaraikani, PhD, Associate Professor, Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology, Intra College Road, Kattankulathur, Chennai 603203, India. tamilant@srmist.edu.in

Abstract

The concept of inflammatory bowel disease (IBD), which encompasses Crohn's disease and ulcerative colitis, represents a complex and growing global health concern resulting from a multifactorial etiology. Both dysfunctional autophagy and dysbiosis contribute to IBD, with their combined effects exacerbating the related inflammatory condition. As a result, the existing interconnection between gut microbiota, autophagy, and the host's immune system is a decisive factor in the occurrence of IBD. The factors that influence the gut microbiota and their impact are another important point in this regard. Based on this initial perspective, this manuscript briefly highlighted the intricate interplay between the gut microbiota, autophagy, and IBD pathogenesis. In addition, it also addressed the potential targeting of the microbiota and modulating autophagic pathways for IBD therapy and proposed suggestions for future research within a more specific and expanded context. Further studies are warranted to explore restoring microbial balance and regulating autophagy mechanisms, which may offer new therapeutic avenues for IBD management and to delve into personalized treatment to alleviate the related burden.

Key Words: Inflammatory bowel disease; Gut microbiota; Autophagy; Crohn's disease; Ulcerative colitis

Core Tip: Further research is needed into the intricate interplay between the gut microbiota, autophagy, and inflammatory bowel disease with the aim of implementing possible new treatment protocols and/or specific public health policies to both alleviate the burden of inflammatory bowel disease and improve outcomes for affected patients.

Citation: Subramanian A, Jahabardeen A, Thamaraikani T, Vellapandian C. More on the interplay between gut microbiota, autophagy, and inflammatory bowel disease is needed. *World J Gastroenterol* 2024; 30(27): 3356-3360
URL: https://www.wjgnet.com/1007-9327/full/v30/i27/3356.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i27.3356

TO THE EDITOR

Inflammatory bowel disease (IBD) is a term that refers to a highly prevalent group of chronic diseases characterized by inflammation in the gastrointestinal tract, posing a significant health challenge worldwide[1]. Despite extensive research, its multifactorial origins and complex pathogenesis remain incompletely understood. Several variables, including the host's immune system, the makeup of the intestinal microbiota, genetic predisposition, and environmental variables, affect the onset and course of IBD[2].

However, it is known that the interconnection between the gut microbiota, autophagy, and the host's immune system is a key nexus in IBD development. In this connection, nutritional and other possible influences on gut microbiota and their impact on IBD, in addition to their potential to target the microbiota and modulate autophagic pathways for IBD therapy, are important topics to be discussed and researched in more detail.

The onset of IBD often coincides with dysregulated autophagy, leading to the accumulation of pathogens and the breakdown of immune tolerance, resulting in chronic intestinal inflammation[3]. At the same time, the composition and function of the gut microbiota are also disrupted in individuals with IBD, thereby contributing to the ongoing inflammatory response[4]. A better understanding of these interactions may provide new insights for developing more effective treatments in the evolving landscape of microbiome and immunology research, as well as assist in a more accurate subclassification of IBD phenotypes. In line with this, recent scientific focus on this subject has converged on the interplay between gut microbiota, autophagy, and IBD, recognizing their pivotal roles in the development and progression of the disease[5].

The present manuscript briefly discussed the intricate relationships between these three entities, gut microbiota, autophagy, and IBD, within a concise framework. By exploring their interactions, mechanisms, and therapeutic implications, we also aimed to provide additional insights into potential avenues for future research, especially aimed at better outcomes for IBD patients.

Gut microbiota and IBD

First, dysbiosis, characterized by an imbalance in microbial composition, is closely linked to IBD pathogenesis. Changes in gut microbial diversity and function influence the host's responses, including immune and metabolic responses, aimed at restoring the balance in host-microbe interactions. This host response encompasses elements such as antimicrobial peptides, reactive oxygen species, immunological mediators, mucus, and other modifications that influence the structure and functioning of the gut microbial community, effectively altering the local ecosystems within the gut immune responses, mucosal integrity, and inflammation, which may exacerbate IBD symptoms[6]. The human gastrointestinal microbiota, comprised of trillions of microorganisms, plays a pivotal role in host health, including nutrient metabolism, immune modulation, and defense against pathogens[7]. A recent study has identified reduced microbial diversity and changes in the abundance of bacterial taxa in IBD patients compared to healthy individuals, thus highlighting the intricate relationship between gut microbiota dysbiosis and IBD pathophysiology[8].

As a result, mindful dietary choices, among other healthy habits, are essential in IBD management. By selecting foods that promote healthy gut microbiota, support the integrity of the mucosal barrier, and directly influence the immune response, individuals can play an active role in reducing the impact of IBD on their daily lives[9].

Autophagy in gut homeostasis

Autophagy is an essential cellular function shared by all eukaryotic species, which is essential to the preservation of regular physiological processes. In addition, it is relevant in a variety of circumstances, including famine, decreased availability of growth factors, and elevated energy requirements. Under these circumstances, autophagy is activated to provide energy and sustain vital metabolic functions. Dysfunctional autophagy, in turn, has been implicated in IBD onset and progression, leading to exacerbation of inflammation and impairing immune regulation[5,10].

The intricate mechanisms of autophagy involve several major pathways, including macro-autophagy, micro-autophagy, and chaperone-mediated autophagy. Macro-autophagy, the most extensively studied pathway, involves the formation of autophagosomes that engulf cellular components for degradation[11]. Dysregulated autophagy in IBD is associated with impaired clearance of intracellular pathogens and dysfunctional immune responses, thereby perpetuating chronic inflammation and mucosal damage[5,10].

Intersection of gut microbiota, autophagy, and IBD

The dynamic interplay between the gut microbiota and autophagy significantly influences IBD pathophysiology, potentially compromising pathogen clearance, while disrupting microbial balance and causing chronic inflammation in the gut[5,10]. Deciphering these reciprocal effects is crucial to understanding the molecular mechanisms underlying IBD progression. In light of this, examining how imbalances in gut microbiota composition or autophagy may enhance the inflammatory response should offer important insights for prospective treatment approaches. Moreover, by focusing on

the complex relationships between autophagy and the gut microbiota, new approaches to the management and treatment of IBD may become possible.

In this regard, emerging evidence suggests bidirectional interactions between the gut microbiota and autophagy pathways in modulating IBD pathogenesis. Dysbiosis-induced changes in microbial composition and metabolites influence autophagic activity, while dysregulated autophagy compromises mucosal barrier function and immune homeostasis, thereby exacerbating gut inflammation[5].

A study revealed that mutations in autophagy-related genes like the autophagy-related 16 like 1 single nucleotide and nucleotide-binding oligomerization domain-2 genes have been strongly linked to Crohn's disease. The autophagy-related 16 like 1 single nucleotide plays a crucial role in the autophagic process. Mutations in these genes impair autophagy, leading to an inadequate response to gut microbiota and promoting chronic inflammation[12,13]. Clinical trials have shown that fecal microbiota transplantation can help restore healthy microbial imbalance in IBD patients, leading to significant improvement in the composition of gut microbiota and a reduction in inflammatory markers[14]. Several preclinical studies have monitored changes in gut microbiota composition following treatment with autophagy-inducing agents[15]. A deeper understanding of the crosstalk between the gut microbiota and autophagy pathways should be promising and imminently needed within this research scope.

Therapeutic implications and future perspectives

Addressing defective autophagy holds promise in modulating the development of IBD, thus restoring intestinal homeostasis, rebalancing the gut microbiota, and improving the clearance of intracellular pathogens. Innovative therapies targeting autophagy pathways, such as dietary interventions[16] and gene therapies[17], may offer potential avenues for advancements in personalized IBD management and treatment.

Genetic polymorphisms in autophagy-related genes have been implicated in IBD susceptibility, highlighting the therapeutic potential of modulating autophagic pathways in disease management[18]. Furthermore, microbiota-based therapies, including fecal microbiota transplantation[19] and targeted probiotics[20], show promise in restoring gut microbial balance and alleviating IBD symptoms. Within this perspective, more effective and individualized therapeutic strategies are imminently needed for IBD patients, taking into account the relationships among gut bacteria, autophagy, and the immune system.

Suggestions for further research in the field of IBD

Table 1 summarizes our suggestions for future research for more promising management and treatment of IBD cases in the short, medium, and long term.

Final consideration

In reaction to the above discussion, further research into targeted interventions and personalized treatment approaches is warranted to alleviate the burden of IBD and improve patient outcomes. Therefore, the continuity and proposition of new clinical trials and investigations focusing on different interconnected aspects, such as autophagy modulation, microbiota-based treatments, and novel techniques are also essential to the goal of developing more potent medicines and effective treatments for IBD. The growing understanding of the relationships among gut microbiota, autophagy, and IBD is bringing us one step closer to a time when patients will no longer have to endure many of the severe consequences of this difficult and multifaceted condition.

Table 1 Potentially promising research proposals related to inflammatory bowel disease

Suggestions	Scope	Possible research methods and expected outcomes
(1) Additional experimental and human studies on IBD pathophysiology	Considering more specifically the interrelationship between gut microbiota, autophagy, and IBD	(1) Usage of genetically engineered mice (IL-10 knockout mice, TNFΔARE mice) to mimic human IBD; (2) Use CRISPR/Cas9 technology to create targeted mutations in autophagy-related genes to study their effects on gut inflammation; (3) Recruit a diverse cohort of IBD patients including those with Crohn's disease and ulcerative colitis, as well as healthy controls, to better understand the pathophysiology of IBD; and (4) Employ bioinformatic tools to model the complex interactions between genes, proteins, and microbiota in IBD
(2) Development of more effective medicines and therapies for IBD patients	Considering more specifically the interrelationship between gut microbiota, autophagy, and IBD	(1) Utilizing primary intestinal epithelial cells, immune cells, and patient-derived organoids to test drug efficacy and toxicity; (2) Developing monoclonal antibodies targeting specific cytokines (TNF-α, IL 12/23) involved in IBD inflammation; and (3) Developing formulations of beneficial bacteria or prebiotic fiber to restore gut microbiota imbalance
(3) Development of new and more effective personalized treatment approaches	Considering more specifically the interrelationship between gut microbiota, autophagy, and IBD	(1) Developing microbiome-based biomarkers for IBD diagnosis, prognosis, and treatment response; and (2) Utilizing biomarkers to stratify patients and tailor treatments based on individual profiles to enhance personalized therapeutic intervention
(4) Periodic epidemiological monitoring studies on the burden, years of life lost, or years	Expanded scope	Performing data collection, analysis, and interpretation, ensuring reliable results for analyzing the distribution patterns

living with disability in cases of IBD		
(5) Assessment of the quality of life of affected patients according to new medicines used or treatment protocols	Expanded scope	Using appropriate quality of life measurement tools (Short form health survey or EuroQol 5D questionnaire) designing a robust study, collecting, analyzing, and interpreting the results
(6) More comprehensive pharmacovigilance studies involving affected patients	Expanded scope	Designing a structured approach to monitor, assess, and understand the safety profiles of medications
(7) Studies on scientific misinformation related to IBD	Expanded scope	Utilizing a systematic approach to identify, analyze, and understand the spread and impact of misinformation related to IBD

CRISPR/Cas9: Clustered regularly interspaced short palindromic repeats/associated protein 9; EuroQol 5D: European quality of life-five dimensions; IBD: Inflammatory bowel disease; IL: Interleukin; TNF-α: Tumor necrosis factor-alpha; TNFΔARE: Tumor necrosis factor, adenylate-uridylate-rich element deletion.

ACKNOWLEDGEMENTS

We thank the SRM Institute of Science and Technology for supporting this study.

FOOTNOTES

Author contributions: Subramanian A contributed to the study conception and design and writing of the original draft; Jahabardeen A and Vellapandian C contributed to the writing of the manuscript; Thamaraikani T contributed to the writing of the manuscript and supervision.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Country of origin: India

ORCID number: Arunkumar Subramanian 0000-0002-2707-2501; Afrarahamed Jahabardeen 0009-0000-6840-9230; Tamilanban Thamaraikani 0000-0002-3240-0645; Chitra Vellapandian 0000-0002-3052-9517.

S-Editor: Fan M
L-Editor: Filipodia
P-Editor: Zheng XM

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