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ABOUT COVER

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REVIEW

Gut microbiome and metabolic-associated fatty liver disease: Current status and potential applications

Gong-Jing Guo, Fei Yao, Wei-Peng Lu, Hao-Ming Xu

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Abstract

Metabolic-associated fatty liver disease (MAFLD) is one of the most common chronic liver diseases worldwide. In recent years, the occurrence rate of MAFLD has been on the rise, mainly due to lifestyle changes, high-calorie diets, and imbalanced dietary structures, thereby posing a threat to human health and creating heavy social and economic burdens. With the development of 16S sequencing and integrated multi-omics analysis, the role of the gut microbiota (GM) and its metabolites in MAFLD has been further recognized. The GM plays a role in digestion, energy metabolism, vitamin synthesis, the prevention of pathogenic bacteria colonisation, and immunoregulation. The gut-liver axis is one of the vital links between the GM and the liver. Toxic substances in the intestine can enter the liver through the portal vascular system when the intestinal barrier is severely damaged. The liver also influences the GM in various ways, such as bile acid circulation. The gut-liver axis is essential in maintaining the body's normal physiological state and plays a role in the onset and prognosis of many diseases, including MAFLD. This article reviews the status of the GM and MAFLD and summarizes the GM characteristics in MAFLD. The relationship between the GM and MAFLD is discussed in terms of bile acid circulation, energy metabolism, micronutrients, and signalling pathways. Current MAFLD treat-

ments targeting the GM are also listed.

Key Words: Metabolic-associated fatty liver disease; Gut microbiota; Current status; Application

Core Tip: Metabolic-associated fatty liver disease (MAFLD) is a highly prevalent metabolic disease worldwide. In this review, we provide an overview of the current status and potential applications of the gut microbiota (GM) in MAFLD, focusing on key aspects such as bile acid circulation, energy metabolism, and microelement disorder, as well as signal pathways and GM metabolites implicated in MAFLD development and treatments, with a particular emphasis on targeting the microbiome.

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INTRODUCTION

Metabolic-associated fatty liver disease (MAFLD), originally known as non-alcoholic fatty liver disease (NAFLD), is one of the most common chronic liver diseases worldwide. Liver inflammation and fibrosis are the pathological processes implicated in MAFLD. MAFLD can develop into non-alcoholic steatohepatitis (NASH), which can then progress to liver cirrhosis, hepatic failure, and liver cancer[1]. The rate of MAFLD has been on the rise, with a global rate of 25%-30%, due to lifestyle changes, excessive calorie intake, and unbalanced diet structures. In certain groups, such as patients with Type 2 diabetes, the rate of MAFLD even exceeds 70%[2,3]. MAFLD poses a threat to human health and leads to substantial social and economic burdens. The gut microbiota (GM) lives in the human intestinal tract. In the past 10 years, there has been an exponential growth of studies on the relationship between GM and human health and disease in databases such as PubMed. The GM consists of over 10^{14} microorganisms[4], and its genome comprises of over 3 million genes, whereas the human genome consists of approximately 23000 genes[5]. Therefore, the GM is considered as one of the "new organs" in human beings. Steady-state GM plays a role in digestion, energy metabolism, vitamin synthesis, the prevention of pathogenic bacteria colonisation, and immunoregulation[6,7]. The gut-liver axis is one of the vital links between the GM and the liver. The intestine and liver both originate from the ventral foregut endoderm. When the intestinal barrier is severely damaged, toxic substances in the intestinal tract enter the portal vein through the superior and inferior mesenteric veins and then flow into the liver. Meanwhile, the liver influences the intestinal microecology in various ways. For example, the liver secretes bile acids, which enter in the enterohepatic circulation to alter the intestinal microecology [8]. In recent years, the development of 16S sequencing and integrated multi-omics analysis has helped to further understand the role of the GM and its metabolites in MAFLD. It is reported that *Akkermansia muciniphila* can improve liver function, reduce oxidative stress, inhibit inflammation, and reverse the metabolic disorder caused by high-fat diets [9]. Some secondary bile acid-producing bacteria, such as *Lactobacillaceae* and *Lachnospiracea*, have cholesterol-reducing potential[10]. Specific bile acids produced by bacteria can regulate GM structure and restore GM balance[11]. Numerous studies have confirmed that GM metabolites play a significant role in the onset and progression of MAFLD when they are present in the intestine or enter the circulation. GM can mediate the fermentation of dietary fibres, leading to the production of short-chain fatty acids (SCFAs) as the primary metabolites of this process. Among the SCFAs, butyric acid can improve the MAFLD induced by high-fat diets *via* activating peroxisome proliferator-activated receptor α to inhibit liver inflammation and enhance the expression of glucagon-like peptide-1 receptor[12]. Furthermore, SCFAs can also activate G-protein-coupled receptor and induce the release of glucagon-like peptide-1 (GLP-1) and peptide YY, resulting in metabolically balanced feedback regulation[13]. SCFAs can not only regulate glycolipid metabolism, inhibit fat synthesis, and reduce liver fat content but also escalate intestinal barrier function, thereby improving MAFLD[14]. Therefore, the main aims of this research field are to observe and characterize the GM in MAFLD, investigate the impact of GM and its metabolites on the onset and progression of MAFLD, and explore the potential of targeting the GM for MAFLD treatment.

This article primary focuses on elucidating the impact of the gut-liver axis on MAFLD. It provides an overview of the existing clinical MAFLD cases and commonly utilized animal models. The review involves the important aspects such as bile acid circulation, energy metabolism, microelement disorder, and other relevant factors such as signal pathways and GM metabolites implicated the development of MAFLD. Then, current MAFLD treatments utilizing GM as the target are presented. Table 1 summarizes the key characteristics of GM in both clinical MAFLD cases and commonly used MAFLD animal models.

Table 1 Studies presenting gut dysbiosis in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis

			Alterations of GM (↑/↓)			
Country	Subjects	Year	Phylum	Family	Genus/Species	Ref.
United States	Obese patients without NASH (n = 25), NASH (n = 22), Controls (n = 16)	2013	↑*Bacteroidetes*; ↑ *Proteobacteria*; ↓ *Firmicutes*; ↓*Actinobacteria*	↓*Bifidobacteriaceae*; ↑*Alcaligenaceae*; ↓ *Clostridiales family XI*; ↑*Campylobacteraceae*; ↓*Lachnospiraceae*; ↑*Enterobacteriaceae*	↑*Prevotella*; ↑*Escherichia*	[69]
Canada	NAFLD (n = 33) *vs* Controls (n = 17)	2013	↓*Bacteroidetes*	/	↑*Clostridium coccoides*	[124]
Canada	NAFLD (n = 30) *vs* Controls (n = 30)	2013	↑*Proteobacteria*; ↑ *Firmicutes*; ↓ *Bacteroidetes*	↑*Kiloniellaceae*; ↑*Pasteurellaceae*; ↑ *Lactobacillaceae*; ↑*Lachnospiraceae*; ↑ *Veillonellaceae*; ↓*Ruminococcaceae*; ↓ *Porphyromonadaceae*	↑*Lactobacillu*; ↑*Robinsoniella*; ↑*Roseburia*; ↑ *Dorea*; ↓*Oscillibacter*	[125]
Hong Kong	NASH (n = 16) *vs* Controls (n = 22)	2013	↓*Firmicutes*	/	↓*Faecalibacterium*; ↓*Anaerosporobacter*; ↑ *Parabacteroides*; ↑*Allisonella*	[126]
China	NAFLD (n = 53) *vs* Controls (n = 32)	2015	↑*Firmicutes*; ↑ *Proteobacteria*	↑*Peptostreptococcaceae*; ↑*Lactobacillaceae*; ↓*Ruminococcaceae*; ↓ *Porphyromonadaceae*	↑*Escherichia*; ↑*Lactobacillus*; ↑*Streptococcus*; ↑*Anaerobacter*; ↓*Prevotella*	[127]
United States	Controls (n = 26), obese (n = 11), NAFLD (n = 13)	2015	↑*Actinobacteria*	/	↓*Erysipelotrichia*; ↑*Prevotella*; ↓*Alphaproteobacteria*; ↑*Clostridia*; ↓*Verrucomicrobia*; ↑ *Fusobacteria*; ↑*Epsilonproteobacteria*; ↑ *Gammaproteobacteria*	[67]
France	NASH (n = 35) *vs* Controls (n = 22)	2016	↑*Proteobacteria*	↑*Enterobacteriaceae*; ↓*Ruminococcaceae*	↑*Ruminococcus*; ↓*Prevotella*; ↑*Escherichia*; ↓ *Anaerospacter*; ↓*Coprococcus*; ↓*Eubacterium*; ↓*Faecalibacterium*; ↑*Bacteroides*	[128]
Italy	NAFLD (n = 61) *vs* Controls (n = 54)	2017	↑*Actinobacteria*; ↓ *Bacteroidetes*	↓*Rikenellaceae*	↑*Bradyrhizobium*; ↑*Anaerococcus*; ↑*Peptoniphilus*; ↑*Ruminococcus*; ↓*Oscillopira*; ↑*Dorea* ; ↑*Blautia*; ↑*Propionibacterium acnes*	[129]
China	NAFLD (n = 43) *vs* Controls (n = 83)	2016	↑*Bacteroidetes*; ↓ *Firmicutes*	↑*Bacteroidaceae*; ↓*Lachnospiraceae*; ↑ *Prevotellaceae*; ↓*Ruminococcaceae*; ↓ *Lactobacillaceae*; ↓*Peptostreptococcaceae*	↓*Coprococcus*; ↓*Anaerosporobacter*; ↓ *Anaerotruncus*; ↓*Ruminococcus*; ↓*Lactobacillus*	[130]
China	NAFLD (n = 25) *vs* Controls (n = 22)	2017	↑*Proteobacteria*; ↓ *Bacteroidetes*	↑*Lachnospiraceae*; ↑*Enterobacteriaceae*; ↓*Prevotellaceae*; ↓*Ruminococcaceae*; ↑ *Erysipelotrichaceae*; ↑*Streptococcaceae*	↑*Fusobacteria*; ↓*Prevotella*; ↑*Blautia*; ↑ *Escherichia*; ↑*Shigella*; ↑*Fusobacteria*; ↑ *Escherichia Shigella*	[131]
Canada	NAFLD (n = 39) *vs* Controls (n = 28)	2018	↓*Firmicutes*; ↓ *Bacteroidetes*	↑*Lactobacillaceae*	↓*Ruminococcus*; ↓*Faecalibacterium*; ↓ *Coprococcus*	[132]
Italy	Obese, NAFL and NASH (n = 61) and Controls (n = 54)	2016	/	/	↑*Lactobacilli*; ↓*Bifidobacteria*; ↑*Lactobacilli mucosae*; ↓*Bifidobacteria longum*; ↓ *Bifidobacteria adolescent*; ↓*Bifidobacteria bifidum*	[133]
Brazil	NASH (n = 13) *vs* Controls (n = 10)	2017	/	/	↑*Bacteroides*; ↑*Lactobacilli*; ↓*Ruminococcu*; ↓ *Bifidobacterium*; ↑*Prevotella*; ↓*Faecalibacterium*	[134]
China	NAFLD (n = 30) *vs* Controls (n = 37)	2018	/	↑*Lactobacillaceae*; ↑*Veillonellaceae*; ↑ *Peptostreptococcaceae*; ↑*Coprobacillaceae*; ↑*Erysipelotrichaceae*; ↓*Parapre-votellaceae*; ↓*Victivallaceae*	↑*Porphyromonas*; ↑*Clostridium*; ↑*Blautia*; ↑ *Dorea*; ↑*Peptococcus*; ↑*Peptococcaceae_rc4-4*; ↑*Mitsuokella*; ↑*Slackia*; ↑*Succinivibrio*; ↓ *Odoribacter*; ↓*Coprococcus*; ↓*Proteus*	[135]
Germany	NAFLD (n = 90) *vs* Controls (n = 21)	2020	↓*Bacteroidetes*	↓*Ruminococcaceae*; ↑*Lactobacillaceae*; ↑ *Veillonellaceae*	↑*Dorea*	[136]
United States	NAFLD (n = 44) *vs* Controls (n = 29)	2020	↓*Bacteroidetes*	/	↓*Prevotella*; ↓*Gemmiger*; ↓*Oscillospira*	[137]

NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

GM AND METABOLIC DISORDERS IN MAFLD

Bile acid metabolism disorders

Primary bile acids are synthesized in the liver before being secreted into the gall bladder and released into the duodenum after a meal. Bacteria metabolize primary bile acids in the intestinal tract into secondary bile acids, which are then reabsorbed into the portal vein. While most bile acid molecules are captured by the liver and undergo recirculation, a small fraction of them persists in the blood as signalling molecules. Bile acid synthesis in hepatocytes involves the oxidation of cholesterol mediated by cytochromes P450 enzymes. The synthesis mainly occurs through the classic and

alternative pathways, producing cholic and chenodeoxycholic acids, which are subsequently conjugated to taurine and glycine, respectively, to form conjugated bile acids. Synthesized primary bile acids are deposited into the gallbladder *via* the bile salt export pump. Gall bladder contraction triggered by eating promotes bile acid secretion into the intestinal tract [15]. Primary bile acids increase the permeability of the intestinal mucosa, resulting in endotoxemia and aggravating MAFLD. Thus, the bile acid level is elevated in the liver tissue, serum, and urine of MAFLD patients. Meanwhile, there is a significantly higher proportion of hydrophobic and cytotoxic bile acids[16]. Patients with NASH exhibit increased synthesis of bile acids compared to other conditions. The ratio of primary bile acids to secondary bile acids is also higher than in healthy individuals[17]. Bile salt hydrolases produced by *Bacteroides*, *Clostridium*, *Lactobacillus*, *Bifidobacterium*, and *Listeria* in the GM can deconjugate conjugated bile acids to form free bile acids. *Clostridium*, *Fusobacterium*, *Peptococcus*, and *Pseudomonas* species have the ability to catalyze the desulfuration of bile acids. *Bacteroides*, *Eubacterium*, *Clostridium*, *Escherichia*, *Eggerthella*, *Peptostreptococcus*, and *Ruminococcus* are implicated in the dehydroxylation of primary bile acids to produce secondary bile acids[18]. Moreover, it was found that *Clostridium leptum* is positively related to taurocholic acid and negatively related to cholic acid and chenodeoxycholic acid. This indicates that *Clostridium leptum* may promote the transformation from primary bile acids to secondary bile acids, thereby reducing the damage caused by primary bile acids to the liver[19].

About 95% of primary and secondary bile acids can be reabsorbed in the intestine and transported back into the liver through the portal veins. However, lithocholic acids present in secondary bile acids are primarily excreted with the feces. Hepatocytes synthesize new bile acids to compensate for the bile acids lost in the enterohepatic circulation. After reabsorption, bile acids are conjugated to the farnesoid X receptor (FXR) and G protein-coupled bile acid receptor (GPBAR; also named TGR5), thereby promoting the secretion of fibroblast growth factor 19 (FGF19) by intestinal cells. FGF19 conjugates to fibroblast growth factor receptor 4 (FGFR4), activating c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). These two signaling pathways decrease the genetic expression of cholesterol 7 α-hydroxylase (CYP7A1), which inhibits bile acid synthesis through negative feedback[20]. The effect of choline on lipid metabolism may be mediated by activating FXR to participate in liver lipid metabolism, thereby reducing the synthesis of cholesterol and triglycerides. The effect of bile acids on glucose metabolism has been established, as evidenced by the presence of insulin resistance and hyperglycemia in FXR gene-deficient mice, and the administration of oral dietary cholic acid to activate FXR can inhibit the expression of the gluconeogenesis gene in mice, reduce fasting glucose, and increase insulin sensitivity. Choline alleviates metabolic inflammation induced by tumor necrosis factor-alpha (TNF-α) and lipopolysaccharides (LPS), while bile acids such as cholic acid, deoxycholic acid, and chenodeoxycholic acid can inhibit the release of monocyte chemotactic protein-1 induced by TNF and LPS, suggesting that bile acids have anti-inflammatory effects[21]. Choline is a pleiotropic hormone-like signaling molecule with both metabolic and endocrine functions. It plays an important role in regulating cholesterol and triglyceride metabolism, insulin resistance, metabolic inflammation, and liver steatosis *via* activation of choline-specific receptors that are widely distributed in the body.

GM can regulate the synthesis and reabsorption of bile acids. Germ-free animals exhibit reduced excretion of bile acids in feces, accompanied by a significant increase in the total bile acid content in the gall bladder and small intestine[22,23]. Probiotics supplements or fecal microbiota transplantation can distinctly reduce the total bile acid content in the liver, gall bladder, and cecum germ-free animals[24]. In aseptic conditions, tauro-β-muricholic acid, a primary bile acid, accumulates due to its inability to undergo further metabolism. It can be used as an antagonist to inhibit intestinal FXR expression, thereby downregulating FXR expression. The expression of liver CYP7A1 promotes liver bile acid synthesis and is regulated *via* the enterohepatic circulation[23]. There is an increased expression of bile acid transporters in the ileum and colon of germ-free animals. This leads to decreased bile acid excretion in the feces, resulting in highly efficient bile acid reabsorption. The key enzymes in bile acid synthesis, such as CYP7A1, CYP7B1, and CYP27A1, can all be regulated by the GM, mainly *via* induction of the FXR signaling pathway[25]. Moreover, bile acids also have ab effect on the GM. Amphipathic bile acids directly perform the anti-bacterial function by breaking the cell membrane of bacteria, which is critical to maintaining the steady state of the bacterial flora. Studies revealed that bile duct ligation could arouse bacterial translocation in rat's mesenteric lymph nodes as early as after one week. Three weeks after bile duct ligation, the translocation was found to be expanded to tissue such as the liver, spleen, and lung. In addition, gram-negative bacteria in the animal cecum and endotoxin levels in the blood were significantly elevated. The villi of the distal ileum were flattened, and Peyer's patches increased in size[26]. Bacteria overgrowth in the small intestine, bacterial translocation, and endotoxemia can be effectively inhibited by taking bile acids orally[27]. Secondary bile acids can inhibit the growth of *Clostridium difficile*[28]. In addition, bile acids can enrich the bacteria that utilize bile acids. For example, bacteria with bile salt hydrolase activity, such as *Lactobacillus reuteri*, can resist cytotoxicity resulting from bile salts[29]. An in vitro culture experiment revealed that bile acids were required for the growth of *Bilophila wadsworthia*[30]. Studies have shown that diets rich in milk fat altered the bile acid profile, mainly by increasing the total amount of bile acid, and the abundance of *Bilophila wadsworthia* also increased with the increase in bile acids.

Choline metabolism disorders

Choline is a quaternary amine rich in methyl groups that exists in tissues in either free or esterified forms. In the liver, choline exists in the form of phosphatidylcholine (PC). Choline has been recognized as an essential nutrient by the National Academy of Sciences (NAS) since 1998. The biological functions of choline mainly include neurotransmitter synthesis, lipid metabolism, and cell membrane signal transduction. Choline can also serve as a methyl donor for the synthesis of PC in the liver[31]. PC, in turn, is indispensable for the synthesis and secretion of very low-density lipoprotein (VLDL). Moreover, choline also prevents abnormal lipid accumulation by mediating liver lipid transport. Therefore, the lack of choline may lead to hepatic steatosis[32]. For over 50 years, researchers have recognized the association between choline deficiency and accumulation of fat in the liver. Choline-deficient diets are often used in animal experiments to induce MAFLD. The administration of diets deficient in choline and vitamin B12 to weanling rats

induces fatty liver and renal cortical necrosis, resulting in high deaths rate within 10 d[33,34]. Research has demonstrated that patients with MAFLD exhibit varying degrees of decreased choline levels in their plasma, which is associated with the degree of liver damage[31,35].

Choline has two primary sources. Approximately 70% of choline is obtained from dietary sources, while the remaining 30% is synthesized by the GM. Among 79 gut microbiota strains screened from the human gastrointestinal tract, eight strains have been identified to significantly affect choline metabolism (*Anaerococcus hydrogenalis*, *Clostridium asparagiforme*, *Clostridium hathewayi*, *Clostridium sporogenes*, *Edwardsiella tarda*, *Escherichia fergusonii*, *Proteus penneri*, and *Providencia rettgeri*). However, genetic analysis has revealed that the gene set responsible for anaerobic choline metabolism is widely distributed among the three main bacterial groups present in the human gut, including *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. This metabolic pathway contributes to the bioavailability of choline in the human body and subsequently affects serum choline concentration. On the other hand, choline levels in the diet may also affect the gut microbiota. In patients with choline deficiency, choline supplementation has been shown to decrease the abundance of *Gammaproteobacteria*. This reduction in *Gammaproteobacteria* can alleviate the inhibition of key enzymes in choline metabolism, thereby reducing the occurrence of MAFLD[36-38]. As a result, GM disruption can alter choline metabolism and reduce the host's capacity to efficiently utilize choline, leading to a relative deficiency of choline and increased production of substances such as trimethylamine N-oxide (TMAO). In turn, this can lead to the occurrence of hepatic steatosis[32,39,40]. Bacterial species such as *Escherichia coli* and *Desulfovibrio desulfuricans* within in GM have the capability to utilize choline and convert it into methylamine. When there is a disruption in the GM involved in the metabolism of choline, it can lead to a deficiency of choline and potentially contribute to the development of MAFLD[40]. In the presence of abundant MAFLD-associated intestinal bacteria, there is an increase demand for choline by these bacteria. This leads to choline deficiency in the host, exacerbating the risk of MAFLD and potentially progressing to NASH[41]. Reduced choline utilization may lead to decreased PC synthesis in the body, thereby inducing fatty acid synthesis and increasing triglyceride (TG) production. Meanwhile, it decreases the surface activity of lipid droplets lacking PC. Large lipid droplets are easier to form. Therefore, it is difficult for lipoprotein lipase (LPL) to decompose lipid droplets[9,42]. GM disruption can alter the body's reservoir of choline, thereby inducing choline deficiency and decreasing VLDL secretion, which leads to the accumulation of fat in the liver[43]. Moreover, TMAO and GM are closely associated with choline metabolism. GM can produce enzymes that catalyze the transformation of dietary choline into methylamine. After metabolism, methylamine is transformed into TMAO by GM-produced trimethylamine-lyase. TMAO can regulate protein activity and stability, increase foam cell production, inhibit cholesterol transport, aggravate liver fat deposition, and even induce liver inflammation[31,32,44]. Trimethylamine lyases in GM can decompose dietary choline into TMA, which enters the liver through the portal vein and is oxidated into TMAO. TMAO can upregulate the expression of sterol regulatory element-binding protein-1c (SREBP-1c). SREBP-1c is a critical transcription factor in the regulation of liver lipid metabolism, which promotes TG synthesis, aggravating hepatic steatosis[45]. TMAO also upregulates glucose metabolism and increases serum inflammatory factors for insulin resistance (IR) promotion[46,47]. It affects lipid metabolism and cholesterol's steady state by reducing the transformation of cholesterols into bile acids[35]. TMAO contributes to the progression of MAFLD through various mechanisms. GM metabolites also include secondary bile acids and ethanol, which have been discussed in the previous section.

Lipid metabolism disorders

The GM has the ability to generate energy from indigestible substances (*e.g.*, *Firmicutes* can ferment resistant starch to provide energy for intestinal epithelial cells)[48]. Therefore, GM is critical in the development of obesity, and it disruption can lead to obesity-related MAFLD[49]. Obesity can increase the level of proinflammatory cytokines secreted by macrophages and promote adipose tissue infiltration, leading to the development of hepatic steatosis[50]. The intestines of obese people are rich in *Firmicutes*. *Firmicutes* can ferment indigestible dietary fiber (polysaccharide) and produce additional energy from the intestine content, which promotes the progression of obesity and MAFLD. The fecal microbiota of obese mice caused by high-fat fodder was transplanted to mice fed with regular fodder, and it was found that mice transplanted with fecal microbiota on a high-fat diet had more fat deposition than mice transplanted with fecal microbiota on a regular diet. A further study found that structural changes in the GM could lead to greater lipid absorption by the body, promoting the biosynthesis of fatty acids. However, it was proven that some probiotics, such as *Lactobacillus*, could reduce liver fat deposition by reducing fatty acid absorption in the host intestine[51,52].

Fasting-induced adipocyte factor (FIAF) is a lipoprotein lipase inhibitor, and inhibition of the FIAF gene can significantly reduce body fat deposition. LPL is the key regulatory factor of fatty acid released from lipoprotein in skeletal muscles, the heart, and adipocytes. Under physiological conditions, GM can inhibit FIAF gene expression, promote LPL expression, and decrease TG accumulation in the cells[50,53,54]. Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a regulatory factor that serves in maintaining energy balance in the cells, playing a vital part in energy balance. AMPK can be directly phosphorylated by acetyl-CoA carboxylase, which promotes fatty acid oxidation in the tissue and further reduces fat deposition. GM disruption can lead to a reduction in the levels of AMPK in skeletal muscles and the liver, which subsequently leads to inhibition of fatty acid oxidation and excessive accumulation of fat in the liver [50]. Besides, the GM can induce or inhibit angiopoietin-like protein 4 (AGTPL-4) through bile acids to influence LPL activity, thereby affecting fat deposition inside the liver and steatosis outside the liver[42]. A study[55] revealed that giving mice fodders rich in saturated fatty acids, cholesterols, and sugar could increase lipid accumulation in their livers and cause a significant rise in the relative abundance of *Firmicutes* in mice's intestines. *Firmicutes* are important in the fermentation of resistant starch and dietary fibers and for energy use. The fermentation products are present in the form of SCFAs. On the one hand, SCFAs are essential energy substances of intestinal epithelial cells, which can enhance energy production by promoting sugar and fat synthesis[56]. On the other, SCFAs can alter fatty acid oxidation by inhibiting AMPK, leading to the accumulation of fatty acids in the liver[57]. Moreover, SCFAs are the ligands of G protein-coupled

receptors 41 (GPR41). After conjugating to GPR41, SCFAs can mediate leptin production by stimulating GRP41 in mouse adipocytes to regulate energy metabolism[58].

IR

Insulin is an important hormone that regulates the steady state of glucose levels in the body. The activation of insulin receptors through phosphorylation initiates the body's biological response, inclusing increased glucose transport in skeletal muscles and adipose tissues, glycogen synthesis, and lipogenesis. IR reduces the biological effect of insulin, causing hyperinsulinemia (HIS). In turn, HIS leads to abnormal glucose transport and increased glycogenolysis and fatty acid synthesis, promoting MAFLD. Therefore, IR is one of the vital causes of MAFLD[59]. The composition and relative abundance of GM bacteria differ between obese individuals and those who are slim. The GM of obese individuals is mainly composed of *Firmicutes* and *Actinobacteriota, while Bacteroidete are* also present but less predominant. As mentioned above, *Firmicutes* with high relative abundance can enhance energy consumption by utilizing more indigestible substances in the intestinal content[60]. An imbalanced ratio of gut bacteria can also disrupt intestinal permeability, leading to increased absorption of LPS. Administration of antibiotics in high-fat diet (HFD) mice has been shown to decrease blood LPS concentration. Furthermore, this reduction in blood LPS concentration contributes to a decrease in adipose tissue inflammation and oxidative stress, which helps prevent adipose tissue hypertrophy and improves glycolipid metabolism parameters of HFD mice[61].

LPS are cell wall components found in gram-negative bacteria and are considered to be a key trigger of IR. Studies[62, 63] have shown that IR caused by intestinal endotoxins is mainly mediated by Toll-like receptor 4 (TLR4). In other words, LPS can activate the TLR4 located on the surface of insulin target cells. TLR4 can stimulate hepatocytes to produce inflammatory factors. Stimulating the production of proinflammatory kinase (*e.g.*, JNK) can inhibit the phosphorylation of insulin receptor substrates, thereby inhibiting the insulin signal transduction pathway. Moreover, GM disruption can accelerate the above processes to trigger IR. Reduced insulin sensitivity leads to a decrease in the rate of blood glucose utilization. HIS occurs when islet β cells are in the compensatory hypersecretory state. Further, HIS can disrupt the islet signaling pathway in the liver, forming a vicious circle. IR can alter the regulation of fat by insulin, enhancing steatosis and increasing free fatty acids in the serum. Fatty acids can cause hepatotoxicity. On one side, they can trigger MAFLD *via* various mechanisms, such as causing mitochondrion swelling to increase their permeability, inflammatory invasion, hepatocyte degeneration and necrosis, and induction of cell apoptosis[54]. On the other hand, the liver can transform free fatty acids into TG. Excessive fat will be deposited in the liver when the fat synthesized in the liver exceeds the hepatocyte's ability for oxidative utilization and synthetic lipoprotein transport, thereby promoting the development of MAFLD[61].

Increased endogenous ethanol

The GM can produce and metabolize ethanol. In the relatively hypoxic environment of the intestine, pyruvic acids produced through carbohydrate decomposition can be metabolized by the GM into acetaldehyde, which is further reduced into ethanol[64]. When there is intestinal bacteria overgrowth (Small intestinal bacteria overgrowth often exists in MAFLD) or excessive carbohydrate intake, ethanol metabolism mediated by the GM becomes active[65]. A current study[66] suggested that the primary product of *Enterobacteriaceae* (*e.g. Escherichia*) metabolism is ethanol. Other GM, such as *Bacteroides*, *Bifidobacterium,* and *Clostridium*, may also produce ethanol. The ethanol metabolized by GM is also called endogenous ethanol. Under normal conditions, the liver efficiently eliminates endogenous ethanol from the bloodstream of the portal vein through the action of liver alcohol dehydrogenase, catalase, and the ethanol oxidation system. However, in the intestines of MAFLD patients, the abnormal increase of ethanol-producing bacteria promotes and increased production of ethanol. Some patients with MAFLD have a preference for carbohydrates. Due to these two factors, reactive oxygen species are constantly provided to the liver, inducing liver oxidation and triggering inflammation, which is the "second strike" to the liver[66]. A study on children with MAFLD[67] revealed that the relative abundances of *Gammaproteobacteria* and *Prevotella* in these children were significantly higher than in healthy children. For this reason, the production of endogenous ethanol was also distinctly enhanced. Besides, an animal experiment[68] proved that administering antibiotics to alter the GM could reduce the ethanol concentration of the air exhaled by obese mice. A similar conclusion was also confirmed in NASH patients[69], as the air exhaled by NASH patients also had higher ethanol concentrations than healthy individuals. Besides, it was observed that the relative abundance of *Escherichia* in the GM increased significantly, which also confirmed that the increase in endogenous ethanol caused by GM disruption is related to MAFLD.

GM disruption leads to an increase in the relative abundance of bacteria producing ethanol, thereby increasing the ethanol content in the intestines. Ethanol activates various cytokines in the intestinal epithelial cells to increase intestinal wall permeability. Meanwhile, ethanol and acetaldehyde, which are metabolized products, enter the liver through the portal vein. These products can either directly stimulate hepatocytes or activate liver TLR to produce multiple cytokines and inflammatory mediators, resulting in inflammatory liver injury. In addition, the acetaldehyde produced by ethanol through intestinal metabolism can damage the expression of tight-junction proteins between intestinal epithelial cells to alter the intestinal barrier function, leading to bacterial translocation and endotoxemia (Figure 1).

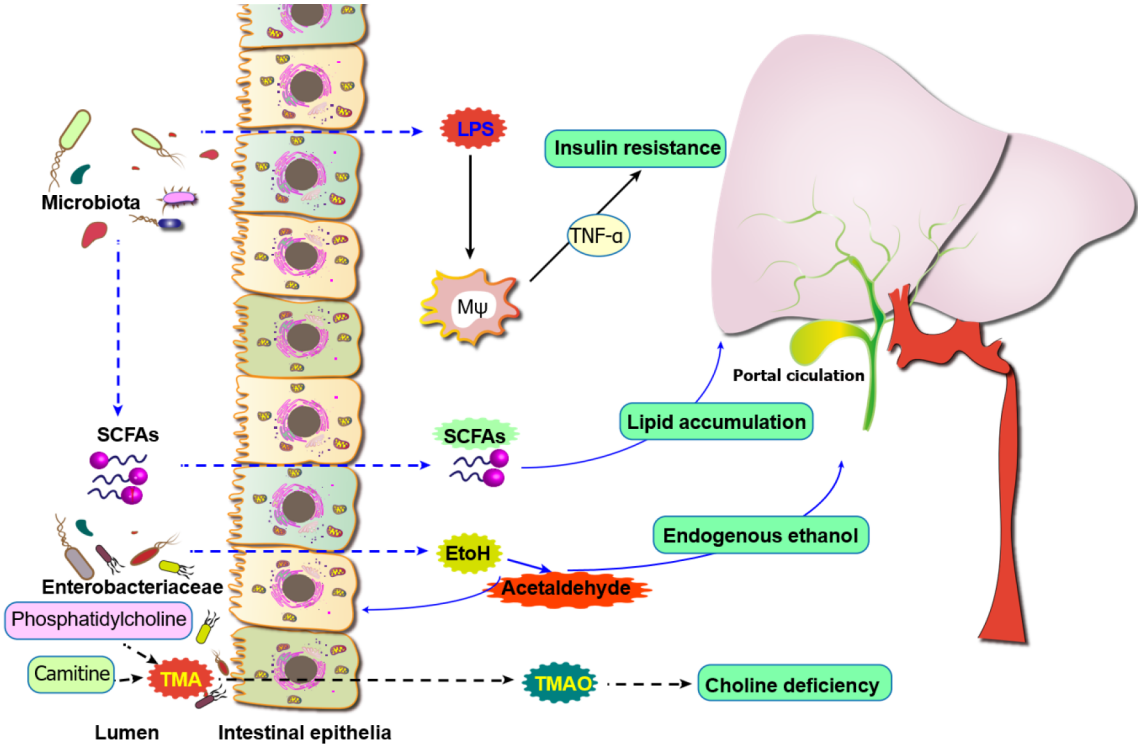

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Figure 1 Gut microflora can affect several factors related to the development of metabolic-associated fatty liver disease. These effects lead to the production of free fatty acids, insulin resistance, and impaired bile secretion in the liver, respectively. In addition, changes in intestinal microflora may lead to increased intestinal permeability, and microbial-derived compounds are transferred from the intestine to the liver through the portal vein, resulting in changes in pro-inflammatory signals, metabolism, and toxicity. Finally, ethanol and its toxic derivative acetaldehyde aggravated hyperoxidative stress and choline deficiency in hepatocytes. EtOH: Ethanol; LPS: Lipopolysaccharides; SCFAs: Short chain fatty acids; TNF-α: Tumor-necrosis factor; TMAO: Trimethylamine N-oxide.

THE INFLUENCE OF GM ON RELEVANT SIGNALING PATHWAYS

FXR signaling pathway

The FXR signaling pathway is one of the members of the nuclear receptor superfamily, and FXR's primary function is to regulate bile acid metabolism and enterohepatic circulation. The synthesis, metabolism, and reabsorption of bile acids are regulated by the negative feedback of FXR-relevant signaling pathways in the liver and ileum. Activating FXR can adjust the metabolic state of blood fat, blood glucose, and cholesterol and improve IR[70,71]. Obeticholic acid is an FXR agonist, which inhibits bile acid synthesis and enhances bile salt excretion through the FXR/FGF15/19 signaling pathways, thereby reducing bile acid reabsorption by the liver. Meanwhile, obeticholic acid can regulate the GM, improve intestinal mucosa barrier function, reduce inflammation, decrease the production and translocation of intestinal endotoxin, maintain gut-liver axis balance, and alleviate liver inflammation[70]. GM can also activate FXR in various ways (*e.g.*, *via* increasing fatty acid oxidation). Activated FXR improves glucose metabolism by inhibiting gluconeogenesis and glycogenolysis, reducing fat synthesis, and enhancing skeletal muscle insulin sensitivity. GM disruption can inhibit the transduction of the FXR signaling pathway, leading to an escalation of fatty acid synthesis and the generation of lipid toxicity. This, in turn, further deteriorates hepatic steatosis and promotes the occurrence and progression of MAFLD[72-74].

GPBAR signaling pathway

The mechanism of the GPBAR signaling pathway is similar that of FXR, and these two pathways are closely related to each other[75]. After reabsorption, bile acids induce ileal cells to secrete FGF19 by conjugating to the FXR and GPBAR of ileal cells. FGF19 further conjugates to FGFR4, which reduces CYP7A1 gene expression by activating the JNK and ERK signaling pathways. Therefore, bile acid synthesis is inhibited in negative feedback[23]. GM disruption can alter FXR expression and the transduction of the GPBAR signaling pathway, leading to the production of proinflammatory factors. For example, GPBAR can activate cyclic adenosine monophosphate and epidermal growth factor receptor kinase pathways, resulting in the activation of protein kinase C, which leads to the activation of nuclear factor- κB (NF-κB). Activated NF-κB enhances the expression of numerous proinflammatory factors, such as Il-1β, IL-6, and TNF-α. As a result, this activates the inflammatory immune response of the liver, promoting the occurrence and progression of MAFLD[76,77].

TLR signaling pathway

TLR is essential in the gut-liver axis, especially in maintaining the intestinal mucosa barrier. A damaged intestinal mucosa

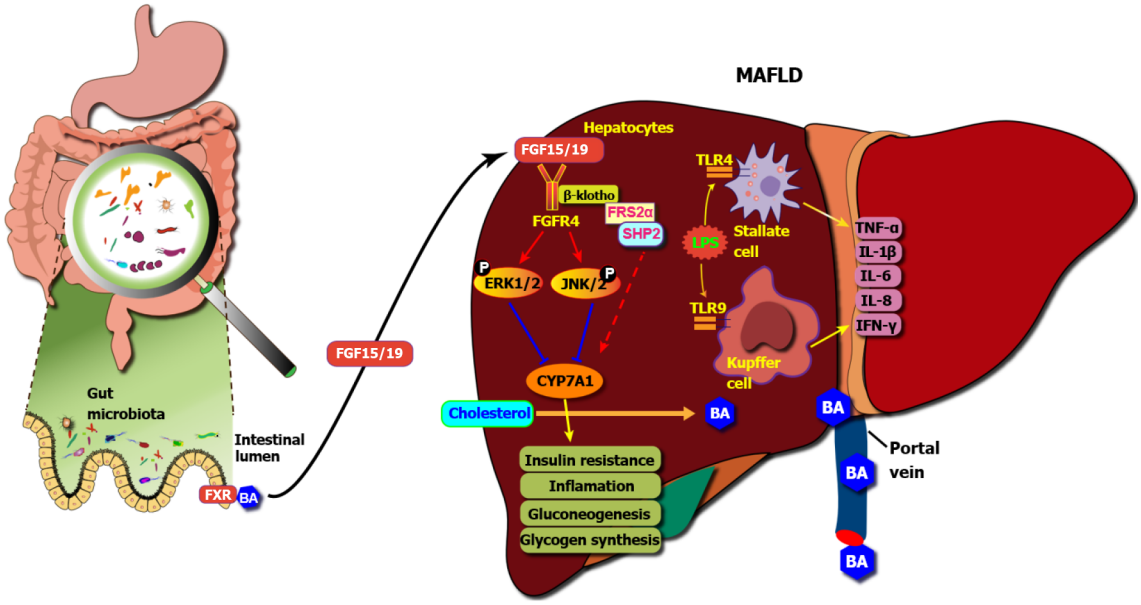

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Figure 2 Mechanisms showing the role of gut microbiota in metabolic-associated fatty liver disease. FXR: Farnesoid X receptor; TGR5: Takeda G protein-coupled; MAFLD: Metabolic-associated fatty liver disease; BA: Bile acid; LPS: Lipopolysaccharides; TNF-α: Tumor-necrosis factor alpha; TLR: Toll like receptor.

barrier leads to increased permeability, which induces GM transposition. Therefore, a growing number of bacterial metabolites, bacterial substances, and other compounds can enter the liver through the portal vein, causing inflammation, oxidative stress, and lipid deposition. This eventually leads to fat liver injury, which can progress rapidly to liver fibrosis, also called "intestinal leakage"[78]. Bacterial flora translocation increases the endotoxin level in the portal vein or the liver. Pathogen-associated molecular patterns accumulate in the portal vein circulation, promoting the development of liver inflammation[79]. Besides, the increased abundance of pathogenic bacteria caused by GM disruption (or distinct abnormal relative abundance of opportunistic pathogens) lead to the excessive production of LPS. Subsequently, LPS stimulates endothelial cell TLR4 and dendritic cell TLR9 and induces the production of inflammasomes (*e.g.*, NLRP3) and proinflammatory factors (*e.g.*, IL-1β). This further damages intestinal mucosa permeability and reduces liver insulin sensitivity, thereby increasing visceral and subcutaneous fat and promoting the occurrence and progression of MAFLD [80] (Figure 2).

Immunoregulation

Liver inflammation is the critical driving factor of MAFLD development, and the gut-liver immune axis plays a vital role in the process. LPS, peptidoglycan (PGN), and bacterial deoxyribonucleic acid (DNA) can be translocated into the liver through the injured intestinal barrier, causing immune cell hyperactivation. LPS can tale advantage of the injured intestinal barrier to enter the liver *via* portal blood flow and induce the activation of inflammasomes[81]. PGN is one of the components of the bacterial cell wall. It is a macromolecule polymerized by acetylglucosamine, acetylmuramic acid, and amino acid short-chain peptide, which plays a role in insulin tolerance[82]. PGN and TLR2 can activate relevant NF-κB and TNF-α signaling pathways after conjugating to nucleotide oligomerization domain (NOD) 1 or NOD2, resulting in liver inflammation. NOD1 can also detect nutrient overload by sensing changing in bacterial microorganisms and promoting the translocation of PGN to regulate the energy metabolism in the gut-liver axis[83]. Bacterial DNA can directly activate immune cells such as macrophages, natural killer cells, B lymphocytes, and dendritic cells. It can also conjugate to TLR9 inside lysosomes *via* endocytosis, activating the NF-κB pathway and secreting inflammatory factors, such as IL-1β, IL-6, and TNF-α[84].

The bacterial flora therapy of MAFLD

Antibiotics: Research[85] has shown that short-term use of antibiotics can reduce circulating endotoxins and serum transaminases, improving the clinical symptoms of MAFLD patients. Among the antibiotics, the application of rifaximin has received the greatest attention. After rifaximin treatment, the BMI index, transaminase level, and hepatic steatosis degree of MAFLD patients decreased significantly. Even clinical research revealed that rifaximin could reduce the fermentation of carbohydrates and sterols by altering the GM structure, lowering serum inflammatory factors, and improving IR. Antibiotics cocktail (ampicillin, neomycin, metronidazole and vancomycin) can regulate free and conjugated secondary bile acid levels to decrease liver inflammation[86], inhibit intestinal FXR to reduce hepatic steatosis [73], and inhibit the activation of liver macrophage to lower liver inflammation[87]. However, antibiotics play a dual role. Short-term antibiotic treatment can exert therapeutic effects, while long-term application may result in bacterial drug resistance and increase the risk of secondary infection.

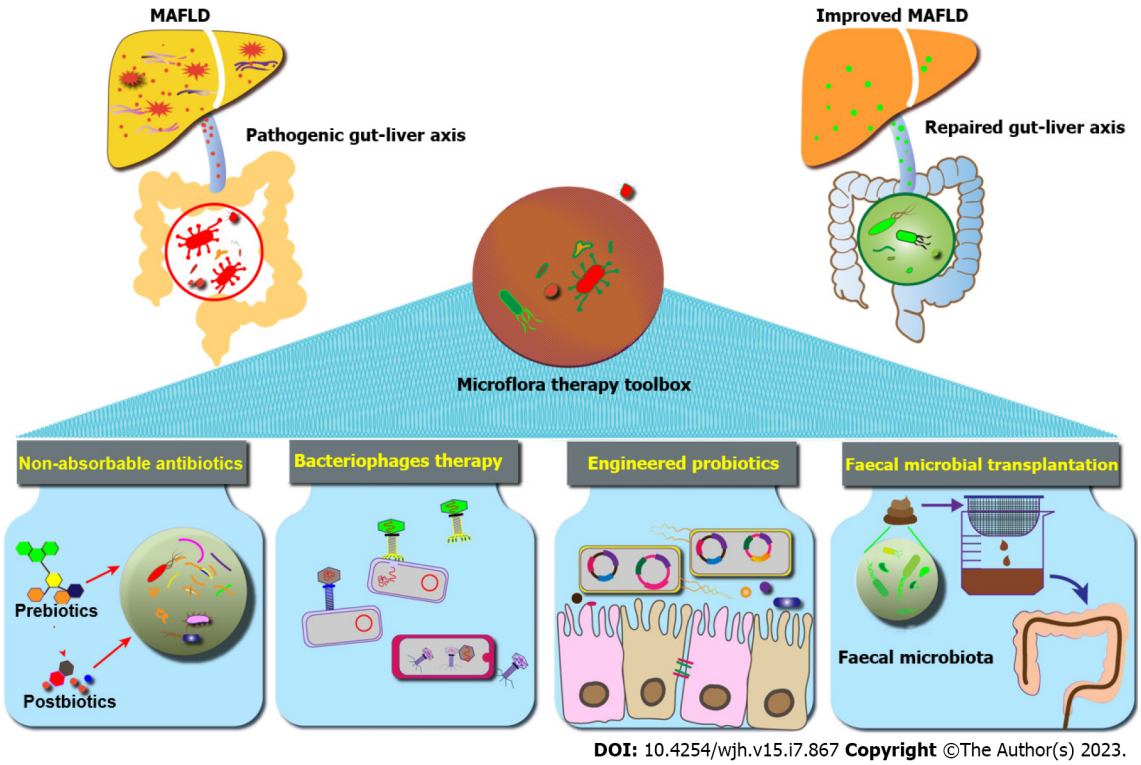

Figure 3 Therapeutic interventions for metabolic-associated fatty liver disease based on microbiota. Intestinal-centered therapy including antibiotics, bacterial metabolites, probiotics, engineered bacteria, bacteriophages, and fecal microbial transplantation can specifically interfere with intestinal microflora to re-establish the interface between the liver and the microbiome. MAFLD: Metabolic-associated fatty liver disease.

Probiotics: Clinical experiments on MAFLD patients[88] demonstrated that *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* could significantly reduce the patient's serum transaminase level. After taking *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for three months, the transaminase level of MAFLD patients improved[89]. The application of *Clostridium butyricum* in clinic settings and MAFLD animal models has shown promising potential in preventing hepatic steatosis[90, 91]. In addition, multiple probiotic formulations present better therapeutic effects than one specific bacterial strain. For example, VSL3 (consisting of eight probiotic bacterial strains: *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Lactobacillus bulgaricus*) has better therapeutic effects than any single bacterial strain[90,92,93]. Studies targeting children with MAFLD [68,94,95] revealed that after VSL3 treatment, patients' fatty liver disease condition and BMI were distinctly improved. Follow-up research showed that the total quantity and activity of GLP-1 increased after VSL3 treatment. Meanwhile, VSL3 has been found to regulate plasmic peroxide, such as malondialdehyde and 4-hydroxynonenal, leading to therapeutic effects and relieving chronic liver disease. It achieves this by protecting the intestinal barrier and reducing endotoxemia and oxidative/nitroso stress. An animal experiment also verified[96] that probiotics can lower the weight of mice and improve GM disruption. Probiotic intervention can increase the adundance of intestinal anaerobic bacteria (*e.g.*, *Actobacillus* and *Bifidobacterium*). However, this decreases the abundance of *Escherichia* and *Enterococcus*, enhancing the integrity of the intestinal mucosa barrier. Highly expressed TLR4 in the liver improves serum inflammatory factors, liver histology, serum liver enzyme, metabolic index, and glucose metabolism. Evidence indicates that probiotics can decrease liver and systematic inflammation by inhibiting the LPS/TLR4 signal transduction inflammatory cascade.

Prebiotics: Fructo-oligosaccharides (FOS) is an indigestible fermentable dietary fiber compound that lowers liver oxidative stress and inflammation by improving intestinal permeability and the integrity of close junctions[97]. Lactulose is another prebiotic that enhances the growth of Bifidobacteria and Lactobacillus and inhibits endotoxic gram-negative bacteria. After taking lactulose for six weeks, the inflammation and liver injury of HFD obese mice was reduced, which was related to lowered LPS level in the circulation[98]. Clinical research[99] has shown that the serum ALT and AST levels of NASH patients decreased significantly after receiving Bifidobacteria and FOS treatments. Combining multiple probiotic bacterial strains (Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bifidobacteria breve, Bifidobacteria longum, and Streptococcus thermophilus) and FOS in combination with lifestyle interventions were more beneficial than lifestyle changes alone for MAFLD patients[100]. Combining Bifidobacteria longum and FOS with lifestyle interventions can significantly decrease NASH activity index and liver fat accumulation[101]. The result of a meta-analysis revealed that combining prebiotics could distinctly lower hepatic steatosis and the levels of ALT, AST, LDL, TG, and TC. Moreover, it was also helpful for reducing levels of inflammatory factors such as TNF-α and IR[102]. However, the administration of inulin diet, a soluble fiber, to mice with TLR5 gene knock-out led to an increase in the mice's bilirubin level, indicating that excessive inulin intake may cause liver injury and

even liver cancer[103]. Research has also highlighted that acetate, the fermentation product of inulin in the colon, can provide excess substrate for fat synthesis in the liver, escalating the production of lipids in the liver[104].

Fecal microbiota transplantation

The earliest fecal microbiota transplantation (FMT) treatment can be traced back to the book called *A Handbook of Formulas for Emergencies (Zhou Hou Bei Ji Fang)* written by Ge Hong from the Eastern Jin Dynasty (266 A.D. - 317 A.D.) in China. In the book, FMT treatment, also called "Huang Long Soup," for food poisoning and severe diarrhea was first recorded. Later, Li Shizheng also recorded in *the Compendium of Materia Medica (Bencao Gangmu)* that FMT by oral administration can treat severe diarrhea, fever, vomiting, and constipation[105]. FMT in modern medicine started in the year 1985. Ben Eiseman performed FMT by enema for patients with severe pseudomembranous colitis using feces from the patient's family member, and three out of the four patients were cured[106]. FMT was officially written into the clinical guidance for recurrent *Clostridium difficile* infection treatment in 2013[107]. As research progresses, numerous pieces of evidence support the potential efficacy of FMT in treating GM-related liver disease and metabolic disorders such as MAFLD. Studies have shown[108,109] that transplanting the GM of slim or obese mice can induce the recipient to have a phenotype similar to that of the host. The bacterial flora from slim mice can make obese mice lose weight. Six weeks after overweight patients with metabolic syndrome received bacterial flora from the slim individuals, the sensitivity of their liver and peripheral insulin was significantly enhanced[110]. Several studies have demonstrated[111-114] that the therapeutic effects of FMT on patients with T2DM and ulcerative colitis were related to GM steady state, normal blood fat level, and IR improvement. Feces from HFD-responsive and non-responsive mice were transplanted into germ-free mice. Mice receiving bacterial flora from the responsive group developed steatosis and exhibited increased relative abundance of *Barnesiella* and *Roseburia*. In contrast, the non-responsive group showed an increased relative abundance of *Allobaculum* in their bacterial flora[55]. In addition, FMT could significantly restore the GM disruption in NASH mice models induced by HFD by increasing the relative abundance of probiotics (*e.g.*, *Christensen* and *Lactobacillus*) and mitigate endotoxemia, hepatic steatosis, and inflammation[115]. In a RCT admitting 75 MAFLD patients, Xue *et al*[116] divided the patients into an FMT group (47 individuals) and a non-FMT group (28 individuals). The patients from the non-FMT group took oral probiotics, while the FMT group received three FMT enemas within three days. Both groups received a healthy diet and conducted exercise regularly for over 40 min. After treatment for one month, it was found that FMT lead to a reduction in liver fat deposition by improving the GM disruption, lowering the incidence of fatty liver disease. Moreover, the GM reconstruction effect of FMT on thin-type MAFLD patients was better compared to obese MAFLD patients. FMT can be administered through various methods to meet the requirements of different patients, including *via* nasogastric tubes, nasojejunal feeding tubes, gastroscopes, coloscopes, colonic catheters, retention enema, and capsules. However, FMT may pose certains risks. For example, the GM condition of different providers may affect the therapeutic effect, infection may occur during the transplantation, and it is uncertain how the GM can be effectively colonized in the patient's intestine. All these problems require further exploration.

Phage therapy

Phages are viruses that specifically infect and kill bacteria. They have the ability to adapt and evolve, enabling them to overcome the developing defensive mechanism of bacteria. Phages do not have the same mechanism as antibiotics. Thus, antibiotic resistance does not affect phages, and bacteria with high antibiotic resistance can still be inhibited by phages [117]. By studying the changes in bacterial composition and relative abundance, bacteria can be targeted for eradication using phages specific to that bacteria, after determining the mechanism by which a specific bacterium affects the onset or progression of MAFLD or whether the two are causally related. Its adverse effects on MAFLD can be eliminated without affecting the normal function of other bacteria[118]. For example, using phages for the targeted eradication of high-alcohol-producing *Klebsiella pneumoniae* (HiAlc-Kpn, found in over 60% of MAFLD patients, can produce enormous amounts of alcohol and is the leading cause of the bacterial auto-brewery syndrome) can effectively mitigate the bacterial auto-brewery syndrome of MAFLD model mice[119]. An analysis was conducted on feces samples of NASH patients and healthy people. The *Enterococcus faecalis* level in the feces samples of alcoholic hepatitis patients is 300 higher than in healthy subjects. The relative abundance of *Enterococcus faecalis* significantly increased in approximately 80% of feces samples from patients with alcoholic hepatitis. Further analysis showed that a gene which can encode cytolysin existed in approximately 30% of *Enterococcus faecalis* species[118]. Alcoholic hepatitis mouse models were built using a high-alcohol diet. After being transplanted with a feces sample containing cytolysin, these alcoholic hepatitis mice developed specific hepatocyte injury and died. However, alcoholic hepatitis mice transplanted with samples without cytolysin did not develop liver injury. Targeting the phages specific to *Enterococcus faecalis* can effectively reduce the abundance of this bacterium, especially strains producing cytolysin. This targeted approach has shown promising results in lowering the degree of liver injury in alcoholic hepatitis mice, serving as a protective measure[118]. This is a critical attempt at phage therapy in the gut-liver axis, indicating the potential application value of phage therapy in MAFLD treatment (Figure 3).

In addition to the antibiotics mentioned above, probiotics, FMT, and other therapies that directly target GM, there is growing evidence that modifying dietary habits and increasing physicial exercise both improve MAFLD and the GM disorder in MAFLD. High-fat and high-sugar diets can change the GM structure in different ways[120]. HFD mice have a greater abundance of *Firmicutes,* while mice with a high-sugar diet have a lower relative abundance of *Firmicutes* and *Bacteroides*, which is closely related to the onset and development of MAFLD. High-fructose intake will up-regulate the re-synthesis of fat and inhibit the oxidation of fatty acid β. It can cause hepatic steatosis, induce inflammation through the TLR signaling pathway, and release inflammatory factors. High-fructose intake can also reduce insulin sensitivity. HFD decreases the number of intestinal probiotics *Bifidobacteria* and bacteria that produce butyric acid. It also enhances intestinal permeability, LPS translocation, and chronic systemic inflammation. High saturated fatty acid intake can lower

the GM diversity and increase the ratio of *Firmicutes* to *Bacteroides*, result in weight gain, and increase plasmic insulin and TG content[121]. In MAFLD mice induced by HFD, six-week HFD increases *Firmicutes* abundance and decreases *Bacteroides* abundance. The ratio of *Firmicutes* to *Bacteroides was* significantly enhanced, and the ratio was maitained till the end of the experiment. However, exercise can improve GM disorder resulting from a HFD. This is achieved by changing the ratio of the two bacteria *via* reducing *Firmicutes* abundance and increasing *Bacteroides* abundance[122]. Exercise distinctly alleviates GM disruption caused by HFD and restores the intestinal mucosa barrier function to a certain extent. The relative abundance of some GM reaches a level similar to normal rats. Meanwhile, exercise also significantly downregulated the expression of FXR and CD36 in the liver, indicating improvements in liver lipid metabolism[123]. Research combining exercise and lycium barbarum polysaccharide also found that aerobic exercise restores the close junction of the colon and ileum and improves intestinal mucosa permeability by enhancing ZO-1 expression. Relevant indicators such as intestinal LPS, liver LPS binding protein, and inflammatory factors are also downregulated[123]. However, research on the effects of exercise on GM in MAFLD patients is relatively sparse. Although animal experiments have demonstrated that exercise can improve MAFLD symptoms by restoring GM, more clinical experiments are needed.

CONCLUSION

Generally, with the increase in metabolic diseases such as obesity, the incidence of MADLF also increases yearly. Many clinical studies and animal experiments have demonstrated that the GM is vital in the onset and development of MAFLD, but most studies remain at a phenomenal level. There is no definitive conclusion regarding the "cause" and the "effect" of GM disorder and MAFLD. However, the efficacy of treatments targeting GM, such as probiotics and FMT, has been confirmed in both clinical applications and basic research without reporting severe adverse events. There is also a wide range of opinions on how many bacterial flora therapies are used to improve MAFLD. A healthy lifestyle and good dietary habits are still the foundation of MAFLD treatments. Comprehensive therapies combing bacterial flora therapy certainly have good prospects, but continuous efforts are still needed to design high-quality long-term clinical experiments. Meanwhile, histological studies combining multi-omics, such as metagenome, metabolome, and proteome, are needed. In the future, it is expected that GM can better help in the diagnosis, treatment, and prognosis evaluation.

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FOOTNOTES

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REVIEW

Shifting perspectives in liver diseases after kidney transplantation

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Abstract

Liver diseases after kidney transplantation range from mild biochemical abnormalities to severe hepatitis or cirrhosis. The causes are diverse and mainly associated with hepatotropic viruses, drug toxicity and metabolic disorders. Over the past decade, the aetiology of liver disease in kidney recipients has changed significantly. These relates to the use of direct-acting antiviral agents against hepatitis C virus, the increasing availability of vaccination against hepatitis B and a better understanding of drug-induced hepatotoxicity. In addition, the emergence of the severe acute respiratory syndrome coronavirus 2 pandemic has brought new challenges to kidney recipients. This review aims to provide healthcare professionals with a comprehensive understanding of recent advances in the management of liver complications in kidney recipients and to enable them to make informed decisions regarding the risks and impact of liver disease in this population.

Key Words: Kidney transplantation; Viral hepatitis; Non-alcoholic fatty liver disease; Drug-induced liver injury; COVID-19

Core Tip: Liver disease is a common complication after kidney transplantation and can present in a variety of forms, from asymptomatic biochemical abnormalities to fibrosis/cirrhosis/decompensation/malignancy. Early recognition and referral to a hepatologist are crucial for effective treatment, as they can otherwise lead to impaired quality of life and increased morbidity. Screening of kidney transplant recipients for liver disease, including viral disease, metabolic disorders and drug toxicity, should be prioritised by healthcare providers.

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INTRODUCTION

The efficacy of kidney transplantation (KT) in the treatment of end-stage renal disease (ESRD) has been demonstrated by numerous studies, which have shown that KT is associated with significantly higher survival rates and better quality of life compared to dialysis. As such, KT is now considered the gold standard in the treatment of ESRD[1,2]. Prevalence of chronic kidney disease (CKD) is estimated at 13.4% (11.7%-15.1%) and is on the rise due to increasing rates of diabetes, arterial hypertension, obesity and ageing. This is reflected in the increasing rates of KT; 92532 KT performed in 2021, which is an estimated 40% increase from 2008[2,3]. Advances in organ procurement, surgical techniques, immunosuppression regimens targeting rejection, and prophylactic antibiotic therapies have enabled excellent short-term survival rates after KT, and long-term outcomes are steadily improving over time[4]. This has led to a prolongation of life expectancy of both the graft and the recipient, which is now appreciable well beyond the first year following transplantation[4]. Survival rates of KT recipients may be affected by allograft nephropathy and subsequent failure, as well as a variety of morbidities, including cardiovascular disease (CVD) and infections associated with immunosuppressive drugs [1]. In addition, the incidence of liver abnormalities after KT, which ranges from 20% to 50%, was recently demonstrated by Vieira *et al*[5] to have a significant impact on the survival and quality of life of these patients[5] .

Liver disease after KT occurs in a variety of forms ranging from asymptomatic biochemical abnormalities to severe hepatitis, cirrhosis or malignancy[6]. Causes are heterogeneous and mainly related to hepatotropic viruses, drug toxicity and metabolic disorders[6]. Genetic conditions, such as polycystic kidney disease, which lead to the need for KT are also associated with polycystic liver disease[6]. Significant advances in understanding and treating liver disease in kidney transplant recipients have been made in the last decade. The development of direct-acting antiviral drugs (DAAs) for the treatment of hepatitis C virus (HCV) infection has led to a marked improvement in the treatment of HCV-related liver disease. Hepatitis E has been identified as a causative agent of chronic hepatitis and cirrhosis following solid organ transplantation (SOT). In addition to these advances, the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its impact on SOT has highlighted the need for further research in this field. The impact of coronavirus disease 2019 (COVID-19) on liver function in kidney transplant recipients is not yet fully understood, and the long-term consequences of the virus on liver health in this population are still the subject of ongoing investigation. In addition, the prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide and it is also taking its toll in transplant population due to changes in eating habits, sedentary lifestyle and unavoidable effect of life-long immunosuppression[7]. This review aims to provide healthcare professionals with a comprehensive understanding of recent advances in the management of liver complications in KT recipients, allowing them to make informed decisions about the risks and impact of liver disease in this population (Figure 1).

NON-ALCOHOLIC FATTY LIVER DISEASE

NAFLD is a complex and multifactorial metabolic disorder influenced by a variety of genetic, environmental and lifestyle factors. The pathogenesis of NAFLD is characterised by a cascade of events that result in the accumulation of fat in the liver (hepatic steatosis), which can trigger an inflammatory response that further contributes to liver damage and leads to more severe liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). However, the mechanisms underlying this progression remain unclear and are currently the subject of intense research efforts. Recent studies have highlighted the importance of gut microbiota dysbiosis, oxidative stress and immune system dysfunction in the development and progression of NAFLD[8]. Dysbiosis in the gut microbiota, characterised by a decrease in beneficial bacteria and an increase in harmful bacteria, can lead to increased gut permeability, endotoxaemia and inflammation, contributing to the development of NAFLD. Oxidative stress, an imbalance between reactive oxygen species and antioxidant defences, can also promote the progression of NAFLD by damaging hepatocytes and increasing the release of pro-inflammatory cytokines. Immune dysfunction, characterised by an imbalance between pro-inflammatory and anti-inflammatory metabolic pathways, can lead to an accumulation of immune cells in the liver, thus promoting the progression of NAFLD. In addition, genetic factors have been identified as important contributors to the development and progression of NAFLD. Variants in genes involved in lipid metabolism, insulin resistance and inflammation have been linked to an increased risk of NAFLD[8]. However, the interactions between genetic and environmental factors are complex and not

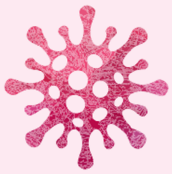
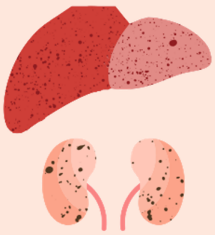
	NAFLD	Viral disease	DILI	ADPKD
				
Progress achieved to date	Diagnostic tools: Non-invasive methods - transient elastography and magnetic resonance elastography These methods can be used without the need for a liver biopsy, which can be risky for patients Risk stratification: Factors that increase the risk of NAFLD progression; obesity, insulin resistance and immunosuppressants (steroids and CNIs) Patient outcomes: Early detection and treatment of NAFLD improve outcomes	DAAs are highly effective drugs with SVR rates of over 97% of patients and good safety profiles HCV D+/R- kidney transplantation Entecavir and tenofovir suppress HBV replication and reduce the risk of liver disease progression HEV may lead to chronic hepatitis and cirrhosis in SOT COVID-19 can lead to various forms of liver injury	Mechanisms of injury: Progress in understanding the mechanisms by which drugs can cause liver damage, *e.g.* mitochondrial dysfunction or oxidative stress- leading to liver cell damage Risk factors: Age, gender, pre-existing liver disease	Disease-modifying therapies: Tolvaptan, a V2R antagonist
High priority topics	Management: Unmet need for effective and safe treatment strategies, including complex medical history, immunosuppressive medications and renal function	Safe and effective HEV vaccine A more equitable distribution of HCV- positive donor organs, combined with increased availability and affordability of DAAs, is essential to expand the donor pool and improve access to life- saving treatments for patients on the waiting list	Accurate and reliable biomarkers for diagnosis and monitoring	Tolvaptan safety, efficacy, and cost- effectiveness in KT recipients Optimal timing and appropriate selection criteria for transplantation

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Figure 1 Progress achieved to date and high priority topics in four main types of liver diseases affecting patients after kidney transplantation. NAFLD: Non-alcoholic fatty liver disease; CNIs: Calcineurin inhibitors; DAAs: Direct acting antivirals; HCV D+/R-: Hepatitis C virus donor positive/recipient negative; COVID-19: Coronavirus disease 2019; HEV: Hepatitis E virus; DILI: Drug induced liver injury; V2R: Vasopresin receptor 2; KT: Kidney transplantation; ADPKD: Autosomal dominant polycystic kidney disease.

fully understood, and more research is needed to fully elucidate the role of genetics in NAFLD.

NAFLD is now considered the most frequent chronic liver disease worldwide, with an estimated 25%-30%of the general population affected, particularly due to the rising prevalence of insulin resistance, obesity and hypertension[7]. Because type 2 diabetes mellitus (T2DM), obesity and dyslipidaemia often coexist with NAFLD, it is considered a hepatic manifestation of the metabolic syndrome[9]. While some experts suggest replacing the term NAFLD with metabolic-associated liver disease (MAFLD) to emphasise the importance of metabolic dysfunction in development and progression of the disease, the term is not yet universally accepted, as it can occur in patients without metabolic disorders[10]. In KT recipients, the high disease burden of NAFLD carries an increased risk of graft dysfunction and patient mortality, making early detection and treatment essential to reducing its impact on transplant outcomes. The increasing prevalence of NAFLD worldwide has led to a growing need for effective diagnostic and therapeutic strategies. Non-invasive diagnostic tools such as imaging and serum biomarkers have been developed to assess the severity of NAFLD and monitor disease progression. Various pharmacological and lifestyle interventions have also been investigated for the treatment of NAFLD, including weight loss, exercise, insulin-sensitising agents and antioxidants[11]. However, the optimal treatment for NAFLD remains uncertain, and more research is needed to find effective treatments that can prevent or reverse disease progression.

NAFLD and CKD

Patients with NAFLD and/or CKD share the same risk factors, including visceral obesity, arterial hypertension, prediabetes or T2DM, systemic insulin resistance, dyslipidaemia and low-grade inflammatory states[12]. Recently, several studies have shown that NAFLD is significantly associated with an increased prevalence of CKD. For example, the prevalence of CKD in patients with NAFLD ranged from 20% to 55%, while the prevalence of CKD in patients without NAFLD was 5% to 30%[13]. In addition, several retrospective and prospective cohort studies have found that the association between NAFLD and an increased incidence of CKD persists after adjustment for age, sex, obesity, hypertension and T2DM[14,15].

Impact of NAFLD on KT recipients

CVD is the main cause of mortality and graft loss after KT[16]. A meta-analysis of 16 studies found that patients with NAFLD had a higher risk of fatal and non-fatal cardiovascular events than patients without NAFLD (OR 1.64, 1.26-2.13) and that patients with severe NAFLD had a higher risk of fatal and non-fatal cardiovascular events (OR 2.58, 1.78-3.75) [17]. In addition, traditional risk factors (T2DM, obesity, hypertension, dyslipidaemia) for CVD and NAFLD may be exacerbated by immunosuppressive drugs, including calcineurin inhibitor and steroids in renal recipients[18]. In 2017, Kemmer and Buggs[19] reported the presence of NAFLD on ultrasound in 22% of KT candidates. Grupper *et al*[20] conducted a study of 341 consecutive KT recipients and showed that 36.4% of kidney recipients had sonographic evidence of NAFLD before transplantation. In the same study, recipients with NAFLD had a higher prevalence of new-onset diabetes before transplantation, and NAFLD was independently associated with cardiovascular mortality after KT (HR 4.4), even after adjustment for known CVD risk factors. It is still unclear whether NAFLD is associated with renal graft dysfunction. Grupper *et al*[20] found no correlation between graft function and NAFLD. However, from the studies already mentioned, NAFLD is significantly associated with a higher incidence of CKD in non-transplanted patients. This means that NAFLD and reduced graft function may also be the case in KT recipients[20]. The results of the above studies emphasise the need to diagnose NAFLD in KT recipients and to undertake aggressive risk factor modification early. The treatment of NAFLD in KT recipients is particularly challenging due to the complex interplay of metabolic disorders, immunosuppression and other comorbidities. Immunosuppressants used to prevent allograft rejection may exacerbate the metabolic disorder and contribute to the development and progression of NAFLD. Therefore, individualised treatment strategies that take into account each patient's unique needs are critical to achieving optimal outcomes according to KT. In addition, physicians caring for patients before and after KT should refer patients to a hepatologist as soon as NAFLD is diagnosed.

VIRAL DISEASES

Hepatitis B virus

The natural course of hepatitis B virus (HBV) infection is a dynamic process that reflects the interaction between HBV replication and the host immune response[21]. In immunocompetent adults, HBV infection is usually acute and self-limiting with more than 90% of patients achieving hepatitis B surface antigen (HBsAg) seroconversion within 6 mo, indicating natural resolution of the infection; however, infection in infancy or childhood often leads to chronicity[21]. Today, an estimated 240 million people are affected by chronic HBV infection[21]. Patients with detectable HBV DNA, especially those over 30 years of age and with a family history of HCC and cirrhosis, are typically considered for antiviral therapyand require lifelong treatment after KT[22,23]. Although modern antivirals such as entecavir or tenofovir result in viral suppression in almost all patients, treatment is long-term, possibly lifelong, and minimises the possibility of seroconversion[24]. Therefore, in chronically infected patients without signs of hepatitis, liver fibrosis or cirrhosis, without extrahepatic manifestations of HBV and with low viral replication rates (as determined by HBsAg and HBV DNA), treatment is not usually recommended by professional society guidelines[22,23]. In immunosuppressive states, *e.g.* due to chemotherapy or after organ transplantation, patients with cleared or inactive HBV may experience an abrupt increase in viral replication, which is called HBV reactivation.

Symptoms of HBV reactivation can range from asymptomatic to severe acute hepatitis, progression to chronic inflammation and fibrosis or HCC, and even acute liver failure. Where HBV is endemic, reported HBV reactivation rates with immunosuppression are as high as 41.5% (resolved HBV) and 70% (chronic HBV infection)[25,26]. As HBV prevalence in KT recipients ranges from 2.2 to 20.9% it is crucial that healthcare professionals understand their patient's status to determine appropriate monitoring or antiviral prophylaxis measures to prevent reactivation[27].

The patient with resolved HBV infection (HBsAg-negative, anti-HBc IgG positive recipient): Up to 30% of KT candidates may have resolved HBV infection [defined as HBsAg-negative and hepatitis B core antibody (anti-HBc) - positive], and 1.4%-9.6% may experience HBV reactivation after KT[27]. The American Association for the Study of Liver Diseases guidelines recommend monitoring every three months for the first year (alanine transaminase and HBV DNA) and antiviral prophylaxis only as an alternative[24]. However, since the risk factors for HBV reactivation are not precisely known, it is not clear which patients should be treated and which should be monitored. In a study by Mei *et al*[28] 52 patients with resolved HBV infection were retrospectively analysed. Five (9.6%) cases of HBV reactivation occurred, and anti-HBcAg titre (P = 0.042) and age (P = 0.037) were identified as risk factors for HBV reactivation. Interestingly, ATG treatment, steroid pulse doses and low-dose rituximab were not associated with HBV reactivation[28]. Shaikh *et al*[27] found that only 3.4% of KT recipients who did not receive antiviral prophylaxis experienced HBV reactivation, and delayed graft function was identified as a significant risk factor. Still, there were no significant adverse graft-related outcomes among those who experienced reactivation[27].

The patient with chronic HBV infection (HBsAg-positive recipient): Chronic HBV is defined by persistent HBsAg in serum for at least 6 mo. Although current guidelines recommend prophylactic treatment for HBsAg-positive patients if they require immunosuppressive therapy for transplantation, most evidence is based on patients undergoing chemotherapy[22,23]. A systematic review and meta-analysis by Thongprayoon *et al*[29] included a total of 87623 KT patients and found significant association between HBsAg-positive status and poor outcomes, including mortality (pooled OR = 2.48; 95%CI: 1.61-3.83) and allograft failure (pooled OR = 1.46; 95%CI: 1.08-1.96). There was also a significant negative correlation between study year and risk of allograft failure, suggesting a possible improvement in

patient and graft survival in HBsAg-positive recipients over time[29]. A recent study by Mo *et al*[30] confirmed high rates of viral reactivation in a real-world study of HBsAg-positive KT recipients, with inappropriate antiviral agents (all other than lifelong prophylaxis) (HR = 7.34, 95%CI 1.51-35.69, P = 0.01) and high levels of HBV DNA (≥ 1000 IU/mL) pre-transplant being the main risk factors (HR = 4.39, 95%CI 1.08-17.81, P = 0.04). However, the study found no difference in patients' or graft outcomes between patients who experienced reactivation and those who did not[30].

The kidney graft from donors with resolved HBV infection (HBsAg-negative, anti-HBc IgG positive donor): Donors with resolved HBV infection may alleviate organ shortage in KT. A study by Yamada *et al*[31] found a low risk of reactivation - 45 cases of KT from donors with resolved HBV infection to HBV-naive recipients were analysed, and one patient (2.2%) became seropositive for anti-HBc, and one patient (2.2%) had detectable HBV-DNA levels after transplantation. In the same study, the presence of covalently closed circular DNA in transplanted organs from donors with resolved HBV infection and the capability of HBV replication in kidney cell lines was demonstrated[31]. Accordingly, further research is needed to fully evaluate the safety and efficacy of this approach.

Hepatitis C virus

HCV infection is a global health problem affecting an estimated 71 million people worldwide[32]. The prevalence of HCV tends to be higher in patients with ESRD and in KT recipients than in the general population[33]. Although transplantation of HCV-infected patients offers a clear survival advantage over dialysis, HCV positivity has often been a barrier to KT in the past. Possible reasons for this include concerns about impaired survival of grafts and patients associated with certain HCV-related causes, such as increased rates of liver fibrosis and glomerulonephritis[34]. Highly effective, all-oral DAAs have revolutionised HCV treatment with cure rates of > 95%[35]. DAAs not only improve outcomes in HCV-positive KT patients, but also open new opportunities to increase scarce graft availability by safely transplanting HCV-positive organs into HCV-negative recipients[36]. The focus of interest today is the efficacy and safety of DAAs after KT and the challenges of using HCV-positive grafts in HCV-negative recipients.

DAA therapy for KT recipients: Sofosbuvir, a second-generation DAA approved in 2013, paved the way for efficient interferon/ribavirin-free treatment of HCV. Today, DAAs have proven their efficacy and safety both in clinical trials and in practice[37-39]. Chute *et al*[37] reported sustained viral response (SVR) rates of 97% in 418 of KT recipients, most of whom were treated with sofosbuvir-based therapies[37]. In the phase-3, open-label, single arm MAGELLAN-2 study, which evaluated a 12-wk course of the pangenotypic regimen of glecaprevir/pibrentasvir SVR was achieved in 98% of patients[38]. In addition to confirming the efficacy of the different DAA regimens used (23 patients, 100% SVR rate), Alkadi *et al*[40] found that there were no significant changes in renal function or calcineurin inhibitor levels during or after therapy[40]. In another study, DAAs were found to reduce HCV prevalence from 1.97% to 0.43% among recipients of KT when used over a five-year period[41]. Current DAA regimen guidelines for KT recipients are readily available through the American Association for the Study of Liver Diseases/Infectious Diseases Society of America (AASLD/IDSA) and the European Association for the Study of the Liver (EASL) guidelines[35,42].

ESRD and advanced liver disease: The prevalence and clinical-epidemiological profile of HCV infection in ESRD have changed over time. In particular, patients today tend to be older than in the past; more deceased donors are used for transplantation; there are fewer co-infections with HBV, but a higher percentage of cirrhotic patients are treated. Additionally, decompensation was found to be more frequent in recent years as well as patient survival rate being lower than before[32]. Since cirrhosis is an important predictor of poor survival after KT, it is advisable sess the stage of liver fibrosis in all KT candidates. In patients with established cirrhosis and portal hypertension in whom antiviral HCV treatment fails or is not an option, combined liver and KT must be considered[43].

Utilisation of HCV-positive donors: One of the most important advances in the last decade to increase transplantation rates has been the practise of KT from HCV-positive donors to HCV-negative patients (HCV D+/R-), followed by DAA therapy. After the pioneering studies THINKER and EXPANDER, which demonstrated good graft function and found little evidence of adverse outcomes after HCV D+/R- KT, this practice steadily increased in the United States[44,45]. United Network for Organ Sharing (UNOS) data showed and found increasing rates (0.3% to 6.9%) of HCV D+/R- from 1/1/2017 to 12/12/2020[46]. A growing number of real-world studies confirm the safety and efficacy of the HCV D+/R- approach and highlight its advantages, including shorter waiting times, access to younger donors with excellent allograft function, and similar survival compared to HCV D-/R- KT[46,47]. Substantial additional administrative work may be required outside of clinical trials for insurance approval of DAA therapy. A study by Edmonds *et al*[47] found that the median time from KT to the first dose of DAA was 45 d[47]. However, even delayed initiation of DAA treatment, with a median of 70 d after KT, had no negative impact on SVR rates or liver histology[48].

Understanding gaps and benefits/risks of HCV D+/R- KT may lead to greater acceptance. The majority of patients are willing to accept HCV-positive organs after being educated, citing shorter waiting time, DAA efficacy and faster return to higher functional status[49]. However, Nguyen *et al*[50] revealed disparities in HCV D+/R- KT access based on race/ethnicity, gender, and education level; minorities were 15%-60%, women over 20%, and those with elementary school degrees or less half as likely to receive a HCV nucleic acid amplification technique (NAT) positive kidney compared to those with bachelor's degrees. These findings underscore unequal distribution of breakthrough treatments, which can take years before recognition or reporting occurs[50]. In conclusion, use of HCV-positive kidney grafts for HCV-negative recipients followed by DAA therapy is a potential solution to organ shortage that can improve quality of life of ESRD patients.

Hepatitis E virus

The understanding of hepatitis E virus (HEV) has evolved over the past decade. Genotypes (gt) 1 and 2 are restricted to developing countries and cause large epidemics *via* the faecal-oral route, while gt 3 and 4 are endemic zoonoses in high-income countries. Although HEV usually causes an acute and mild form of hepatitis in imunocompetent hosts, it can lead to more severe and long-lasting infections in individuals who have undergone a SOT[51]. This has become an increasingly important issue because HEV is now considered one of the primary causes of acute viral hepatitis and cirrhosis in transplant recipients. Data on the seroprevalence of HEV in haemodialysis patients are inconsistent, ranging from 0 to 44%, possibly due to significant differences between the geographical regions studied[52].

In 2008, the first report of chronic hepatitis due to gt 3 and 4 was published in SOT[53]. Subsequent studies showed that organ recipients are at increased risk of HEV infection, with prevalence ranging from 0.44% to 24% depending on the organ transplanted and the geographic region[54]. Interestingly, HEV seropositivity was significantly higher in transplant recipients compared to patients on the waiting list (24% *vs* 16.4%, $P = 0.042$)[55]. Still, it is possible that HEV infection is underdiagnosed in SOT recipients, as serological assays with low sensitivity are often used[56]. Clinical course is inconspicuous in most cases with mild but often persistent abnormalities observed in liver function tests[53]. To properly diagnose chronic HEV infection, serum or plasma samples and, if possible, stool samples must be examined using NATs [51]. High clinical suspicion of chronic HEV is important because undetected infection, especially with gt 3, can lead to rapid progression of liver fibrosis and in some cases decompensation and death[57].

KT recipients are particularly susceptible to chronic HEV infections, which can lead to serious complications such as earlier graft rejection. KT recipients with positive ELISA tests or PCR results for HEV are more likely than controls to experience earlier rejection[58]. It is known that HEV infection can cause hepatic and extrahepatic symptoms. A case study of a KT recipient with HEV and membranous nephropathy (MN) illustrates the possible causal relationship between the two conditions. Treatment with ribavirin (RV) as monotherapy proved effective in treating HEV infection and resulted in complete remission of the nephrotic syndrome. This suggests that RV may also have beneficial effects on MN *via* non-specific immunomodulatory mechanisms[59].

Although chronic HEV was originally defined as viral replication lasting longer than 6 mo, in an observational study of SOT recipients, clearance did not occur 3-6 mo after infection, but only within the first 3 mo. These results suggest that SOT patients who are viraemic for longer than 3 mo should be considered for treatment[60]. A recent study that investigated the efficacy of dose reduction of mycophenolic acid in eight KT patients diagnosed with chronic HEV infection showed that only one patient achieved HEV clearance, while most patients required antiviral treatment with RV. The study provided the clinical evidence that reducing mycophenolic acid therapy alone is not sufficient to control viral replication in transplant patients. In addition, rituximab has been identified as a risk factor for chronic HEV that is complicated to treat[61].

Meta-analysis data show that RV is a safe and effective treatment for chronic HEV infection in SOT recipients. RV induces a sustained virological response in 76% of patients and represents a first-line therapy for this patient group[62]. However, it is important to note that the guidelines recommend monitoring HEV RNA for at least six months after completion of RV therapy, as reinfection or relapse can occur even if the virus has been eliminated by therapy. This is especially true for immunosuppressed transplant patients, including those with positive anti-HEV IgG.

Trials in China have shown the efficacy of recombinant HEV genotype 1 vaccine (Hecolin®, Xiamen Innovax Biotech). However, the vaccine is not available outside China and its efficacy against other genotypes is unknown. Further studies in developed countries are needed to assess its efficacy, durability of immune response and cost-effectiveness before it can be recommended[63]. For now, avoiding contaminated food/water and thorough cooking are the only preventive measures.

COVID-19

Since identified in December 2019, SARS-CoV-2 has infected more than 660 million people to date[64]. SARS-CoV-2 causes COVID -19, which typically presents as an upper respiratory tract infection but can lead to severe pneumonia with acute respiratory distress syndrome and multiorgan failure[65]. The virus infects target cells by binding the spike surface glycoprotein (S) to angiotensin-converting enzyme 2 (ACE2), which is mainly expressed in cells of the alveolar epithelium type II[66]. However, it was also found in the heart, ileum, kidney, bladder and liver, with cholangiocytes being the major ACE2-expressing cell population, with a 20-fold higher expression rate than in hepatocytes[67]. This could potentially make the liver susceptible to SARS-CoV-2 virus, but evidence for specific viral hepatotropism is limited.

Abnormalities in liver function tests have been observed in up to 78% of hospitalized COVID-19 patients, with some individuals developing acute liver injury (ALI) and reports of liver failure due to SARS-CoV-2 infection[68,69]. Given the significantly higher expression of ACE2 receptors on cholangiocytes, one might generally expect a cholestatic laboratory pattern to predominate in patients with COVID-19, but this is not the case. Therefore, it has been hypothesised that liver injury is not primarily due to direct virus-induced damage to hepatocytes, but rather to indirect causes such as hepatotoxic drugs, systemic inflammatory responses and respiratory distress syndrome-induced hypoxia[70]. Some cases of acute de novo autoimmune hepatitis have also been reported following COVID-19, suggesting possible immunogenicity due to molecular mimicry of SARS-CoV-2[71]. Cholangiopathy resembling secondary biliary cholangitis, a possible chronic complication of COVID-19, has also been reported in critically ill patients[72]. In addition, the presence of cirrhosis among patients infected with SARS-CoV-2 is associated with a higher risk of death[73]. Major hepatic histopathological findings in patients with COVID-19 are nonspecific and mainly consist of hepatic steatosis, congestion of hepatic sinuses, vascular thrombosis, and portal/periportal tract lymphocytic infiltrates[74].

COVID-19 and Kidney transplant recipients: KT recipients are often older, have multiple comorbidities and require chronic immunosuppression, and as such represent a high risk group for more serious COVID-19 disease. Since the onset

of the pandemic, a large amount of data on recipients of SOT and KT have been reported, mainly from case series, small cohorts and larger registries. However, large multicentre studies with appropriate control groups are lacking. So, it remains a challenge how to appropriately treat KT recipients with COVID-19 and to understand what factors influence the outcomes. Clinical presentation, including abnormalities in liver function tests, has been shown to be similar in SARS-CoV-2 infected KT patients and in the general population[75].

In a multicentre cohort study the incidence of hepatitis was 20.2% among KT recipients, who received some form of antiviral therapy for SARS-SoV-2[76]. As in immunocompetent patients, the liver disease in KT recipients is usually mild and transient, characterised mainly by elevated levels of AST and ALT[76]. AST abnormality is more pronounced in patients with a severe form of the disease, and higher AST values have been shown to be a marker of poor outcome[77,78]. Early results from national and international registries and multicentre studies found a variable mortality rate of 18% to 32% among KT recipients hospitalised for COVID-19[77,79].

One distinctive feature that must be considered when assessing the possible aetiology of liver damage in patients with COVID-19 is the possibility of drug-induced liver injury (DILI) associated with certain treatments. Data on adverse events are sparse and often cannot be attributed with certainty exclusively to drugs for COVID-19, as a variety of other medications such as antibiotics and antipyretics are widely used in the treatment of such patients. To date various medications have been tried in treatment of SARS-CoV-2 infection – glucocorticoids, antimalarials, immunomodulatory agents (JAK2 inhibitors, IL-6 and IL-1 inhibitors), monoclonal antibodies, antiviral drugs and all of them may theoretically exhibit hepatotoxicity[80,81]. Table 1 Lists the hepatic contraindications and risk of drug induced liver injury (DILI) for drugs approved for the treatment of SARS-CoV-2 infection in the European Union and the United States of America [80-82].

DRUG-INDUCED LIVER INJURY

DILI, defined as liver injury caused by various drugs, herbs or other xenobiotics, results in abnormalities in liver tests or liver dysfunction, where other causes can be reasonably excluded[83]. The incidence of DILI is increasing worldwide due to the development of new drugs in the West and herbal preparations in Asia[83]. The classes of drugs most commonly associated with DILI include antibiotics, anticonvulsants and psychotropic drugs[84]. Polypharmacy, a common occurrence in KT recipients, increases the risk of drug interactions that may contribute to a higher incidence of DILI[84].

The severity of DILI can be associated with the dose of the consumed substance, *e.g.*, acetaminophen overdose/poisoning. However, most medications cause DILI in a non-dose dependent way, *i.e.*, idiosyncratically. Recent research proposed a 3-step model of DILI in which direct cell stress, direct mitochondrial inhibition and/or specific immune reactions can lead to mitochondrial permeability transition. Various environmental (age-related changes of pharmacokinetics, induction/inhibition of CYP450 enzymes, impaired antioxidant defence, immunological sensitisation, pre-existing liver disease, concomitant infections (*e.g.* HBV, HCV), mitochondrial dysfunction) and genetic factors may influence each sequence of the proposed model. In KT recipients, there are few potential environmental risk factors, *e.g.* drug interactions (antibiotics, antifungal and antiviral prophylaxis, immunosuppressive therapy), immunological sensitisation, malnutrition, infections and mitochondrial dysfunction due to diabetes, as well as all possible genetic risk factors that make them more susceptible to developing DILI than the general population. Previous research reported the occurrence of suspected DILI in 23% of KT recipients with possible causality in 57% of cases, and the associated drugs were antimicrobials, immunosuppressants and diuretics[84].

Assessing causality of DILI remains a challenge due to the lack of reliable biomarkers for hepatotoxicity. Several scoring systems are available, but there are discrepancies between scales. The best agreement seems to be between the Roussel-Uclaf Causality Assessment Method (RUCAM) scale and the Digestive Disease Week-Japan (DDW-J) scale, which includes an *in vitro* drug lymphocyte stimulation test (DLST) and is based on the RUCAM scale. Newer methods such as bile acid metabolomics could be used to assess the severity of DILI[85].

Despite some advances in the diagnosis and prognosis of DILI, the mainstay of treatment is to assess the need for immediate discontinuation of the suspect drug. N-acetylcysteine is currently a worldwide accepted treatment for acetaminophen hepatotoxicity. Bicyclol is a substance that may attenuate ALI by several mechanisms, including induction of autophagy, inhibition of oxidative stress and inactivation of the NLRP3 inflammasome. The results of the research in China show a satisfactory safety profile of the substance with a potentially favourable clinical outcome[86,87].

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASES

Autosomal dominant polycystic kidney disease (ADPKD) is a prevalent genetic disorder causing uninhibited cyst growth in the kidneys, liver, and pancreas. It is one of the top causes of ESRD with an estimated occurrence rate ranging from 1:400 to 1000 live births due to mutations in PKD1 and PKD2 genes[88]. In addition, patients may present with other abnormalities, such as cerebral aneurysms, cardiac valve malformations, colonic diverticulosis, hernias of the abdominal wall and inguinal region, and cysts of the seminal vesicles[88]. Given the frequency of the disease, patients with ADPKD make up a large percentage of KT recipients. In rare cases, patients with ADPKD are referred for simultaneous liver and KT due to an enlarged liver leading to early satiety, abdominal pain and cachexia. In the majority of cases, however, synthetic liver function remains intact, so that most patients receive only a KT. After transplantation, liver cysts may continue to grow but rarely cause problems. Nevertheless, cyst complications must be included in the differential

Table 1 Hepatic contraindications and risk of drug-induced liver injury among SARS-CoV-2 treatment drugs

Drug	Liver Contraindication	Risk of DILI
Systemic corticosteroids	Caution in liver failure	+
Remdesivir	ALT > 5 × ULN	++
Tocilizumab	ALT > 5 × ULN	++
Sarilumab	ALT > 1.5 × ULN	++
Anakinra	Efficacy and safety in patients with AST/ALT ≥ 1.5 × ULN not been evaluated.	+
	Not recommended with severe hepatic impairment (Child-Pugh C)	
Baricitinib	Not recommended with severe hepatic impairment (Child-Pugh C)	+
Tofacitinib	Not recommended with severe hepatic impairment (Child-Pugh C)	+
Nirmatrelvir/ritonavir	Not recommended with severe hepatic impairment (Child-Pugh C)	+
Lopinavir/ritonavir	Not recommended with severe hepatic impairment (Child-Pugh C)	++
Molnupiravir	Limited experience of the use with any degree of hepatic impairment.	+
Casirivimab/imdevimab	Not recommended with severe hepatic impairment (Child-Pugh C)	+
Sotrovimab	No data in patients with ALT 5 to < 10 × ULN).	+
Tixagevimab/cilgavimab	Not been evaluated in patients with hepatic impairment	+
Regdanvimab	Not been evaluated in patients with hepatic impairment	+

ALT: Alanine transaminase; AST: Aspartate aminotransferase; ULN: Upper limit normal; +: Uncommon; ++: Common; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

diagnosis of both fever of unknown origin in KT recipients with ADPKD (due to cyst inflammation) and abdominal pain (*e.g.* rupture).

For patients with symptoms of liver enlargement that persist after KT, there are several treatment options: surgical resection, fenestration or sclerotherapy of cysts, or drug treatment[89,90]. Liver transplantation and combined liver/KT have been performed in patients with severe, symptomatic disease. Sirolimus appears to reduce the volume of the polycystic liver, possibly through an antiproliferative effect. Liver volume was significantly lower in seven KT patients receiving sirolimus-mycophenolate-prednisone than in nine recipients receiving tacrolimus-mycophenolate-prednisone [91].

Recently, tolvaptan, an antidiuretic antagonist, was approved as the first therapy for ADPKD after the TEMPO and TEMPO-R trials demonstrated significantly less eGFR loss compared to the placebo group[92]. It is expected that more and more patients with ADPKD will be treated with tolvaptan in the future. Tolvaptan is a V2R antagonist that blocks vasopressin signalling, which plays an important role in cyst growth in ADPKD due to the resulting intracellular increase in cyclic adenosine monophosphate[92]. The main adverse effect of tolvaptan is liver toxicity, requiring frequent monitoring, and polyuria is a logical consequence of V2R blockade. However, adherence to tolvaptan appears to be well-feasible in the majority of patients[93]. Additional ongoing studies will determine whether the benefits are long-term, whether they can be observed in patients with advanced kidney disease and whether they can be translated into quality of life and cost/effectiveness parameters[94]. Tolvaptan may also be considered for patients after KT according to preliminary reports, taking into account the possible adverse effects, including hepatotoxicity, hypernatremia and alteration of serum tolvaptan concentration when used concomitantly with cyclosporine[95].

Other, rare cystic diseases, such as Autosomal Recessive Polycystic Kidney Disease, Autosomal Dominant Tubulointerstitial Kidney Disease and Carolli disease should also be considered in KT recipients[96].

OTHER LIVER DISEASES

Iron overload

Iron overload is a common problem in ESRD patients and transplant recipients, often resulting from the use of iron supplements to treat anaemia, the use of iron-based phosphate binders, and disruption of iron utilization due to chronic inflammation associated with CKD. Anaemia, defined as a hemoglobin concentration < 130 g/L in men and < 120 g/L in women, is a frequent complication of CKD[97]. The prevalence is 50%-70% in ESRD patients before transplantation and decreases to 51% at 6 mo and 37% at 2 years after KT[98]. Erythropoietin deficiency and impaired iron homeostasis, including absolute and functional iron deficiency, are major contributors to CKD-associated anaemia. Standard therapies for the treatment of anemia include exogenous substitution of erythropoietin, iron supplementation, and blood transfusion. The availability of erythropoiesis-stimulating agents has reduced the need for blood products and the risk of

blood-borne infections, iron overload, and allosensitization. Recently, inhibitors of the hypoxia-inducible factor prolyl hydroxylase (HIF-PHI) have emerged as a new therapeutic option for anaemia. HIF-PHIs mediate both the erythropoietin and iron metabolism pathways. HIF-PHIs stabilize hypoxia-inducible factor (HIF), which stimulates endogenous erythropoietin production and affect the transcription of several iron metabolism and transport genes, leading to a decrease in ferritin levels and hepcidin levels[99-102]. HIF-PHIs can reduce the need for iron supplements by mobilizing stored iron[101]. Several randomized control trials in CKD patients regardless of dialysis status of roxadustat, vadadustat, and daprodustat showed no inferiority compared to ESA therapy with a similar safety profile[99,102-105]. These promising results could have significant clinical relevance as iron supplementation could be reduced.

Adequate iron stores are necessary for normal functioning of ESA and maintenance of stable hemoglobin levels. However, iron overload can lead to accumulation of iron in various organs. Iron-related side effects, mostly mediated by the formation of reactive oxygen species, can affect the liver (secondary hemochromatosis, cirrhosis), the heart (heart failure and arrhythmias), and endocrine organs (hypogonadism, diabetes mellitus). Excess iron can aggravate inflammation and shift the immunoregulatory balance, negatively affecting the immune system.

Serum ferritin and serum transferrin saturation (TSAT) are commonly used tests for iron status in CKD patients, including transplant recipients. Hepcidin measurement is not clinically more useful or better than TSAT and ferritin. Serum ferritin levels are affected by inflammation as it acts as an acute phase reactant. Serum ferritin levels ≤ 30 μg/L indicate iron deficiency. However, the ferritin value at which the iron stores in the bone marrow are filled is controversial. Currently, the 2012 KDIGO guidelines recommend iron supplementation therapy based on the combination of TSAT (≤ 30%) and ferritin (≤ 500 μg/L)[106].

CONCLUSION

Liver disease is a frequent occurrence following KT, estimated to affect 20%-50% of cases, and has a substantial impact on the survival and quality of life of these patients. Symptoms vary from asymptomatic, transient elevations of liver enzymes to the development of fibrosis and chronic liver failure. In severe cases, liver failure can have an acute onset or, in the chronic form, progress to cirrhosis with clinical indications of decompensation, or even the development of malignant disease such as HCC. Vigilant monitoring of KT recipients is crucial for the early detection of liver disease, allowing for timely intervention and improved outcomes. The incidence of viral diseases such as HBV and HCV has declined due to the effectiveness of antiviral medications and HBV vaccination programs. Globally, the prevalence of metabolic diseases such as NAFLD is rising, especially in transplant patients who are vulnerable due to lifelong immunosuppressive therapy. HEV and, more recently, SARS-CoV-2 have also been implicated as potential causes of liver disease. Additionally, healthcare professionals should be aware of the possibility of DILI, as many immunosuppressive and adjunct drugs can cause liver damage. Patients should be monitored closely for signs of drug toxicity, and medications should be adjusted accordingly. Screening KT recipients for liver disease is a priority for healthcare professionals. Early diagnosis and referral to a hepatologist are critical for managing underlying liver problems, which can adversely impact quality of life and general health.

FOOTNOTES

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MINIREVIEWS

Solid-Tubulocystic carcinoma: A new variant of intrahepatic cholangiocarcinoma

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Abstract

A new variant of intrahepatic cholangiocarcinoma (iCCA) has been recognized in recent years presenting predominantly as a large hepatic mass in young woman with the characteristic expression of inhibin by immunohistochemistry. This variant iCCA was originally termed as cholangioblastic variant of iCCA, and subsequently proposed to be renamed as inhibin-positive hepatic carcinoma or solid-tubulocystic variant of iCCA to better reflect its immunohistochemical profile or morphologic spectrum. The tumor histologically is composed of small to medium sized cells with scant to moderate amount of eosinophilic cytoplasm heterogeneously organized in solid, tubular, and cystic growth patterns. The tumor cells are positive for biliary markers, inhibin and albumin, and have a novel recurrent gene fusion, *NIPBL::NACC1*. Awareness of this new iCCA variant and its clinicopathologic features will aid in the diagnostic work-up and avoid confusion with other primary and metastatic hepatic neoplasms.

Key Words: Cholangiocarcinoma; Intrahepatic; Solid-tubulocystic; Cholangioblastic; Inhibin

Core Tip: Solid-tubulocystic variant of intrahepatic cholangiocarcinoma (iCCA) is a recently recognized iCCA variant, which previously was termed as cholangioblastic iCCA. This new variant iCCA predominantly presents in young woman characterized by a heterogenous microscopic appearance with small to medium sized tumor cells with eosinophilic cytoplasm organized in solid, tubular, and cystic growth patterns. One of the defining features is the diffuse expression of inhibin. Recurrent *NIPBL::NACC1* gene fusion has been identified in this iCCA variant. Compared to typical iCCAs, patients with this variant iCCA may have a better prognosis with 25% of the cases reported died of disease in 5 years.

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INTRODUCTION

Cholangiocarcinoma (CCA) is a highly aggressive adenocarcinoma arising from the biliary tree and can be divided into intrahepatic CCA (iCCA), perihilar CCA and distal CCA. Perihilar CCA arises from the second-order bile ducts to the insertion of the cystic duct whereas distal CCA is confined to the common bile duct below this insertion[1,2]. Collectively perihilar and distal CCA are referred as extrahepatic CCA. The most recent WHO classification of the digestive system tumours classified iCCA into two main subtypes based on their histologic features, small duct and large duct[3]. Other recognized rare variants of iCCA include adenosquamous carcinoma, squamous carcinoma, mucinous carcinoma, mucoepidermoid carcinoma, signet-ring cell carcinoma, clear cell carcinoma, ductal plate malformation-like pattern carcinoma, lymphoepthelioma-like carcinoma and sarcomatous carcinoma[3,4]. Recently, a new variant of inhibin-positive iCCA has been reported, which was termed as solid-tubulocystic or cholangioblastic variant iCCA[5-10]. This novel variant has a characteristic gene fusion, *NIPBL::NACC1*, which was first recognized by Argani *et al*[7], and subsequently confirmed in another case report by González *et al*[5].

This mini review summarized the unique clinical, morphologic, and molecular features of this newly identified and underrecognized inhibin-positive solid-tubulocystic/cholangioblastic variant iCCA. Awareness of this new variant would aid practicing physicians to recognize this rare variant iCCA.

CLINICAL FEATURES

The inhibin-positive solid-tubulocystic variant iCCA presents with a median age of 28 years (range: 15-54 years) and predominantly occurs in woman (82%)[5-9,11-13]. The majority of the patients presents with non-specific abdominal distention and pain as well as nausea, vomiting and discomfort. On imaging the tumor is characterized by a heterogenous appearance with both solid and cystic areas and tends to have a well-demarcated border[5]. The median size at presentation is 16 cm (range: 6.9-32 cm). Of the patients reported with available follow-up information, half of the patients developed recurrence or metastasis during follow-up which were treated with adjuvant chemotherapy, and 25% (4 cases) died of disease all within 5 years of diagnosis.

MORPHOLOGIC FEATURES

The inhibin-positive solid-tubulocystic/cholangioblastic variant iCCA has distinct and characteristic morphologic features with multiple different patterns recognized within each tumor. Grossly, the tumors are well-circumscribed and the cut-surfaces vary from a solid tan-white to tan-yellow surface with areas of hemorrhage and degeneration[5]. Tumor necrosis is often seen particularly in larger tumors. Areas with a multicystic surface creating a spongiform appearance in some cases can be seen[5]. These cystic structures often have smooth inner surface and are filled with clear to tan-yellow fluid. In some cases, fibrous bands can be seen within the tumor.

As one could expect from the heterogenous gross appearance, the histologic features of this tumor vary in different areas within the same tumor which is both useful in their diagnosis but can also create confusion and a potential misdiagnosis. The solid areas are characterized by tumor cells with scant to moderate amount of eosinophilic focally granular cytoplasm with round to oval nuclei with a finely granular chromatin and occasional small nucleoli (Figures 1A and B). These tumor cells are organized in solid sheets to trabecular growth patterns and tubular/pseudoglandular structures. Both the cytologic morphology and architectural configuration are reminiscent of well-differentiated neuroendocrine tumors (WD-NET) and acinar cell carcinomas (ACC) which are differential diagnoses of these tumors. In other areas a tubular architecture is seen with cystic dilatation. The cysts are lined by tumor cells with similar cytologic features as the solid areas, and pink colloid-like secretions can be seen within the lumen in some cases mimicking thyroid follicular neoplasm. In some cases, the compact solid areas have a more crowded pattern with tumor cells showing only scant cytoplasm. Given this appearance these tumor cells are considered as a more "primitive" appearance and referred as

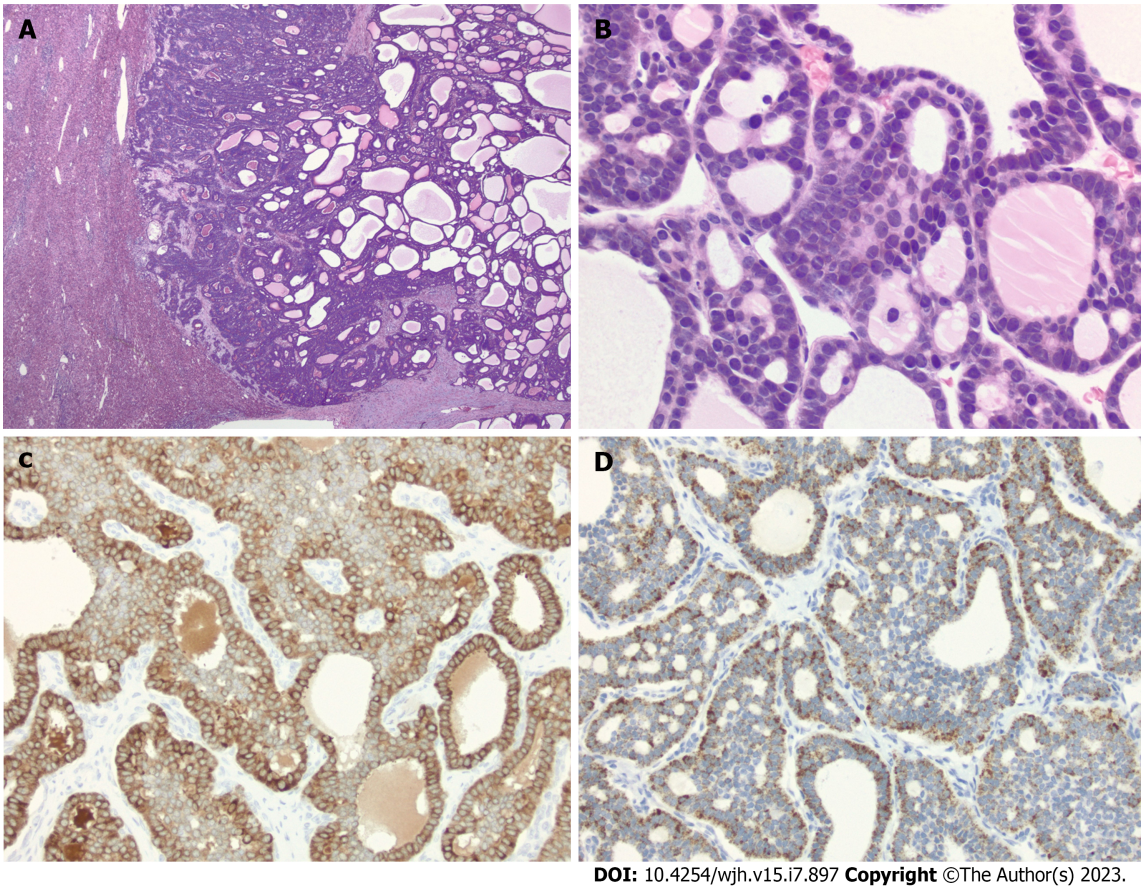

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Figure 1 Solid-tubulocystic variant of intrahepatic cholangiocarcinoma. A: Representative picture of a resected solid-tubulocystic variant of intrahepatic cholangiocarcinoma (right) in a non-cirrhotic liver (left). The tumor is well demarcated from the background liver (20x, Hematoxylin-eosin stain); B: Representative picture showing the tumor with solid, tubular, and cystic growth patterns (200x, Hematoxylin-eosin stain); C: Representative picture showing the tumor cells are positive for inhibin (200x, immunohistochemical stain); D: Representative picture showing tumor cells are positive for albumin (200x, in situ hybridization).

blastema-like areas. Therefore, these lesions have been termed as "cholangioblastic variant of iCCA" given the presence of blastema-like areas and the expression of neuroendocrine markers, a common feature of other primitive tumors[6]. However, in our opinion these tumors do not have the characteristics of other primitive epithelial tumors, nor recapitulate the developing ductal plate[5]. In light of this and the histologic features, we prefer to use the term of solid-tubulocystic variant of iCCA as proposed by Wen KW and colleagues[8], instead of cholangioblastic variant of iCCA.

IMMUNOPHENOTYPE

Perhaps given the rarity of this variant iCCA, an extensive immunophenotype has been reported in the case report and small case series in the literature with the most characteristic finding being the diffuse and strong expression of inhibin (Table 1) (Figure 1C)[5-9,11,14]. As expected, the tumor is diffusely positive for cytokeratin (CK) AE1/AE3, CAM5.2, CK7 and CK19, whereas being negative for CK20 and CDX2, consistent with a biliary phenotype. It is also negative for hepatocellular markers, such as HepPar1 and arginase-1. Of note, albumin by in situ hybridization is positive in tumor cells (Figure 1D), which is consistent with primary hepatic carcinoma - iCCA. One of the pitfalls is the focal to diffuse expression of multiple neuroendocrine markers including CD56, synaptophysin and chromogranin, which varies from a weak to strong positivity, but INSM1, a more specific neuroendocrine marker is so far reported to be negative.

MOLECULAR FEATURES

Recently, a novel fusion in this variant of iCCA involving the exon 8 of *NIPBL* and exon 2 of *NACC1, NIPBL::NACC1* has been identified in 3 cases[7]. An additional case was identified by the authors in the Cancer Genome Atlas harboring an identical fusion (case TCGA-ZH-A8Y6). The digital slide of this case available online (www.cbioportal.org) shows the characteristic morphologic features of solid-tubulocystic variant of iCCA. Most recently, a subsequent study reported one case to harbor this fusion[5]; hence to date 5 cases have been identified with this novel fusion. However, it remains unclear if this fusion is specific for this tumor type. To date this exact fusion has only been reported in 2 other malignancies both reported as "noncolorectal and nonpancreas gastrointestinal primary[15]." Unfortunately, no

Table 1 Aggregated selected immunophenotype[5,6,7,8,11], *n* (%)

Antibody	Positive	Negative
Inhibin	14 (100)	0
Cytokeratin AE1/AE3	6 (100)	0
CAM5.2	4 (100)	0
Cytokeratin-7	14 (100)	0
Cytokeratin-19	12 (100)	0
Cytokeratin-20	0	6 (100)
CDX2	0	3 (100)
Arginase-1	0	5 (100)
HepPar1	0	13 (100)
Glypican-3	1 (12.5)	7 (87.5)
Glutamine synthetase	3 (100)	0
Albumin by *in situ* hybridization	6 (100)	0
Synaptophysin	10 (76.9)	3 (23.1)
Chromogranin	9 (64.3)	5 (35.7)
CD56	5 (71.4)	2 (28.6)
INSM1	4 (100)	0
Beta-catenin	0	7 (100)
CD10	0	3 (100)
Pax8	0	2 (100)
Gata3	0	1 (100)
Mammaglobin	0	1 (100)
GCDFP-15	0	1 (100)
ER	0	2 (100)
PR	0	2 (100)
S100	0	3 (100)

CDX2: Caudal-related homeobox gene 2; INSM1: Insulinoma associated protein 1; GCDFP-15: Gross cystic disease fluid protein 15; ER: Estrogen receptor; PR: Progesterone receptor; HMB45: Human melanoma black 45; pCEA: Polyclonal carcinoembryonic antigen; mCEA: Monoclonal carcinoembryonic antigen; SF-1: Steroidogenic factor 1; WT-1: Wilms tumor 1; TTF-1: Thyroid transcription factor 1; AFP: Alpha fetoprotein; CA125: Cancer antigen 125; CA19.9: Cancer antigen 19.9; SALL4: Sal-like 4; Oct3/4: Octamer binding transcription factor 3/4; PLAP: Placental-like alkaline phosphatase; hCG: Human chorionic gonadotrophin; RCC: Renal cell carcinoma; SOX9: SRY-box transcription factor 9.

description of the morphology of these two tumors is available. Although this fusion appears to be specific for this tumor at this time, given its characteristic morphology and immunohistochemical profile, molecular testing may not be necessarily needed in our opinion for diagnostic purposes.

The *NIPBL* (Nipped-b homolog) gene codes the protein delangin[16], a cohesin loading factor, which is essential for the chromatin loading of cohesin, an important ring-shaped protein complex for the structural organization of chromosomes and for the repair of DNA double-strand breaks[17]. Delangin also plays a role in translocating cohesin along the chromatin fibers which is thought to be the underlying pathogenesis of Cornelia de Lange syndrome[18,19]. Additionally, *NIPBL* plays a crucial role in myeloid differentiation[20]. Loss-of-function of *NIPBL* has been associated with an increased number of myeloid progenitors and a decrease of mature myeloid cells, and has been reported in acute megakaryoblastic leukemia[21,22]. In solid tumors, variant in *NIPBL* occurs in tenosynovial giant cell tumor, specifically a *NIPBL::ERG* fusion[23]. Interestingly, NIPBL overexpression has been shown to correlate with poor prognosis and chemotherapy resistance in non–small cell lung cancer, and knockdown of NIPBL in non–small cell lung cancer cell or breast cancer cell lines induces impaired proliferation, cell cycle arrest, apoptosis and autophagy[24-26]. NAC1 coded by *NACC1* is a member of the Bric-a-Brac Tramtrack Broad (BRB) family of protein complex which is also known as pox virus and zinc finger (POZ). This protein complex has multiple cellular functions involving in cellular proliferation, apoptosis, protein degradation, transcription and cellular morphology, among others[27-29]. NAC1 is associated with development of endometrial carcinomas[29], and tumor recurrence[28], and the development of chemoresistance in ovarian cancers[30-

32]. Although both *NIPBL* and *NAC1* genes have a crucial role in cellular proliferation and DNA repair, it is unclear to date what is the mechanistic function of their fusion protein. Future studies are needed to understand its biological function of this fusion which could lead potentially to the development of targeted therapies.

DIFFERENTIAL DIAGNOSIS

Metastatic or primary hepatic WD-NETs often share similar histologic pattern, cytologic morphology and positivity of neuroendocrine markers as this solid-tubulocystic variant of iCCA. One of the main clues for a solid-tubulocystic variant of iCCA is the multiple growth patterns present within each case in contrast to WD-NET which tends to have a more homogenous growth pattern. However, immunohistochemistry work-up is essential in their distinction. Although both WD-NET and solid-tubulocystic variant of iCCA can express neuroendocrine markers (synaptophysin and chromogranin), the expression of CK7 and CK19, and albumin by in-situ hybridization in solid-tubulocystic variant of iCCA rules out a diagnosis of WD-NET. Since the morphologic similarity with metastatic ACC, a possibility of ACC needs to be excluded. The negative expression of neuroendocrine markers (synaptophysin and chromogranin) and the positive expression of acinar markers (BCL10, trypsin and chymotrypsin) can help to rule out solid-tubulocystic variant of iCCA. Of note, about 25% of the pancreatic ACCs are positive for albumin by in-situ hybridization[33]. The cystic and glandular architecture containing colloid-like secretions is reminiscent of thyroid follicular neoplasm. The negative expression of TTF-1 or thyroglobulin can help to exclude the possibility of metastatic follicular neoplasm. Although uncommon, a metastatic sex-cord stromal tumor may enter the differential diagnosis given the expression of inhibin and similarity of some of the morphologic features. However, the expression of CK7, CK19, neuroendocrine markers and albumin by in-situ hybridization by solid-tubulocystic variant of iCCA can help to rule out this possibility.

TREATMENT CONSIDERATIONS

Given the rarity of these cases it remains unclear what is the optimal management plan for patients with this new variant iCCA. The majority of the cases reported with available information underwent a complete surgical resection ranging from segmentectomies to lobectomies, and one case was deemed not a surgical candidate given the presence of widely metastatic disease[5-9]. Two patients were treated before resection, one with neoadjuvant chemotherapy and one with transarterial chemoembolization[6]. The non-surgical candidate was treated with cisplatin and gemcitabine[9]. Most of the patients were treated with adjuvant chemotherapy after resection with a similar regimen, cisplatin and gemcitabine [7]. The previously reported pediatric patient was treated with a different regimen which included capecitabine[5].

CONCLUSION

Inhibin-positive hepatic carcinoma with solid-tubulocystic features (solid-tubulocystic carcinoma) is a recently recognized iCCA variant which predominantly presents in young woman with a large size at presentation. They have a characteristic morphology of round to oval cells with scant to moderate amount of eosinophilic cytoplasm which are organized in multiple different growth patterns including solid, tubular, and cystic. This novel variant is associated with a characteristic fusion gene, *NIPBL::NAC1*, being the first iCCA with a described recurrent gene fusion. Although much remains unknown of this variant based on the reported cases to date, they are associated with relatively favorable outcome with a 25% mortality rate compared to the 7%-20% 5-year survival rate of typical iCCAs[2]. Further studies are needed to fully explore the biological function of this fusion gene and its role in the tumorigenesis and to continue to characterize this rare tumor.

FOOTNOTES

Author contributions: González IA reviewed the literature and drafted the manuscript; Luo W provided some of the histological images and edited the manuscript; Zhang X provided overall intellectual input, reviewed the literature, and edited the final version of the manuscript; all authors approved the final version to be published.

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ORIGINAL ARTICLE

Case Control Study

Liver stiffness in pregnant women with intrahepatic cholestasis of pregnancy: A case control study

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Abstract

BACKGROUND

Intrahepatic cholestasis of pregnancy (ICP) is a rare but severe complication for both the mother and the unborn child. The diagnosis is primarily based on elevated serum levels of bile acids. In a large ICP cohort, we here study in detail liver stiffness (LS) using transient elastography (TE), now widely used to non-invasively screen for liver cirrhosis within minutes.

AIM

To specifically explore LS in a large cohort of women with ICP compared to a control group with uncomplicated pregnancy.

METHODS

LS and hepatic steatosis marker controlled attenuation parameter (CAP) were measured in 100 pregnant women with ICP using TE (Fibroscan, Echosens, Paris, France) between 2010 and 2020. In 17 cases, LS could be measured postpartum. 450 women before and 38 women after delivery with uncomplicated pregnancy served as control group. Routine laboratory, levels of bile acids and apoptosis marker caspase-cleaved cytokeratin 18 fragment (M30) were also measured.

RESULTS

Women with ICP had significantly elevated transaminases but normal gamma-glutamyl transferase (GGT). Mean LS was significantly increased at 7.3 ± 3.0 kPa compared to the control group at 6.2 ± 2.3 kPa ($P < 0.0001$). Postpartum LS decreased significantly in both groups but was still higher in ICP (5.8 ± 1.7 kPa *vs*

4.2 ± 0.9 kPa, $P < 0.0001$), respectively. In ICP, LS was highly significantly correlated with levels of bile acids and M30 but not transaminases. No correlation was seen with GGT that even increased significantly after delivery in the ICP group. Bile acids were mostly correlated with the liver apoptosis marker M30, LS and levels of alanine aminotransferase, aspartate aminotransferase, and bilirubin. In multivariate analysis, LS remained the sole parameter that was independently associated with elevated bile acids.

CONCLUSION

In conclusion, LS is significantly elevated in ICP which is most likely due to toxic bile acid accumulation and hepatocyte apoptosis. In association with conventional laboratory markers, LS provides additional non-invasive information to rapidly identify women at risk for ICP.

Key Words: Intrahepatic cholestasis of pregnancy; Transient elastography; Bile acids; Liver stiffness; High risk pregnancy

Core Tip: Intrahepatic cholestasis of pregnancy (ICP) is a rare but severe complication in both mothers and unborn children. In a large ICP cohort, we studied liver stiffness (LS) in detail using transient elastography, which is now widely used for non-invasive screening of liver cirrhosis within minutes. LS is significantly elevated in pregnancies with ICP, most likely owing to toxic bile acid accumulation and hepatocyte apoptosis. Interestingly, no correlation was observed with γ-glutamyl transferase. In association with conventional laboratory markers, LS provides a novel non-invasive tool to rapidly identify women at risk for pregnancy complications.

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INTRODUCTION

Approximately 3% of pregnant women have liver disorders that can cause severe problems for the mother and unborn child, *e.g.*, liver failure, preterm labor, and stillbirth[1-3]. Despite intensive research on pregnancy-related liver complications in recent decades, treatment options are still insufficient, and no effective screening tests for early assessment have been established[4-6]. Intrahepatic cholestasis of pregnancy (ICP) with elevated serum bile acid levels higher than 20 μmol/L is the most common pregnancy-specific liver disease. Its etiology is complex and consists of genetic, endocrine (circulating estrogen and progesterone), and environmental factors (reduced vitamin D and selenium levels in winter). Severe forms with bile acids levels higher than > 40 μmol/L are associated with abnormal fetal echocardiography, meconium-stained amniotic fluid, spontaneous preterm labor and fetal asphyxia. Moreover, women with a total serum bile acid level of 100 μmol/L have an increased risk of stillbirth. The incidence varied between 0.05% and 27.6% for all pregnancies[7-12].

ICP typically presents in the third trimester as nocturnal pruritus of the soles and palms[13]. ICP also increases the risk of gestational diabetes and pre-eclampsia[14]. Long-term consequences of ICP include a higher risk of cancer of the liver and biliary tree, diabetes mellitus, thyroid disease, autoimmune diseases (psoriasis, inflammatory polyarthropathies, and Crohn's disease), and cardiovascular disease[15]. Treatment with ursodeoxycholic acid has been proven to significantly improve itching, blood levels, and fetal outcomes in numerous studies; however, pregnancy termination remains the only causal therapy[2,7]. A rapid diagnosis of ICP is essential to protect mothers and children from (long-term) complications [16]. The diagnosis is normally based on elevated serum levels of bile acids. Unfortunately, these blood tests usually take several hours, even at maximum care facilities and are not available at any time. To date, no screening tests are available for liver disease during pregnancy, except serological testing for viral hepatitis in the third trimester.

Despite the scarcity of ICP data, enormous progress has been made in the molecular understanding of cholestatic liver disease in recent decades[10]. Hepatocytes and cholangiocytes cooperatively produce bile, which is a mixture of organic and inorganic compounds[17]. Cholestasis usually describes the impairment of bile flow caused by defects in hepatocytes, which form and secrete bile, and/or defects in the secretory machinery of cholangiocytes[17]. The detergent properties of bile render it highly toxic to cells and tissues[17]. In addition to drugs, inflammation, liver disease, and hormones, several gene mutations have been discovered that can cause cholestasis and ICP[8-10,18-21].

Measurement of liver stiffness (LS) using elastographic techniques has become the gold standard for the noninvasive diagnosis of liver fibrosis and cirrhosis and it often avoids invasive liver biopsies[22]. Transient elastography (TE) (FibroScan, Echosens, Paris, France), the first elastographic technique, is an ultrasound-based technique that uses a transducer probe to create an elastic shear wave[23]. Pulse-echo ultrasound is used to measure shear wave velocity, which is directly associated with LS expressed in kilopascals (kPa). TE requires only a few minutes, is highly accurate, and has a lower sampling error than biopsy, thus allowing for repetitive measurements[24]. LS values below 6 kPa are

considered normal, while the generally accepted cutoff values for liver fibrosis (F3) and cirrhosis (F4) are 8 and 12.5 kPa [25]. However, LS is not only elevated by the fibrosis stage but also by other important confounding factors, including physiological conditions such as food and alcohol intake, or pathological confounders such as inflammation or pressure elevation[22]. Of note, all these confounders and artifacts will always increase LS but never decrease it, which is the most important reason, while normal LS has a very high negative predictive value in excluding liver pathologies[24].

Therefore, liver elastography is an ideal diagnostic tool to address hepatic complications during pregnancy. In the first elastography study of > 500 pregnant women without liver disease, we recently demonstrated that LS increased significantly in the third trimester and was an independent predictive factor for pre-eclampsia[26]. These findings were independently confirmed in a smaller study in Denmark[27]. Moreover, in pregnant women with cirrhosis, LS predicts hepatic decompensation after delivery[28]. The aim of the present study was to specifically explore LS in a large cohort of women with ICP compared with a control group with uncomplicated pregnancies.

MATERIALS AND METHODS

Study design and patient cohort

The study protocol (435/2006 and S201/2015) of this observational, prospective, case-control study was approved by the Ethics Committee of the University of Heidelberg and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The study design is shown in Figure 1. Briefly, between February 2010 and March 2020, 652 women were recruited at the Department of Gynecology at the University of Heidelberg or Salem Medical Center in Heidelberg during prenatal ultrasound or who presented to the prenatal outpatient department with prenatal complications or in the ward. Postpartum examinations occurred 24 h after delivery. Inclusion criteria were age ≥ 18 years and an intact pregnancy at weeks 9 to 42 or status postpartum. The healthy control cohort was obtained from our previous study[26]. ICP was diagnosed based on typical clinical symptoms, such as pruritus, and laboratory markers, such as elevated transaminase and serum bile acid levels > 20 μmol/L. The exclusion criteria were as follows: No signed informed consent; other pregnancy complications, such as preeclampsia or HELLP syndrome; and no valid LS measurements.

Laboratory parameters

Routine blood parameters were measured at the General Laboratory of University Hospital Heidelberg and Limbach Laboratory in Heidelberg. In 100 patients, serologically detected caspase-cleaved (M30) and total (M65) cytokeratin 18 levels as markers of liver apoptosis was measured as described previously using ELISA (Peviva, Bromma, Sweden)[29]. We also measured total bile acids not only in women suspected of having ICP but also in 60 women in the control group for comparative purposes.

LS and controlled attenuation parameter

LS and controlled attenuation parameter (CAP) were measured using TE (FibroScan, Echosens, Paris, France). M or XL probes were used according to the manufacturer's specifications and placed in the right lobe of the liver at the intercostal position, as described previously[30]. LS and CAP values were calculated as the medians of at least 10 consecutive measurements. In parallel to liver elastography in the control group, routine abdominal ultrasound was performed to exclude liver pathologies, such as liver cirrhosis, liver congestion, or liver tumors. In addition, the degree of liver steatosis was graded (0–3) and the spleen size was determined. Cutoff values from a recent meta-analysis were used[31]. Valid LS measurements were obtained for all the women.

Statistical methods

Statistical analyses were performed using the SPSS Statistics [version 23.0 (IBM, New York, United States), Excel 2016 (Microsoft, Redmond, United States), and GraphPad Prism 6 (GraphPad Software, San Diego, United States)]. For group comparisons, means and standard deviations were calculated, and an independent samples t-test was used. The Spearman rank-order correlation coefficient was calculated to conduct correlation analysis. Univariate and multivariate binary logistic regression analyses were used to identify the independent predictors of pregnancy complications, and receiver operating characteristic (ROC) analysis was performed.

RESULTS

Patient characteristics

For a better comparison, Table 1 presents only the characteristics of women (control and ICP) in the third trimester and after delivery. Almost all women with ICP (98 of 100, 98%) were in their third trimester, which is consistent with the literature[32]. In the control cohort, 228 of 450 patients (50.7%) were in the third trimester. A smaller number of women were followed up 1 d after delivery for both controls and ICP (n = 38 and n = 17). Supplementary Table 1 shows patient characteristics of the entire cohort in all trimesters. Accordingly, differences between the controls and patients with ICP remain. Women with ICP were significantly younger, and by definition, bile acids were significantly increased by a factor of approximately 6 (P < 0.0001). Women with ICP also had significantly elevated transaminase [predominantly alanine aminotransferase (ALT)] and bilirubin levels, but not elevated levels of alkaline phosphatase (AP) and gamma-glutamyl transferase (GGT). The levels of caspase 3-cleaved CK18, liver apoptosis marker M30, and uncleaved CK18 (M65), repres-

Table 1 Patient characteristics for the third trimester and after delivery

Parameters	Normal range[1]	ICP		Control	
		3. trimester, *n* = 98	Postpartal, *n* = 17	3. trimester, *n* = 228	Postpartal, *n* = 38
Age (years)		31.0 ± 4.2[a]	28.3 ± 3.5[c]	32.2 ± 5.2	32.9 ± 4.3
Bile acids (μmol/L)	2-5	39.0 ± 29.4[d]	23.7 ± 11.6[a]	6.8 ± 6.9	2.3 ± 1.2
AST (U/L)	< 35	116 ± 106[d]	88.2 ± 80[a]	26 ± 27	28 ± 6
ALT (U/L)	< 35	211 ± 202[d]	127 ± 106[b]	18 ± 26	15 ± 8
GGT (U/L)	< 40	23 ± 22	48 ± 40[a]	30 ± 55	18 ± 18
AP (U/L)	35-105	178 ± 68	182 ± 73	150 ± 95	283 ± 344
Bilirubin total (mg/dL)	< 1.3	0.65 ± 0.27[d]	0.52 ± 0.56	0.47 ± 0.26	0.65 ± 0.35
M30 (U/L)	< 200	432 ± 220[a]	293 ± 75[a]	339 ± 207	296 ± 50
M65 (U/L)	< 400	1180 ± 571[d]	777 ± 426[d]	680 ± 343	486 ± 89
Creatinine (mg/dL)	< 1.3	0.69 ± 0.38[a]	0.88 ± 0.39[a]	0.57 ± 0.12	0.61 ± 0.12
Urea (mg/dL)	< 50	19.8 ± 6.8[a]	25.5 ± 6.4[b]	16.9 ± 4.5	19.4 ± 5.3
Uric acid (mg/dL)	3.5-7.2	5.6 ± 2.4[d]	6.3 ± 2.8	3.9 ± 0.9	4.7 ± 1.0
Total protein (g/L)	66-83	67.6 ± 4.2[b]	65.2 ± 6.8	64.7 ± 4.4	66.3 ± 9.5
Albumin (g/L)	38-59	35.6 ± 2.7	36.3 ± 12.7	35.7 ± 2.7	
Leukocytes (1/nL)	3.7-10.0	8.8 ± 2.4[d]	12.0 ± 3.4[a]	10.8 ± 3.0	15.5 ± 4.8
Erythrocytes (1/pL)	4.1-5.1	3.9 ± 0.4	3.6 ± 0.6	4.0 ± 0.4	3.7 ± 0.5
Hemoglobin (g/dL)	12-16	11.4 ± 1.1	10.3 ± 1.5	11.7 ± 1.3	10.8 ± 1.5
Hematocrit (%)	36-43	33 ± 3	30 ± 6		
MCV (/pL)	80-96	86.1 ± 4.4	88.0 ± 3.5		
Platelets (1/nL)	150-360	228 ± 72	241 ± 61	227 ± 68	237 ± 61
Haptoglobin (g/L)	0.3-2.0	0.8 ± 0.4	1.0 ± 0.8		
Quick (%)	70-120	123.3 ± 5.1	119.4 ± 21.1	123.0 ± 13.0	124.5 ± 4.4
INR	< 1.1	0.91 ± 0.04	0.90 ± 0.03	0.90 ± 0.03	0.90 ± 0.03
Grade of steatosis in United States (0-3)	0	0.42 ± 0.50[a]	0.14 ± 0.38	0.18 ± 0.42	0.00 ± 0.00
Spleen size (cm)	< 11	11.4 ± 1.9	11.3 ± 1.1	11.1 ± 1.5	11.1 ± 1.7
Liver stiffness (kPa)	< 6	7.3 ± 3.0[c]	5.8 ± 1.7[d]	6.2 ± 2.3	4.2 ± 0.9
CAP (dB/m)	< 290 (S3)	206 ± 49[c]	213 ± 43	228 ± 39	224 ± 46

[1]Normal range for women.
[a]$P < 0.05$ *vs* controls in the corresponding group (3. trimester or postpartal).
[b]$P < 0.01$ *vs* controls in the corresponding group (3. trimester or postpartal).
[c]$P < 0.001$ *vs* controls in the corresponding group (3. trimester or postpartal).
[d]$P < 0.0001$ *vs* controls in the corresponding group (3. trimester or postpartal).
ICP: Intrahepatic cholestasis of pregnancy; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; AP: Alkaline phosphatase; MCV: Mean corpuscular volume; INR: International normalized ratio; CAP: Controlled attenuation parameter; M65: Soluble cytokeratin 18; M30: Caspase-cleaved cytokeratin 18 fragment.

entative of liver necrosis, were significantly elevated. However, M65 showed a twofold increase in women with ICP, whereas M30 was only slightly higher in this group. Interestingly, although all liver-related parameters decreased after delivery, GGT was the only marker with postpartum elevation compared with controls.

LS is significantly increased in women with ICP

As shown in Figure 2A and Table 1, LS was significantly higher in women with ICP, both before and after delivery. Moreover, in both groups, we observed an increase in LS as pregnancy progressed. In the ICP group, mean LS was significantly increased at 7.3 ± 3.0 kPa compared with the control group at 6.2 ± 2.3 kPa ($P < 0.0001$). An increase in LS to > 6 kPa was observed in 24.9% of healthy pregnant women and 44.2% of the ICP group, whereas an elevated LS of > 8

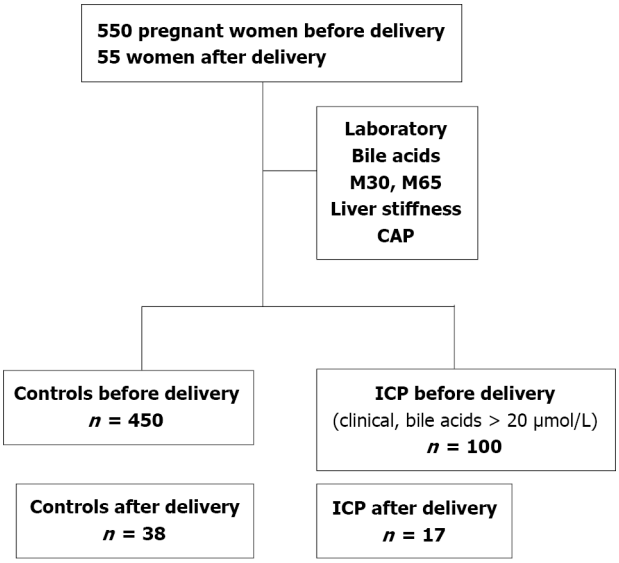

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Figure 1 Study design. ICP: Intrahepatic cholestasis of pregnancy.

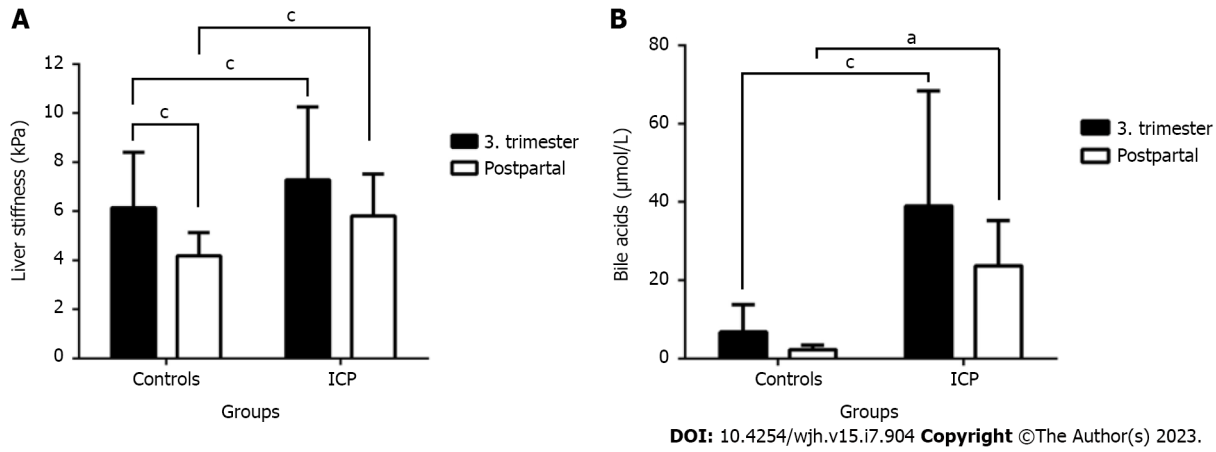

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Figure 2 The 3. trimester and postpartal in the intrahepatic cholestasis of pregnancy and control group ([a]*P* < 0.05, [c]*P* < 0.001). A: Liver stiffness (mean and standard deviation); B: Bile acids (mean and standard deviation). ICP: Intrahepatic cholestasis of pregnancy.

kPa was observed in 7.1% of the control and 15.6% of the ICP group. LS of > 12.5 kPa considered above the cutoff value for cirrhosis was measured in 3.0% of the control and ICP groups. In confirmation to our initial study[26], however, postpartum LS decreased significantly in both groups to 5.8 ± 1.7 kPa in the ICP group and 4.2 ± 0.9 kPa in the control group, respectively. Finally, hepatic steatosis, as measured by the CAP, was normal in most women. It slightly but significantly increased in the ICP group during delivery, from 206 to 213 dB/m, whereas no significant changes were observed in the controls (Table 1). In summary, although LS generally increases during pregnancy, the liver is significantly stiffer in women with ICP before and after delivery than in controls without hepatic complications.

Parameters associated with LS elevation

Next, we performed Spearman's rho correlation analysis with clinical and laboratory parameters to identify potential confounders associated with elevated LS. Table 2 shows the results of the ICP, control, and total cohorts. In the ICP cohort, only a few parameters were significantly correlated with LS, namely, serum levels of bile acids and the liver apoptosis marker M30. Bilirubin levels hardly met the level of significance, whereas leukocyte count and Quick's test results were negatively correlated. No association between LS and bile acid levels was observed in the control group, and M30 Levels were weakly but significantly correlated with LS. In contrast, as described recently[26], LS significantly correlated with the duration of pregnancy, onset of gestational diabetes, body weight, mean arterial diastolic pressure, and AP and M65 levels in the total cohort. Interestingly, AST levels, which are usually highly associated with LS in liver diseases[33], were significantly correlated in the total cohort but not in the ICP or control groups alone. No correlation was observed between GGT and ALT levels in the ICP cohort. Finally, no association was observed between markers of hemolysis or anemia. In conclusion, in women with diagnosed ICP, bile acids are tightly associated with elevated LS and markers of liver apoptosis, but not with conventional liver function tests, except for bilirubin.

Table 2 Spearman Rho correlation with liver stiffness for women with intrahepatic cholestasis of pregnancy, controls and all

Parameter	Spearman rho correlation with liver stiffness		
	ICP, *n* = 100, r	Control, *n* = 450, r	All, *n* = 550, r
Bile acids total (μmol/L)	0.368[c]	0.085	0.438[d]
M30 (U/L)	0.881[b]	0.313[b]	0.385[c]
Quick (%)	-0.276[b]	0.210[a]	-0.010
Leukocytes (1/nL)	-0.223[a]	0.012	-0.203[b]
Bilirubin total (mg/dL)	0.213[a]	-0.091	0.124
MAD (mmHg)	-0.379	0.137[b]	0.154[c]
Gestational diabetes (1 or 0)	0.376	0.269[d]	0.301[d]
Pruritus (1 or 0)	0.353	-0.015	0.246[d]
Spleen size (cm)	0.340	-0.032	0.014
Uric acid (mg/dL)	0.308	0.261[a]	0.411[d]
AST (U/L)	0.146	0.103	0.327[d]
Creatinine (mg/dL)	0.281	0.057	0.163
Body weight (kg)	0.241	0.301[d]	0.317[d]
Platelets (1/nL)	-0.119	0.000	-0.074
AP (U/L)	0.237	0.329[c]	0.378[d]
Urea (mg/dL)	0.234	-0.064	0.041
Hemoglobin (g/dL)	-0.112	-0.022	-0.104
CAP (dB/m)	0.097	0.083	-0.006
M65 (U/L)	0.357	0.389[c]	0.452[d]
ALT (U/L)	0.048	0.047	0.273[d]
GGT (U/L)	-0.010	0.114	0.150

[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
[d]$P < 0.0001$.
Note that parameters are sorted first by levels of significance in the intrahepatic cholestasis of pregnancy group (p) and then the absolute correlation coefficient in descending order. Most relevant parameters are on top. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; AP: Alkaline phosphatase; ICP: Intrahepatic cholestasis of pregnancy; CAP: Controlled attenuation parameter, MAD: Mean arterial diastolic pressure; M65: Soluble cytokeratin 18; M30: Caspase-cleaved cytokeratin 18 fragment.

LS is independently associated with elevated bile acids in ICP

Figure 2B shows the levels of bile acids in both controls and women with ICP before and after delivery. Bile acid levels, which are a major criterion for the diagnosis of ICP were about six times elevated in the ICP cohort and promptly decreased after delivery. A slight but significant decrease was observed in the control group, suggesting that pregnancy causes bile acid elevation. Notably, bile acid levels were markedly elevated in the ICP cohort after delivery. Supplementary Table 2 lists the parameters associated with elevated bile acid levels. Bile acids are significantly associated with clinical features of pruritus and gestational diabetes. Among the laboratory parameters, bile acids were mostly correlated with the liver apoptosis marker M30 and the levels of ALT, AST, and bilirubin. Haptoglobin levels, leukocyte counts, and coagulation test results (Quick) were negatively associated with them. No association was observed between liver steatosis (CAP), M65 levels, AP, or splenic size. In the total cohort, bile acids were best associated with ALT, AST, and LS, in descending order. In multivariate analysis, LS remained the only parameter independently associated with elevated bile acid levels (Supplementary Table 3). In conclusion, in patients with ICP, bile acids are closely associated with liver damage in the form of apoptosis, and LS is independently associated with bile acid levels. In the third trimester, a LS of 6.5 kPa significantly discriminates between ICP and controls ($P = 0.033$) although with a modest AUROC of 0.65 (0.58–0.72, $P = 0.033$).

DISCUSSION

In this study, we noninvasively measured LS through TE and steatosis using CAP in a large cohort of pregnant women with diagnosed ICP, primarily through elevated bile acids. Our data clearly show that LS is higher in women with ICP than those in controls. Although LS decreased rapidly after delivery, as described recently[26,27], it remained significantly higher in women with ICP despite identical follow-up observation times. Women with ICP predominantly had elevated ALT levels, followed by those with elevated AST and bilirubin levels. In addition to AP levels, no differences were observed in GGT levels. Although all parameters, including LS, improved after delivery, GGT levels increased significantly in the ICP cohort. In addition, hepatic fat content, as measured using CAP, although within the normal range, was lower in patients with ICP than in controls. Finally, the liver apoptosis marker M30 showed the highest association with bile acid levels in univariate regression analysis, whereas LS remained the strongest independent predictor of bile acid levels > 20 mol/L in multivariate regression analysis.

In confirmation of earlier reports[26-28], the present study demonstrates that noninvasive assessment of LS through elastography is feasible and well accepted in pregnant women. In contrast to reports from internal medicine departments [34], elastography can be performed in all women. Second, we showed that LS is significantly elevated in women with ICP and higher than that in controls, which is remarkable, as we and others showed that LS is generally and reversibly elevated in the third trimester[26,27]. Consequently, and comparable to the previously reported LS elevation in women with preeclampsia, LS can be considered a feasible and noninvasive tool for screening, identifying, and following women with pregnancy-related liver complications.

What are the confounding factors for LS elevation in women with ICP? In contrast to initial beliefs, LS can be elevated because of many confounding factors, including inflammation, arterial and venous pressure elevation, and physiological conditions such as meal intake[22,24]. Mechanic cholestasis has been demonstrated to reversibly increase LS[35]. Of note, however, continued elevation of LS owing to these confounders has a negative impact on the liver, and the first long-term mortality data demonstrated that LS is one of the best long-term predictors of liver-related and all-cause mortality[22]. LS measurement can also be used to identify and monitor pregnant women with preexisting liver cirrhosis and predict hepatic decompensation[28]. The first study in women with uncomplicated pregnancies showed that elevated LS was significantly correlated with AP, leukocytes, gestational age, and an increase in body weight[26]. In the present study, in women with ICP, the confounders were completely different, and LS was tightly associated with elevated serum levels of bile acids and serum markers of liver apoptosis (M30). This is particularly interesting with regard to the fact that in liver diseases, such as alcoholic liver disease or viral hepatitis, LS elevation is typically associated with transaminase levels, namely, AST but not ALT[33]. Although we show here that LS is an independent predictor of elevated bile acids, the performance of LS in predicting ICP was only moderate and lower than that in the previous smaller ICP cohort[26].

To the best of our knowledge, this is the first study to show an exceptionally strong association between the established serum liver apoptosis marker (M30) with levels and bile acid levels in women with ICP and its association with elevated LS. In the multivariate analysis, LS remained the only parameter independently associated with elevated bile acid levels. In patients with liver disease, the association between liver apoptosis and LS elevation has already been shown both at the histological level[36] and using serum markers, such as M30[29]. The tight association of bile acids with LS and M30 levels in pregnancy is new. Bile acids are synthesized in hepatocytes as essential components for bile formation[9,17]. Owing to their detergent nature, however, they are highly cytotoxic and can disrupt cellular membranes if not protected, *e.g.*, by phospholipids[9,17,19]. Specifically, serum cholic acid becomes the primary bile acid in women with ICP, in contrast to normal pregnant and non-pregnant women, whose proportion is similar to that of chenodeoxycholic acid[37]. Typical examples are cholestatic liver diseases, such as primary biliary cirrhosis, which ultimately cause chronic bile duct inflammation, and later cirrhosis and cancer. Even simple mechanical cholestasis through obstruction of the major bile ducts by biliary stones can cause severe tissue damage.

In the last three decades, many gene mutations have been discovered that can cause cholestasis through the impairment of hepatocyte or cholangiocyte transport proteins relevant to bile formation[8-10,18-20]. These findings have resulted in a group of diseases known as progressive familial intrahepatic cholestasis. The normal GGT levels in our ICP cohort are a strong argument for a genetic cause. Hormonal changes/normalization after delivery with subsequent normalization of bile acid export through the hepatocellular apical membrane are considered important for the role of sex hormones in ICP[9]. In line with this, we observed a postpartum increase in GGT in our ICP cohort, suggesting a reinduction of GGT with the onset of bile flow.

Surprisingly, hepatic steatosis, as measured by CAP, which is now widely explored in patients with fatty liver[38], did not show any conclusive data in women with normal pregnancy or with ICP. The reason for this remains unclear because we expected that at least some women would present with steatosis, which can be a severe complication of pregnancy. We also briefly discuss some of the limitations of our study, which are mostly due to the challenging setting of performing clinical studies during late pregnancy, particularly in women with suspected complications. Serum was not available for all women to allow for the subsequent measurement of markers such as M30 and M65. In addition, we managed to measure LS sequentially before and after delivery in only a few cases in the same person. Another limitation with regard to postpartum follow-up measurements was that we only included women 24 h after delivery. This short time may explain why some parameters did not reach statistical significance.

CONCLUSION

In conclusion, we showed in a large cohort that women with ICP show significantly elevated LS compared with women

with uncomplicated pregnancies. In contrast to the large body of evidence in the liver literature, elevated LS in ICP is primarily correlated with the accumulation of bile acids known to be highly toxic to hepatocytes, and liver apoptosis, as measured through M30 levels, but not with transaminases, bilirubin, or GGT. We also showed for the first time that typically low GGT levels in ICP increase after delivery. Consequently, we believe that screening for LS in pregnancy is not "another diagnostic tool" to further complicate the already intensive surveillance during pregnancy, but could provide a novel non-invasive strategy to early identify women at risk for complications.

ARTICLE HIGHLIGHTS

Research background

Intrahepatic cholestasis of pregnancy (ICP) is a rare but severe hepatic complication for both mother and unborn child. Diagnosis is normally based on elevated serum levels of bile acids. Unfortunately, these blood tests usually take several hours, even at maximum care facilities. So far, there are no screening test for liver disease in pregnancy besides serological testing for viral hepatitis in the third trimester.

Research motivation

Measurement of liver stiffness (LS) through elastographic techniques has become the novel gold standard for the non-invasive diagnosis of liver cirrhosis often avoiding invasive liver biopsies. LS is not only highly correlated to the hepatic fibrosis stage but can also be elevated due to other important confounding factors such as inflammation, congestion or cholestasis. For these reasons, liver elastography could be an ideal diagnostic tool to address hepatic complications during pregnancy.

Research objectives

The aim of the present study was to specifically explore LS in a large cohort of women with ICP before and after delivery compared to a control group with uncomplicated pregnancy.

Research methods

LS and the hepatic steatosis marker controlled attenuation parameter (CAP) were measured in 100 pregnant women with ICP using transient elastography (Fibroscan, Echosens, Paris, France). In 17 cases, LS could be measured after delivery. A large cohort of women with uncomplicated pregnancy served as control group. Routine laboratory, levels of bile acids and the apoptosis marker M30 were also measured.

Research results

In the third trimester, women with ICP show a significantly increased LS at 7.3 ± 3.0 kPa compared to controls (6.2 ± 2.3 kPa, $P < 0.0001$). LS decreases significantly 24 h after deliver and remains higher in ICP (5.8 ± 1.7 kPa *vs* 4.2 ± 0.9 kPa, $P < 0.0001$). In ICP, LS is mainly correlated with levels of bile acids and the apoptosis marker M30. No correlation was seen with GGT and GGT even increased after delivery in women with ICP.

Research conclusions

In conclusion, LS is significantly elevated in ICP which is most likely due to toxic bile acid accumulation and hepatocyte apoptosis. In association with conventional laboratory markers, LS provides additional non-invasive information to rapidly identify women at risk for ICP.

Research perspectives

In the future, elastography should be further validated in order to early identify women at risk for complications. Moreover, elastography studies should be combined with genetic risk assessment, as several mutations of bile transport proteins are involved in the development of ICP.

FOOTNOTES

Author contributions: Nees J, Fluhr H, and Mueller S designed and coordinated the study; Nees J and Ammon FJ performed the experiments; Nees J, Mueller S, and Mueller J analyzed and interpreted the data; Nees J and Mueller S wrote the manuscript.

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Data sharing statement: Data available from the corresponding author at sebastian.mueller@urz.uni-heidelberg.de.

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ORIGINAL ARTICLE

Observational Study

Evaluation of the nutritional status of patients with liver cirrhosis

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Abstract

BACKGROUND

Progressive malnutrition coexists with liver diseases, particularly in patients with cirrhosis. Early diagnosis of malnutrition in patients with advanced stages of chronic liver disease and the implementation of appropriate nutritional treatment for malnourished patients should be an integral part of the therapeutic process.

AIM

To evaluate the nutritional status of patients with various severities of advanced liver fibrosis, using various nutritional status parameters.

METHODS

This study involved 118 patients with liver cirrhosis who were classified into three groups according to their Child-Pugh score. The nutritional status of the patients in each group was assessed using different methods. The average values obtained from the measurements were calculated for each research group. The influence of disease stage on the examined parameters of nutritional status was determined using one-way analysis of variance. To investigate the relationship between the parameters determining nutritional status and the stage of disease advancement, a correlation analysis was performed.

RESULTS

The Child-Pugh A group had the highest mean body weight (76.42 kg), highest mean body mass index (BMI) (26.72 kg/m^2), and largest mean arm circumference (27.64 cm). In the Child-Pugh B group, the mean scores of all examined variables were lower than those of the Child-Pugh A group, whereas the mean body weight and BMI of the Child-Pugh C group were higher than those of the Child-Pugh B group. There was a very strong correlation between the Child-Pugh classification

and subjective global assessment score; a very strong correlation between the Child-Pugh classification and arm circumference; a strong correlation between the Child-Pugh classification and body weight, albumin concentration, fat-free mass index, muscle mass index, phase angle, and BMI; and an average correlation between Child-Pugh classification and fat mass index. Notably, these indicators deteriorated with disease progression.

CONCLUSION

Advanced liver fibrosis leads to the deterioration of many nutritional status parameters. The extent of malnutrition increases with the progression of liver fibrosis. The Child-Pugh score reflects the nutritional status.

Key Words: Liver cirrhosis; Fibrosis; Nutritional status; Malnourishment; Sarcopenia

Core Tip: Early diagnosis of malnutrition in patients with advanced stages of chronic liver disease and the implementation of appropriate nutritional treatments for malnourishment should be an integral part of the therapeutic process. It is important to properly assess the nutritional status of these patients as this can be the basis for therapeutic plans. It is advisable to determine which method of assessing nutritional status is appropriate for patients with cirrhosis since they usually develop specific complications of progressive organ failure.

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INTRODUCTION

Liver fibrosis develops in response to damaging factors with various etiologies (Table 1)[1]. It is a consequence of chronic liver disease[1,2]. Collagen deposition, scarring, and excessive accumulation of extracellular matrix elements have been observed[1]. The formation of scar tissue leads to the loss of contact between hepatocytes, blood vessels and bile ducts, resulting in the impairment of organ function[3]. Cirrhosis is the final stage of advanced fibrosis of the liver parenchyma [4]. The Global Burden of Disease estimates that cirrhosis affects 50 million adults worldwide and causes one million deaths annually[5]. The clinical advancement of the disease and risk of mortality can be assessed using the Child-Pugh scale. The scale considers ascites, hepatic encephalopathy, prothrombin time, serum bilirubin, and albumin and divides patients into three groups: (1) With good prognosis; (2) with moderate prognosis; and (3) with poor prognosis[6,7].

Chronic liver disease with advanced fibrosis and cirrhosis may be accompanied by progressive malnutrition[8]. Malnutrition is caused when the amount of food consumed is less than the required amount (hypoalimentation), often due to an incorrect understanding of the essence of the so-called "liver diet", digestive and absorption disorders that are caused by the slowing down of digestive processes (including secretion of bile acids), metabolic disorders consisting of limited protein synthesis and increased protein catabolism, and acceleration of basic metabolic processes[9]. The complications of liver cirrhosis include hepatic encephalopathy, ascites and decreased concentrations of albumin, coagulation factors, and transport proteins[9,10]. Early diagnosis of malnutrition in patients with advanced stages of chronic liver disease and implementation of appropriate nutritional treatment for malnourished patients should be an integral part of the therapeutic process[11,12] to provide support to the patient, prevent complications, and improve treatment and prognosis[13,14].

Malnutrition is usually diagnosed by assessing the patient's nutritional status. For this purpose, anthropometric measurements and a subjective global assessment (SGA) scale (SGA of nutritional status) are commonly used[15,16]. Laboratory tests, hand grip strength measurements that enable sarcopenia identification and body composition analysis using electrical bioimpedance are carried out for a thorough analysis of the nutritional status[17-21]. Among the numerous methods of assessing nutritional status, it is important to determine which one is the most appropriate for patients with liver fibrosis given the specific complications of progressive organ failure.

This study aimed to assess the nutritional status of patients diagnosed with advanced liver fibrosis at the cirrhosis stage using various methods of nutritional status assessment. In addition, we assessed whether the Child-Pugh score correlates with patients' nutritional status and which methods of assessing nutritional status are appropriate for patients with advanced liver fibrosis.

Table 1 Causes of liver fibrosis leading to cirrhosis

Etiological factor	Diseases causing liver fibrosis
Viral infections	Chronic hepatitis B
	Chronic hepatitis D
	Chronic hepatitis C
	Chronic hepatitis E
Autoimmune	Autoimmune hepatitis
	Primary biliary cirrhosis
	Primary sclerosing cholangitis
	Autoimmune cholangitis
Metabolic	Nonalcoholic fatty liver disease
	Hemochromatosis
	Wilson's disease
	a_1-antitrypsin deficiency
Toxic	Alcoholic liver disease
	Drugs, industrial toxins

MATERIALS AND METHODS

Description of the study group and criteria for patient inclusion

This study was conducted between May and November 2020 and included 118 Caucasian adult patients of Polish nationality who attended the ID Clinic in Mysłowice, Poland. First, informed consent was obtained to ensure voluntary participation. The following inclusion criteria were used: Liver cirrhosis based on clinical symptoms, imaging studies, and transient elastography[22] and no extrahepatic, acute, or chronic disease affecting the nutritional status. Liver cirrhosis was diagnosed by non-invasive transient elastography method using the Fibroscan® 502 Touch device, while in patients with ascites, which is a contraindication to elastography, the diagnosis was established based on clinical data, imaging, and laboratory results. The causes of cirrhosis in the study group were: Alcoholic hepatitis in 49 patients (42%), hepatitis C virus infection in 42 (36%), hepatitis B virus infection in 17 (14%), non-alcoholic steatohepatitis in 10 patients (8%). The study included 55 women and 63 men aged 37-81 years, who were classified into three groups according to the Child-Pugh scale. The Child-Pugh A group included 52 patients (Child-Pugh B: 34 patients; C: 32 patients). The mean age in each group was 58.84 years (Child-Pugh A), 56.94 years (Child-Pugh B), and 58.78 years (Child-Pugh C). The study was reviewed and approved by the Hospital Review Board. The study's planning, conduct, and reporting were in line with the tenets outlined in the Declaration of Helsinki.

Assessment of nutritional status

The nutritional status of patients in each group was assessed using the following methods: (1) Anthropometric measurements: Body weight (kg), height (cm), and body mass index (BMI) (kg/m^2), calculated according to the World Health Organization classification[23]; (2) Measurement and calculation of the circumference of the shoulder muscle [mid-arm muscle circumference (MAMC)]. MAMC = circumference of the arm in the middle of its length (mm) - 3.14 the thickness of the skin fold over the triceps muscle (mm)[12]; and (3) Body composition analysis by the bioelectrical impedance method, using the In Body 770 device. This device has received international certifications from the International Organization for Standardization (ISO), namely ISO 9001: 2015 and ISO 13485: 2016, and medical certification from the International Electrotechnical Commission (IEC), namely EN60601-1 and IEC 60601-1-2.

The following parameters were specified: (1) Fat-free mass index (FFMI) (kg/m^2): The cut-off points for FFMI were based on the criteria for diagnosing malnutrition developed by the European Society for Clinical Nutrition and Metabolism consensus statement: < 15 for women and < 17 kg/m^2 for men[24]; (2) Muscle mass index (MMI): The cutoff points for determining the MMI were established based on the standards of the measuring device[25]; (3) Fat mass index (FMI): The cutoff points for determining FMI were based on the standards of the measuring device[25]; (4) Extracellular water content (ECW) (kL): The cutoff points for determining the ECW were based on the standards of the measuring device[25]; (5) Phase angle (PA) (Xc/R)[26]: The cutoff points for determining the PA were based on the standards provided by the measuring device[22,25]; (6) Grip strength (kg) was measured using a DHD-3 SAEHAN hand dynamometer. The cutoff points for the assessment of weak muscle strength were based on European studies[27]. Grip strength values of 26-32 kg in men and 16-20 kg in women were classified as moderately strong, while those < 26 kg and < 16 kg were classified as weak grip strength[27]; (7) Laboratory albumin test results: Value of 3.5-5.0 g/dL were considered normal albumin concentrations[28]; and (8) A questionnaire survey was conducted using the SGA scale for the subjective assessment of nutritional status[29].

All measurements were taken on each patient on the same day.

Calculations and statistical analyses

Average values obtained from the analyses and measurements were calculated for each group. One-way analysis of variance was used to determine the influence of disease stage on the examined nutritional status parameters. The hypotheses were verified using an F-test for analysis of variance. The significance level of = 0.05 was assumed. Fisher's Least Significant Difference test was used to identify homogeneous groups. Correlation analysis was performed to examine the relationship between the parameters that determine nutritional status and the degree of disease advancement. Statistical analysis was performed using the R statistical package. To obtain transparent conclusions regarding the most appropriate methods of assessing the nutritional status of patients with liver fibrosis, the results of our research were compared with those of other recent studies.

RESULTS

Tables 2 and 3 present the results of the anthropometric measurements of body weight, BMI, and arm circumference, assigned to groups according to the Child-Pugh classification. The results were not significantly different depending on the etiology of cirrhosis.

The Child-Pugh A group had the highest mean body weight (76.42 kg), highest mean BMI (26.72 kg/m^2), and largest mean arm circumference (27.64 cm). In the Child-Pugh B group, the mean scores of all examined variables were lower than those of the Child-Pugh A group, whereas the mean body weight and BMI of the Child-Pugh C group were higher than those of the Child-Pugh B group. The mean circumferences of the arm muscle were as follows: 27.64 cm (Child-Pugh A); 25.95 cm (Child-Pugh B); and 25.20 cm (Child-Pugh C).

Underweight was the most common condition in the Child-Pugh B group; this group had the highest proportion of patients with normal body weight. Overweight and obesity were predominant in the Child-Pugh A group.

Tables 4 and 5 show the results of the body composition analysis: FFMI (kg/m^2), MMI (kg/m^2), FMI (kg/m^2), ECW (L), and PA (Xc/R) (reactance/resistance) of patients assigned to groups according to the Child-Pugh classification.

The highest mean values of FFMI, MMI, FMI, and PA were achieved by patients in the Child-Pugh A group. The mean ECW increased for each subsequent group; however, no significant differences were observed among the groups.

In all, 13.4% of the Child-Pugh A group and 64.7% of the B group had FFMI scores below the cutoff point. In the C group, almost half of the participants had scores below the cutoff. Only one person (1.9%) in the A group had MMIs below the cutoff point. In the B group, 29.4%, and in the C group, 56.2% had MMIs below the cutoff point.

Further, 42.3% of the Child-Pugh A group, 52.9% of the B group, and 59.7% of the C group had FMIs below the cutoff point; 26.9% in Child-Pugh A group, 11.7% in the B group, and 12.5% in the C had FMIs that were exceedingly higher than the cutoff point.

The prevalence of high ECW (above the standard) increased with disease progression. In the Child-A group, half of the patients had above-standard ECW, whereas 70% and over 90% of the Child-B and Child-C groups had above-standard ECW.

Further, 73% of the Child-Pugh A group, 74.6% of the B group, and 100% of the C group had suboptimal PA.

The hand grip strengths of patients in the groups according to the Child-Pugh classification are presented in Figure 1.

In the Child-Pugh A group, patients aged over 70 years had weak or medium grip strength, as expected for this age group. In the B group, men aged 60-69 years had weak grip strength. In the C group, men aged 50-59 years and women aged over 70 years had weak grip strength. Overall, hand grip strength was lower in the B group than in the A group, while the C group had the lowest grip strength.

Honestly Significant Difference Tukey's Test shows the statistically significant differences between the three groups. Significant effects of the Child-Pugh group, gender, and age on handgrip strength have been proven.

Tables 6 and 7 show the albumin concentrations of the patients according to the Child-Pugh classification. The mean serum albumin concentration in the A group was within the reference range. The mean albumin concentration was below the reference range in the B group and significantly lower than the accepted norm in the C group.

Albumin concentrations were below the reference range in 7.6% of the A group. In the B group, 79.4% of the patients had reduced albumin levels. All the participants in the C group had concentrations below the reference range.

Table 8 presents the results of the SGA scale according to the Child-Pugh classification. In the A group, the majority of patients (78.8%) were properly nourished. In the B group, 41.1% of the respondents were properly nourished, 32.3% were at risk of malnutrition, and 26.4% were malnourished. In the C group, 18.7% were properly nourished, but the majority were at risk of malnutrition (37.5%) and malnourishment (43.7%).

The percentages of patients classified as malnourished in each Child-Pugh group, based on BMI, FFMI, MMI, FMI, ECW, albumin level, and SGA scale are presented in Figure 2.

We found that as the percentage of patients classified as malnourished increased with the advancement of the disease according to the Child-Pugh classification, the indicators deteriorated, except BMI and FFMI.

Correlations between the indicators of nutritional status are presented in Figure 3. There was a strong correlation between the Child-Pugh classification and SGA score; a very strong correlation between the Child-Pugh classification and arm circumference; a strong correlation between the Child-Pugh classification and body weight, albumin concentration, FFMI, MMI, PA, and BMI; and an average correlation between the Child-Pugh classification and FMI. The indicators decreased with disease progression.

Table 2 Mean values of anthropometric measurements in groups according to Child–Pugh classification (*n* = 118)

Parameter	A	B	C
	n = 52	*n* = 34	*n* = 32
Body weight (kg)	76.4[1]	64.1[1]	68.9[1]
BMI (kg/m²)	26.7[1]	21.9[1]	23.9[1]
Myocardial circumference (cm)	27.6[1]	25.9[1]	25.2[1]

[1]Mean values are significantly different at $P < 0.05$.
BMI: Body mass index.

Table 3 Nutritional status expressed by body mass index in relation to standards (*n* = 118)

BMI	A		B		C	
	n = 52	%	*n* = 34	%	*n* = 32	%
Underweight	2	3.8	8	24	3	9.3
Correct body weight	18	35	20	59	17	53
Overweight	21	40	6	18	11	35
Obesity	11	21	0	0	1	3.1

BMI: Body mass index.

Table 4 Mean measurement values of body composition components (*n* = 118) in groups according to Child–Pugh classification

Component	A	B	C
	n = 52	*n* = 34	*n* = 32
FFMI (kg/m²)	18.5[1]	16.6[1]	16.6[1]
MMI (kg/m²)	10.1[1]	8.8[1]	8.55[1]
FMI (kg/m²)	7.9[1]	5.25[1]	6.7[1]
ECW (l)	0.388[1]	0.396[1]	0.406[1]
PA (Xc/R)	5[1]	4.4[1]	3.9[1]

[1]Mean values are significantly different at $P < 0.05$.
FFMI: Fat free mass index; MMI: Muscle mass index; FMI: Fat mass index; ECW: Extracellular water; SGA: Subjective global assessment; PA: Phase angle.

DISCUSSION

The effect of liver fibrosis on the patients is reflected by nutritional status measurements. The percentage of underweight individuals, defined by BMI, which was interpreted in our study as possible malnutrition, was higher in the Child-Pugh C group (23.5%) than in the B group (9.3%). Similarly, among 56 patients with liver cirrhosis examined by Łapiński and Łapińska[30], malnutrition determined by BMI was found in 7% of the cohort. BMI assessment is important due to its proven relationship with mortality in patients with chronic liver failure[31]. However, this indicator may not be an appropriate tool for assessing the nutritional status of patients with advanced liver disease given the fact that the body composition is not taken into account, including possible edema and ascites, which are complications of the disease[32, 33]. Fluid retention affects the BMI, resulting in malnourished patients being classified as overweight.

In our cohort, the average circumference of the arm muscle, which reflects the amount of muscle tissue and indicates the risk of sarcopenia, decreased with disease progression. This indicator (in contrast to BMI) does not change with the occurrence of edema[34]. Similar results were obtained by Crisan *et al*[34], who examined the impact of dietary behavior and nutritional status on the outcomes of 101 hospitalized patients with cirrhosis. According to them, malnutrition examined by measuring the circumference of the arm muscle was predominant among the patients with organ decompensation as opposed to those without decompensation[34]. Moreover, in a study conducted by Gnanadeepam *et al* [35], who evaluated the level of weakness in the course of cirrhosis in 81 patients with organ decompensation, arm circumference was correlated with the level of weakness coexisting with the disease.

Table 5 Nutritional status expressed by body composition results in relation to standards (*n* = 118)

Component		A		B		C	
		n = 52	%	*n* = 34	%	*n* = 32	%
FFMI (kg/m²)	Below standard	7	13.4	22	64.7	15	46.8
	Standard	45	86.5	12	35.2	17	53.1
MMI (kg/m²)	Below standard	1	1.9	10	29.4	18	56.2
	Standard	51	98	24	70.5	14	43.7
FMI (kg/m²)	Below standard	22	42.3	18	52.9	19	59.7
	Standard	16	30.7	12	35.2	9	28.1
	Above standard	14	26.9	4	11.7	4	12.5
ECW (L)	Normal	26	50	10	29.4	3	9.3
	Above standard	20	38.4	14	41.1	7	21.8
	Much above standard	6	11.5	10	29.4	22	68.7
PA (Xc/R)	Below standard	38	73	27	74.6	32	100
	Standard	14	26.9	7	20.5	0	0

FFMI: Fat free mass index; MMI: Muscle mass index; FMI: Fat mass index; ECW: Extracellular water; SGA: Subjective global assessment; PA: Phase angle.

Table 6 Mean serum albumin values according to Child–Pugh classification (*n* = 118)

Factor	A	B	C
Albumin concentration (g/dL)	4.181	3.281	2.71[1]

[1]Mean values are significantly different at $P < 0.05$.

Table 7 Nutritional status expressed by the obtained results of albumin concentration in relation to the standards (*n* = 118)

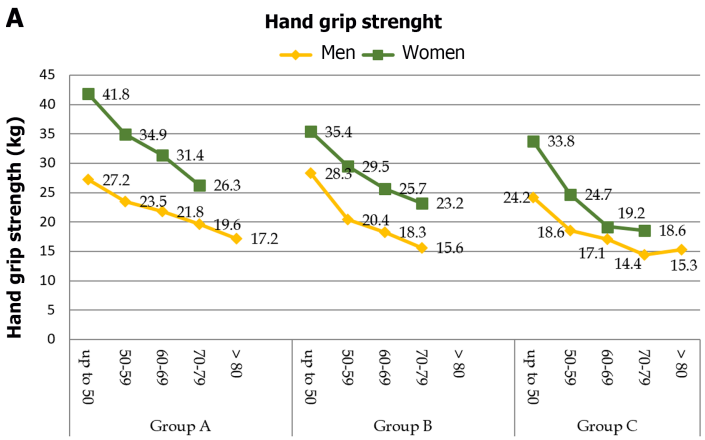
Albumin concentration (g/dL)	A		B		C	
	n	%	*n*	%	*n*	%
Below standard	4	7.6	27	79	32	100
Standard	48	92.3	7	21	0	0

Table 8 Nutritional status expressed by the results of the subjective global assessment scale (*n* = 118)

SGA	A		B		C	
	n = 52	%	*n* = 34	%	*n* = 32	%
Proper nutritional status	41	78.8	14	41.1	6	18.7
Risk of malnutrition	8	15.3	11	32.3	12	37.5
Malnutrition	3	5.7	9	26.4	14	43.7

SGA: Subjective global assessment.

Body composition data indicated a decrease in the average FFMI, skeletal MMI, and PA, with an increase in disease severity and a simultaneous increase in the average ECW. Considering the coexistence of liver diseases with disturbances in the balance between the amount of intracellular and extracellular water and the tendency of water to accumulate outside the cells, the ECW index, without considering water retention in the body, can lead to incorrect estimations of the nutritional status of the patients[36]. In patients with liver diseases, when interpreting the results of body composition analysis, it is advantageous to use the PA, which indicates the ratio of the water level inside the cells to that in the



B

HSD Tukey's test of differences

HSD Tukey's teat		
Group	Hand grip strength mean	*P* value
C	21.44	$P < 0.05$
B	24.55	$P < 0.05$
A	28.01	$P < 0.05$

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Figure 1 Arithmetic mean of the measurement of hand grip strength expressed in kilograms, considering the Child-Pugh classification, age, and sex of the patients. Honestly Significant Difference Tukey's Test of differences (n = 118). A: Arithmetic mean of the measurement of hand grip strength; B: Tukey's Test of differences. HSD: Honestly Significant Difference.

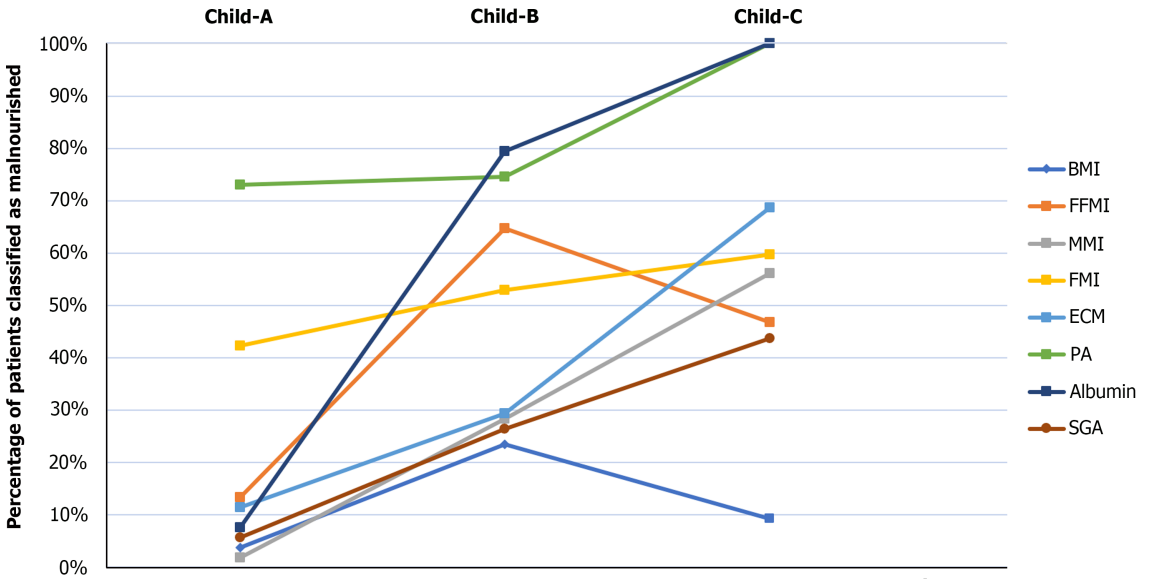

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Figure 2 Percentage of patients classified as malnourished in Child-Pugh groups, based on body mass index, fat free mass index, muscle mass index, fat mass index, extracellular water, albumin concentration, and subjective global assessment score. BMI: Body mass index; FFMI: Fat free mass index; MMI: Muscle mass index; FMI: Fat mass index; ECW: Extracellular water; PA: Phase angle; SGA: Subjective global assessment.

extracellular spaces[37]. Luengpradidgun *et al*[38] also noted that among 30 patients in their study group with cirrhosis and sarcopenia, measurements based on electrical bioimpedance revealed that only six patients had co-occurring malnutrition. These data indicate the need for caution when assessing the nutritional status of this patient group.

The average hand grip strength in the study groups decreased with disease progression. This result was expected because progressive sarcopenia accompanies chronic liver inflammation that eventually leads to fibrosis[38]. Similar results were obtained by Nishikawa *et al*[39], who examined 241 patients with chronic liver disease and classified them according to Child-Pugh scores into groups A and B. Lower grip strength was observed in group B than in group A.

The blood test results indicated a decrease in the mean concentration of serum albumin levels with disease progression, as expected. The deterioration of the organs results in decreased albumin production. Previous studies have indicated the need for including albumin therapy in the treatment plan of patients with advanced liver fibrosis and cirrhosis[40,41].

We used the standardized SGA scale for assessing the nutritional status of the patients; the SGA scale is commonly used in the assessment of patients with liver diseases at risk of malnutrition[12,42]. Aldana Ledesma *et al*[42] compared various tools for assessing malnutrition and sarcopenia in patients with cirrhosis and observed the highest percentage of properly nourished patients in the Child-Pugh A and B groups. This finding is similar to the results of the present study.

When assessing the nutritional status of patients with advanced liver fibrosis, it is necessary to consider their overall condition. The correct assessment method should consider factors such as low protein synthesis, extracellular water accumulation, edema formation, and loss of muscle mass. Furthermore, the extent of hepatic fibrosis, as a result of

	Child	Body mass	SGA	Albumin	FFMI	MMI	FMI	ECW	PA	BMI	Arm muscle circumferene
Child		-0.68	1.00	-0.61	-0.68	-0.62	-0.46	n.s	-0.67	-0.65	-0.87
Body mass	-0.68		-0.71	0.55	0.77	0.66	0.85	n.s	0.58	0.99	0.79
SGA	1.00	-0.71		-0.57	-0.66	-0.59	-0.45	n.s	-0.63	-0.67	-0.85
Albumin	-0.61	0.55	-0.57		0.95	0.99	0.80	0.63	0.96	0.64	0.88
FFMI	-0.68	0.77	-0.66	0.95		0.99	0.92	0.55	0.93	0.84	0.93
MMI	-0.62	0.66	-0.59	0,99	0.99		0.88	0.61	0.96	0.75	0.91
FMI	-0.46	0.85	-0.45	0.80	0.92	0.88		0.52	0.76	0.91	0.78
ECW	n.s	n.s	n.s	0.63	0.55	0.61	0.52		0.57	0.27	0.38
PA	-0.67	0.58	-0.63	0.96	0.93	0.96	0.76	0.57		0.67	0.93
BMI	-0.65	0.99	-0.67	0.64	0.84	0.75	0.91	0.27	0.67		0.82
Arm muscle circumference	-0.87	0.79	-0.85	0,88	0.93	0.91	0.78	0.38	0.93	0.82	

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Figure 3 Correlations between the analyzed indicators of nutritional status. No correlation coefficient means no significant relationship between the tested parameters ($P < 0.05$). SGA: Subjective global assessment; FFMI: Fat free mass index; MMI: Muscle mass index; FMI: Fat mass index; ECW: Extracellular water; PA: Phase angle; BMI: Body mass index.

chronic inflammation, is reflected by the nutritional status of the patients, where the greater the level of organ deterioration, the greater the decline in the nutritional status.

The strengths of our study are the extensive study methods of malnutrition measurement and statistical analysis, which allows choosing the most appropriate measurement tool in the future. Our study also has certain limitations. This study had a relatively small sample size from one medical facility; therefore, further studies with larger sample sizes are recommended to validate our findings.

CONCLUSION

Advanced liver fibrosis leads to a reduction in various nutritional status parameters. Malnutrition among patients worsens with the progression of liver fibrosis, and the level of deterioration of this organ is indicated by the Child-Pugh scores.

Owing to its multifactorial etiology and numerous related complications, the identification of malnutrition in the course of chronic liver diseases is difficult. Therefore, a comprehensive assessment method involving a combination of available clinical, anthropometric, and biochemical methods is required for this patient population. We found that serum albumin concentration, arm circumference, lean body mass, skeletal muscle mass, phase angle, hand grip strength, and SGA score were useful parameters for assessing the nutri-tional status of patients with liver cirrhosis.

ARTICLE HIGHLIGHTS

Research background

Chronic liver disease with advanced fibrosis and cirrhosis may be accompanied by progressive malnutrition. Early diagnosis of malnutrition in patients with advanced stages of chronic liver disease and implementation of appropriate nutritional treatment for malnourished patients should be an integral part of the therapeutic process.

Research motivation

Among the numerous methods of assessing nutritional status, it is important to determine which one is the most appropriate for patients with liver fibrosis given the specific complications of progressive organ failure.

Research objectives

The aim was to assess the nutritional status of patients diagnosed with advanced liver fibrosis at the cirrhosis stage using various methods of nutritional status assessment. We tried to find out which methods of assessing nutritional status are the most appropriate for patients with advanced liver fibrosis.

Research methods

The study group contained 88 patients with advanced liver fibrosis. Patients were classified into three groups according to the Child-Pugh scale. The nutritional status was assessed using many methods: Electrical bioimpedance method, albumin concentration, mid-armmuscle circumference, body mass index (BMI), subjective global assessment (SGA) of nutritional status scale, and hand grip strength. To draw conclusions, proper statistical analyzes were performed.

Research results

There was a strong correlation between the Child-Pugh classification and SGA score; a very strong correlation between the Child-Pugh classification and arm circumference; a strong correlation between the Child-Pugh classification and body weight, albumin concentration, fat-free mass index, muscle mass index, phase angle, and BMI; and an average correlation between the Child-Pugh classification and fat mass index. The indicators decreased with disease progression.

Research conclusions

Malnutrition among patients worsens with the progression of liver fibrosis, and the level of deterioration of this organ is indicated by the Child-Pugh scores. We found that serum albumin concentration, arm circumference, lean body mass, skeletal muscle mass, phase angle, hand grip strength, and SGA score were useful parameters for assessing the nutritional status of patients with liver cirrhosis.

Research perspectives

Another important step in the study of the nutritional status of patients with advanced liver fibrosis seems to be the analysis of patients' diets to prepare individualized recommendations, adequate to progressive malnutrition.

FOOTNOTES

Author contributions: Janota B designed research and wrote the paper; Krupowicz A collected data and wrote the paper; Noras K performed statistical analysis; Janczewska E designed research and supervised the paper.

Institutional review board statement: The study was reviewed and approved by the Hospital Review Board. Planning, conduct, and reporting of the study were in line with the tenets outlined in the Declaration of Helsinki.

Informed consent statement: All study participants provided informed written consent about personal and medical data collection prior to study enrolment.

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SYSTEMATIC REVIEWS

Associations between irritable bowel syndrome and non-alcoholic fatty liver disease: A systematic review

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Abstract

BACKGROUND

Irritable bowel syndrome (IBS) is associated with obesity and metabolic syndrome. IBS and non-alcoholic fatty liver disease (NAFLD) are highly prevalent entities worldwide and may share similar mechanisms including gut dysbiosis, impaired intestinal mucosal barrier and immune system activation.

AIM

To systematically review their association according to the Preferred Reporting Items for Systemic Review and Meta-analyses guidelines.

METHODS

PubMed, EMBASE and Cochrane Database of Systematic Reviews were searched for relevant papers. Manual searches were also performed.

RESULTS

Six studies were included. Both IBS and NAFLD subjects had significantly more metabolic risk factors like hypertension, obesity, dyslipidaemia and diabetes. Our review showed that 23.2% to 29.4% of NAFLD patients had IBS. IBS was significantly higher in NAFLD patients compared with patients without NAFLD (23.2% *vs* 12.5%, $P < 0.01$). A higher proportion of IBS patients had NAFLD (65.8% to 74.0%). IBS patients were three times more likely to have NAFLD compared with non-IBS patients ($P < 0.001$). Two studies showed a significant correlation bet-

ween the severity of IBS and NAFLD. The proportion of NAFLD subjects with IBS increased with NAFLD severity.

CONCLUSION
Further prospective studies are warranted to evaluate the relationship and shared pathways between IBS and NAFLD, potentially leading to the development of future therapeutics.

Key Words: Irritable bowel syndrome; Functional gastrointestinal disorder; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Gut dysbiosis; Metabolic syndrome

Core Tip: The relationship between irritable bowel syndrome (IBS) and non-alcoholic fatty liver disease (NAFLD) is increasingly recognised but their shared mechanisms remain poorly elucidated. We evaluate the association between IBS and NAFLD and discuss the risk factors and possible common mechanistic pathways including the brain-gut-liver axis, gut dysbiosis and translocation, altered hypothalamic-pituitary-adrenal axis and sleep quality.

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INTRODUCTION

The global surge in obesity has heralded the increasing prevalence of non-alcoholic fatty liver disease (NAFLD), which is the hepatic manifestation of the metabolic syndrome. NAFLD has emerged as the most common chronic liver disease and is increasingly recognised as a leading cause of morbidity and mortality[1]. It affects up to 25%-30% of the general population but is highly prevalent (up to 50%-90%) in patients with obesity and features of the metabolic syndrome[1,2]. Irritable bowel syndrome (IBS) is a disorder of gut-brain interaction, characterised by chronic abdominal pain and altered bowel movements without an organic cause[3]. Given the degree of heterogeneity in criteria used for the diagnosis of IBS, the true global prevalence of IBS remains elusive but is estimated to affect 1 in 10 people globally[4,5]. IBS is a complex condition that is caused by a myriad of factors with interplay of genetics, epigenetics, immune activation, gut dysbiosis, abnormal gut-brain interactions, visceral hypersensitivity, altered gut motility, eating behaviours, psychological stressors, and environmental factors[6,7]. To date, studies have found an interesting relationship between obesity and IBS. As NAFLD is closely linked with obesity, insulin resistance and the metabolic risk factors, emerging evidence has shown a possible correlation between NAFLD and IBS due to purported shared underlying pathophysiological links[8].

IBS is significantly associated with a higher prevalence of the metabolic syndrome and a multitude of studies have determined a link and common pathogenic mechanisms between these two conditions. Obesity and metabolic syndrome are found more frequently in IBS patients compared with controls[9]. A cross-sectional study from Japan showed a positive association between IBS and increased prevalence of metabolic syndrome and triglyceride levels. The odds ratio (OR) (95%CI) in IBS subjects were 2.01 (1.13-3.55) and 1.50 (1.03-2.18) respectively as compared with non-IBS subjects[10]. There is a higher occurrence of pre-diabetes and higher low-density lipoprotein (LDL) levels in patients with IBS[11,12]. In a large population-based cohort study, increased bowel movement frequency was associated with elevated risks of cardiovascular disease, diabetes, heart failure and chronic kidney disease[13]. In IBS subjects, an elevated body mass index (BMI) is associated with significantly faster colonic and rectosigmoid transit and higher bowel frequency[14].

Obesity and the metabolic syndrome are linked with insulin resistance, oxidative stress, chronic low-grade inflammation, abnormal lipid metabolism and gut microbiota alterations, all of which play key roles in the pathogenesis of NAFLD and the more progressive non-alcoholic steatohepatitis (NASH)[15,16]. The gut-liver axis is implicated in the development of NAFLD and similar mechanisms have also been shown to be pivotal to the pathogenesis of IBS[17,18]. Several studies have highlighted the correlation between NAFLD and IBS. Lee *et al*[19] demonstrated an increased prevalence of elevated alanine aminotransferase and gamma-glutamyl transferase levels and metabolic syndrome in IBS patients[19].

In this systematic review, we aim to evaluate the association between IBS and NAFLD including the common mechanistic pathways and overlapping risk factors.

MATERIALS AND METHODS

Search strategy and screening

The Preferred Reporting Items for Systemic Review and Meta-analyses (PRISMA) guidelines were used for the purposes

of this systematic review. A two-step approach was adopted to identify studies: (1) A systematic search of electronic databases; and (2) A manual search of direct citations from potentially relevant papers and other peer-reviewed journals.

The following electronic libraries-PubMed, Embase and the Cochrane Database of Systemic Reviews were searched from inception to March 2023 to identify papers studying the associations between IBS and NAFLD. The uses of search strategies were described in Table 1.

Manual searches of direct citations from potentially relevant papers and other peer-reviewed journals were also performed to identify additional studies not included in the systemic search. No filters were used to refine search results. No linguistic or geographical restrictions were imposed.

The titles of all papers retrieved by the literature search were screened for relevance and any studies of obvious irrelevance were excluded. All full manuscripts of relevant papers were then retrieved and subsequently screened in its entirety. The entire screening process was done by two independent researchers to identify studies that met the study selection criteria. Consensus was reached regarding any discrepancies that arose without the need for a third independent reviewer.

Eligibility criteria

Studies that met the following inclusion criteria were included: (1) Observational studies that investigated the relevance between IBS and NAFLD that included cross-sectional, case-control or cohort studies; (2) Population studies that showed the relevance between IBS and NAFLD; and (3) Human studies. Due to predictions of limited number of studies regarding the topic of interest, a planned option of expansion of criteria was put in place to include studies published in the Chinese language, of which all of the authors are also proficient in.

Data extraction

The data extraction process was done independently as per the PRISMA checklist. Publication details were first extracted, which included: (1) Author's name; (2) Year and Country of study; and (3) Methodology (sample characteristics, study design, and modalities used to diagnose IBS and NAFLD). Further data extraction included any risk factors that predisposed patients to the incidence of IBS and NAFLD if present that included risk estimate, including OR with corresponding 95%CIs about the association between IBS and NAFLD.

Risk of bias assessment

All eligible studies were assessed for risk of bias using Cochrane Risk of Bias Assessment (Figure 1).

Search results

The search from PubMed, Embase and Cochrane identified 509 studies along with 2 studies *via* manual search. 441 studies were screened for after the removal of 70 duplicates. 428 studies were excluded and 13 studies were retrieved for and screened in full. Among these 13 studies, 7 studies were excluded for reasons that included (1) Review articles (2) No full papers available; and (3) Non-English or non-Chinese language (Figure 2). The final 6 studies were included in this systematic review (Table 2).

RESULTS

The studies that were included demonstrated associations between NAFLD and IBS. The criteria used to identify IBS patients included ROME III criteria (Ke *et al*[20], Hasanian *et al*[21], Zheng *et al*[22]) and ROME IV criteria (Franco *et al*[23]), whilst Wu *et al*[24] selected IBS patients according to international classification of diseases (ICD)-10 coding[20-24]. Singh *et al*[25], Hasanian *et al*[21], Ke *et al*[20], and Zheng *et al*[22] used ultrasonography for the diagnosis of NAFLD whereas the fatty liver index (FLI) was used by Wu *et al*[24] to assess for NAFLD[20-22,24,25]. Franco *et al*[23] recruited patients with NAFLD based on ICD-10 codes[23]. The mode of diagnosis of IBS was not stated by Singh *et al*[25].

All the included studies demonstrate overlap between IBS and NAFLD, either *via* a substantial proportion of sample population having both IBS and NAFLD, or *via* meta-analysis that showed increased risk of also having IBS or NAFLD should either condition be present. Two studies reported that patients with IBS had increased risk of metabolic syndrome, including higher BMI, dyslipidemia and diabetes mellitus[20,21]. One study demonstrated the same for the IBS and NAFLD overlap group compared to the group that had either IBS or NAFLD[22].

NAFLD in IBS patients

The 100 IBS patients were included in the study by Hasanian *et al*[21] according to the ROME III diagnostic criteria[21]. BMI and waist circumference were obtained. Laboratory investigations included fasting serum glucose levels, lipid profile, liver chemistry profile, international normalized ratio and a complete blood count. NAFLD was diagnosed in 74% of the study population using abdominal ultrasonography. Furthermore, it was noted that a higher prevalence of moderate/severe NAFLD were found among patients with moderate/severe IBS compared to mild IBS (22.4% *vs* 4.8%). This could signify a potential association between higher grades of NAFLD with more severe IBS. Multi-variate analysis affirmed the association of moderate/severe NAFLD with moderate/severe IBS which was independent of other risk factors of IBS (OR: 2.4, 95%CI: 1.3-62.7, P = 0.026). Metabolic syndrome was also found to be independently associated with moderate/severe IBS (OR: 3.1, 95%CI: 1.8-54.6, P = 0.011). The study observed that the most frequent metabolic parameter in IBS patients was high BMI (89%)[21].

Table 1 Search terms used

Database	
PubMed	(Irritable bowel syndrome[Mesh] OR "Irritable bowel syndrome"[tiab] OR "irritable colon*"[tiab] OR IBS[tiab]) AND (Fatty Liver[Mesh] OR (fatty[tiab] AND (liver*[tiab] OR hepat*[tiab]) OR steatohepat*[tiab] OR NAFL*[tiab] OR NASH*[tiab]))
EMBASE	('irritable colon'/exp OR 'irritable bowel syndrome':ab,ti OR 'irritable colon':ab,ti OR 'IBS':ab,ti) AND ('Fatty Liver'/exp OR (fatty:ab,ti AND (liver*:ab,ti OR hepat*:ab,ti) OR steatohepat*:ab,ti OR NAFL*:ab,ti OR NASH*:ab,ti))
CENTRAL	#1 MeSH descriptor: [Irritable Bowel Syndrome] explode all trees
	#2 ('irritable bowel syndrome' OR 'irritable colon' OR 'IBS'):ti,ab,kw
	#3 MeSH descriptor: [Fatty Liver] explode all trees
	#4 fatty:ab,ti AND (liver*:ab,ti OR hepat*:ab,ti) OR steatohepat*:ab,ti OR NAFL*:ab,ti OR NASH*:ab,ti
	#5 (#1 or #2) and (#3 or #4)

CENTRAL: Cochrane Central Register of Controlled Trials.

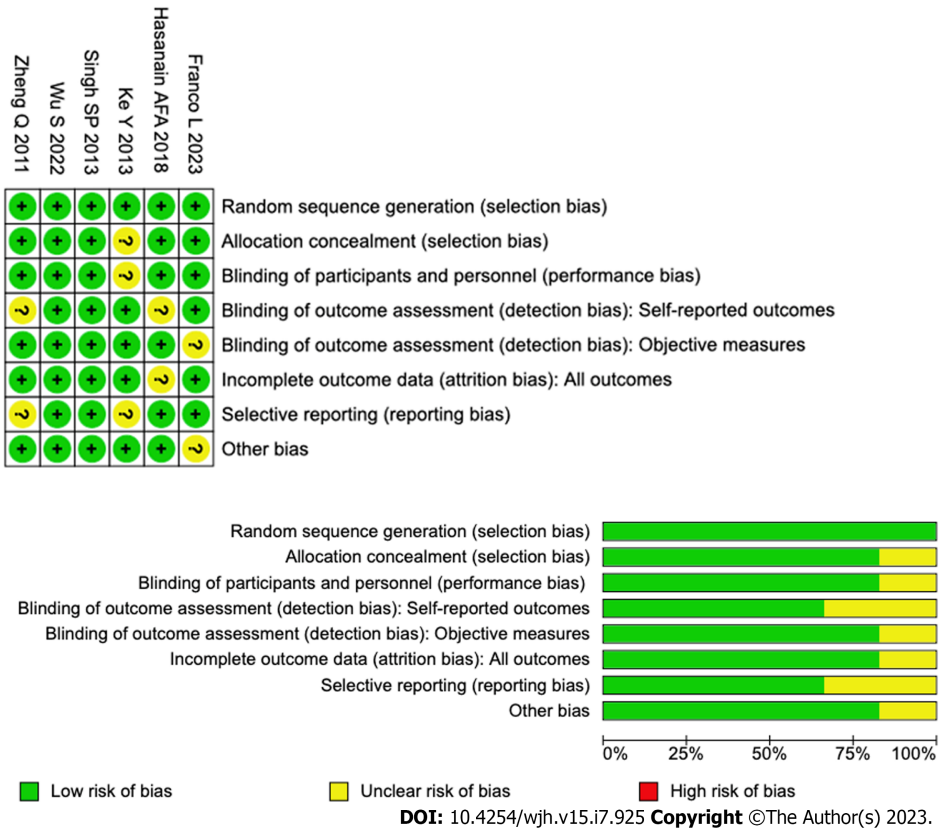

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Figure 1 Cochrane risk of bias assessment.

IBS in NAFLD patients

Ke *et al*[20] aimed to detect the prevalence of IBS in NAFLD patients as well as normal patients. Patients who underwent health examination in Urumqi, China were randomised into 2 groups: NAFLD and normal controls. IBS was diagnosed using the ROME III criteria. NAFLD was diagnosed using ultrasound based on the 2006 Revised Diagnostic criteria by Fatty Liver and Alcoholic Liver Disease Group of Chinese Medical association Hepatology Branch. 65.8% of adults with IBS-like symptoms had NAFLD while the detection rate of IBS was higher in NAFLD patients compared to normal (23.2% *vs* 12.5%, $P < 0.01$), suggesting an association between NAFLD and IBS-like symptoms. NAFLD patients were subsequently subdivided based on severity into: (1) Mild: 212; (2) moderate: 188; and (3) severe: 48. The prevalence of IBS in NAFLD patients increased with the severity of NAFLD as noted by the IBS detection rate for the groups being 11.3%, 27.7% and 58.3% respectively. Further analysis showed that more IBS symptoms were experienced with increasing severity of NAFLD. Multivariate logistic regression analysis demonstrated that IBS-like symptoms were closely related to ethnicity (OR: 0.316, 95%CI: 0.134-0.745, $P = 0.008$), fatty liver (OR: 0.525; 95%CI: 0.278-0.991, $P = 0.047$), BMI (OR: 0.918; 95%CI: 0.844-1.000, $P = 0.049$) and triglyceride levels (OR: 0.855; 95%CI: 0.739-0.988; $P = 0.034$)[20].

Table 2 Summary of study findings

Ref.	Location	Sample characteristics	Study design	IBS diagnosis	NAFLD diagnosis	IBS and NAFLD overlap	Associated risk factors
Ke *et al* [20], 2013	China	No. of patients: 945. NAFLD population: 470 (226 males, 222 females). Total without NAFLD: 475 (198 males, 234 females). Note: DM was in exclusion criteria	Cross-sectional study	ROME III	Ultrasound; Chinese Society of Hepatology, Chinese Medical Association diagnosis criteria for NAFLD	IBS incidence: 104 (23.2%) of NAFLD patients *vs* 54 (12.5%) of patients without NAFLD. NAFLD incidence in patients with IBS symptoms: 65.8% of patients with IBS-like symptoms had NAFLD. Higher detection rate of IBS-like symptoms with more severe NAFLD (P < 0.05). Mild NAFLD (Group A): 24 out of 212 (11.3%). Moderate NAFLD (Group B): 52 out of 188 (27.7%). Severe NAFLD (Group C): 28 out of 48 (58.3%)	Risk factors for IBS-like symptoms: BMI (P = 0.049). Triglycerides (P = 0.034). Fatty liver (P = 0.047). Ethnicity-Han, Uygur (P = 0.008)
Hasanian *et al*[21], 2018	Egypt	100 consecutive patients diagnosed with IBS (49 males, 51 females): 45% IBS-C 23% IBS-D 32% IBS-M. Further divided into: Mild IBS (n = 42); Moderate IBS (n = 43); Severe IBS (n = 15)	Cross-sectional study	ROME III	Ultrasound	74% of IBS patients had NAFLD. Moderate/severe NAFLD significantly associated with moderate/severe IBS. 22.4% moderate/severe IBS patients (95%CI: 15.8%-31.5%) *vs* 4.8% mild IBS patients: (95%CI: 2.7%-7.9%) (P = 0.001)	Predictors of moderate/severe IBS: NAFLD (P = 0.026); Metabolic Syndrome (P = 0.011)
Zheng *et al*[22], 2011	China	No. of patients: 200 (89 males, 111 females) (1) Both NAFLD and IBS: 25; (2) Either NAFLD or IBS: 36; and (3) Neither IBS nor NAFLD: 139	Cross-sectional study	ROME III	Ultrasound; NAFLD diagnosis based on Chinese Society of Hepatology, Chinese Medical Association	25 subjects (12.5%) had both NAFLD and IBS	NAFLD and IBS overlap group compared to group without NAFLD and IBS Overlap had higher: BMI (P = 0.045); TG (P = 0.035); TC (P = 0.038); HDL-C (P = 0.045); LDL-C (P = 0.031); FBG (P = 0.023). NAFLD and IBS overlap group compared to the either NAFLD or IBS group had higher: Hypertension (P = 0.041); Obesity (P = 0.034); Dyslipidemia (P = 0.020); Diabetes (P = 0.037); Digestive system diseases (P = 0.037). GIT sensory threshold: NAFLD and IBS overlap group had lower threshold of the following compared to either NAFLD or IBS group: FSV (P = 0.034); DSV (P = 0.032); MTV (P = 0.035); PSV (P = 0.027)
Franco *et al*[23], 2022	United States	No. of patients: 130 patients with NAFLD (49 males, 81 females)	Cross-sectional study	ROME IV	ICD-10 Code	38 (29.2%) patients with NAFLD fulfilled Rome IV IBS criteria	Increased prevalence of depression (18.4% *vs* 5.4%, P = 0.01) and anxiety (31.6% *vs* 9.8%, P = 0.002) in NAFLD patients with IBS compared to those without IBS. Independent predictors of IBS in NAFLD: Female gender (OR: 5.69, P = 0.001); Depression (OR: 1.23, P < 0.001); BMI (OR: 0.90, P = 0.02)
Wu *et al* [24], 2022	United Kingdom	No. of patients: 396838 (189759 males, 207079 females). NAFLD population: 153203	Prospective cohort study; United Kingdom Biobank	ICD-10 Code	Fatty Liver Index	7129 cases of incident IBS in NAFLD patients (cumulative incidence rate of 1.49 (95%CI: 1.46–1.53) per 1000 person-years). NAFLD patients showed a 13% higher risk of developing IBS (HR = 1.13, 1.05-1.17). Increased risk of IBS in higher FLI quartile	Subgroup analysis showed increased IBS risk for: Age (P < 0.003); Gender (Female) (P < 0.001)

Singh *et al*[25], 2013	India	No. of patients: 632 patients with incidentally detected NAFLD (484 males, 148 females)	Retrospective analysis	Not documented	Ultrasound diagnosis and grading of liver steatosis by two radiologists in a blinded study	186 (29.4%) out of 632 NAFLD patients had IBS	-

IBS: Irritable bowel syndrome; NAFLD: Non-alcoholic fatty liver disease; BMI: Body mass index; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; FBG: Fasting blood glucose; FSV: First sensation volume; DSV: Defaecating sensation volume; MTV: Maximum tolerable volume; PSV: Painful sensation volume; ICD-10: International classification of diseases-10.

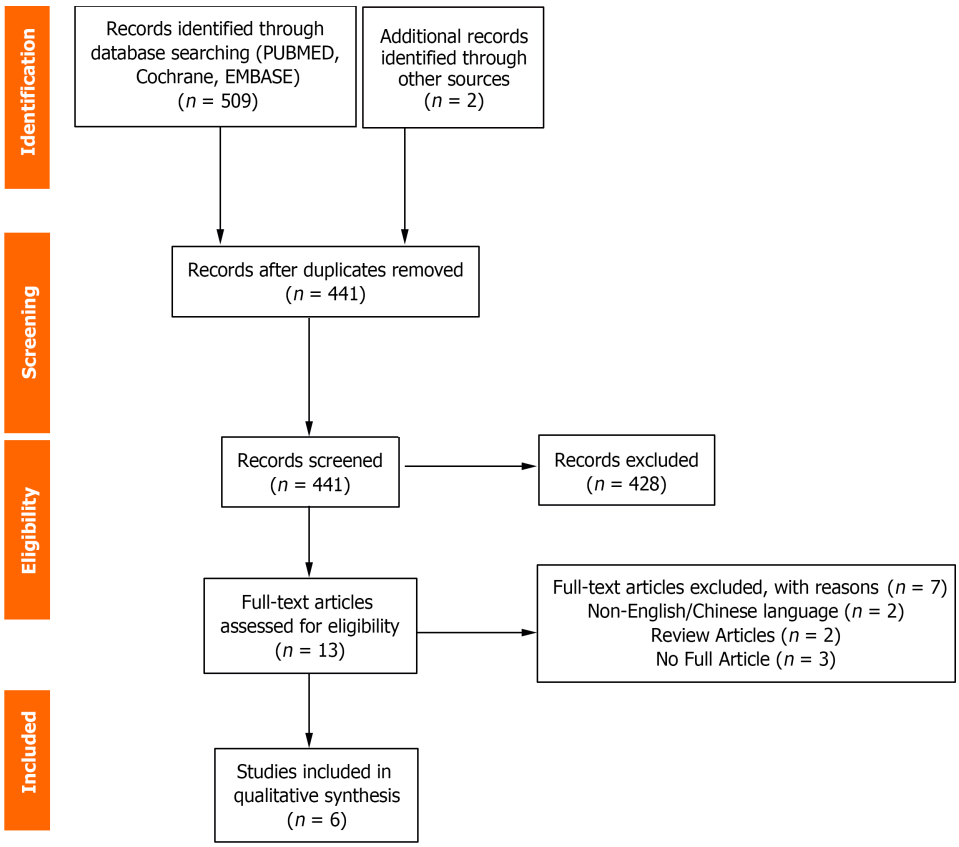

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Figure 2 Preferred Reporting Items for Systemic Review and Meta-analyses flowchart.

A cross-sectional study by Franco *et al*[23] included 130 NAFLD patients of which up to 29.2% of patients had co-existing IBS according to ROME IV criteria[23]. A higher prevalence of depression (18.4%) and anxiety (31.6%) was detected using the Hospital Anxiety Depression Scale in NAFLD patients with IBS compared to those without IBS (5.4% and 9.8%) respectively. Female gender (OR: 5.69, 95%CI: 2.01-16.12, $P = 0.001$) and depression (OR: 1.23, 95%CI: 1.10-1.38, $P < 0.001$) were independent risk factors for IBS[23].

A recent prospective cohort study followed 153203 patients diagnosed with NAFLD using FLI over 12.4 years[24]. IBS patients were determined *via* ICD-10 codes and diagnosis was based on self-report, primary care and hospital admission data. 7129 cases of incident IBS were detected with a cumulative incidence rate of 1.49 (95%CI: 1.46-1.53) per 1000 person-years. NAFLD patients showed a 13% higher risk of developing IBS (HR = 1.13, 1.05-1.17) compared with non-NAFLD patients. The highest FLI quartile was associated with a significant increase in risk of IBS compared to the lowest FLI quartile (HR q4 *vs* q1 = 1.21, 1.13-1.30, $P < 0.001$). This positive association between NAFLD and IBS was also observed by per SD change of FLI (adjusted HR = 1.08, 1.05-1.10) and predominantly in females[24].

Singh *et al*[25] showed that a proportion of NAFLD patients initially presented with IBS, though this was not elaborated upon. Out of 16225 patients with various gastrointestinal complaints, 632 patients with NAFLD were included. These patients attended the clinic not because of NAFLD but rather for a variety of gastrointestinal symptoms and the initial reason for evaluation for 29.4% (186) of these individuals with NAFLD was IBS[25].

Patients with both IBS and NAFLD

Zheng *et al*[22] grouped patients into those with IBS and NAFLD overlap and those without[22]. 200 subjects were recruited and split into 3 groups: (1) Both IBS and NAFLD; (2) either IBS or NAFLD; and (3) neither NAFLD nor IBS. IBS was diagnosed according to the ROME III criteria while NAFLD was diagnosed based on the Chinese Society of Liver Diseases on NAFLD. Out of 200 subjects, 25 (12.5%) had both IBS and NAFLD while 36 (18%) had either NAFLD or IBS. The rest of the 139 (69.5%) subjects had neither IBS nor NAFLD. Results suggested that the combined effect of IBS and NAFLD can implicate the metabolic parameters of patients. The IBS and NAFLD overlap group had a significant higherly incidence of hypertension (8% *vs* 5.56% $P = 0.041$), obesity (12% *vs* 5.56%, $P = 0.034$), dyslipidemia (12% *vs* 2.78%, $P = 0.020$), diabetes (4.0% *vs* 2.78%, $P = 0.037$) and digestive illnesses (24.0% *vs* 11.11%, $P = 0.037$) compared to the group with either IBS or NAFLD. The extent of dyslipidemia was greater in the IBS and NAFLD overlap group compared to the group with either IBS or NAFLD as higher triglycerides (2.34 ± 1.22 *vs* 1.71 ± 0.98, $P = 0.035$), total cholesterol (5.78 ± 1.57 *vs* 4.99 ± 1.06, $P = 0.038$), LDL cholesterol (LDL-C) (3.34 ± 1.12 *vs* 2.90 ± 0.99, $P = 0.023$) were noted. The IBS and NAFLD overlap group had a higher BMI (26.30 ± 3.03 *vs* 25.12 ± 2.59, $P = 0.045$) and higher fasting blood glucose levels (6.02 ± 1.01 *vs* 5.11 ± 0.97, $P = 0.023$)[22].

Anorectal manometry assessment was also performed which suggested that when both IBS and NAFLD were present the multiple risk factors from both conditions had a synergistic effect in irritation of the gastrointestinal tract. There was decreased gastrointestinal volume sensory threshold for patients in the IBS and NAFLD overlap group in comparison with the group that had either IBS or NAFLD as indicated by the decreased first sensation volume (22.56 ± 6.04 *vs* 30.27 ± 5.38, $P = 0.034$), defaecating sensation volume (63.22 ± 5.29 *vs* 78.34 ± 6.41, $P = 0.032$), maximum tolerable volume (82.39 ± 7.45 *vs* 131.78 ± 23.22, $P = 0.035$) and painful sensation volume (132.56 ± 19.29 *vs* 228.32 ± 17.36, $P = 0.027$) in the IBS and NAFLD overlap group[22].

DISCUSSION

The results overall support the possible association between IBS and NAFLD. NAFLD was more prevalent in patients with IBS compared to those without IBS. Individuals with both IBS and NAFLD overlap had more metabolic risk factors including high BMI, hypertension, dyslipidemia, and diabetes. The proportion of NAFLD patients with IBS increased along with the severity. IBS patients were three times more likely to have NAFLD compared with non-IBS patients ($P < 0.001$) with a significant correlation between the severity of IBS and NAFLD.

IBS and NAFLD have been postulated to share similar characteristics which are further discussed (Figure 3).

The brain-gut-liver axis and microbiome

The bidirectional relationship between the gut and the liver is well-established. The gut-liver axis involving gut dysbiosis, intestinal barrier dysfunction, intestinal dysmotility plays a vital role in the pathogenesis of NAFLD[18,26]. Changes in the intestinal microbiota is associated with severity of hepatic fat deposition through several mechanisms: Increasing low-grade mucosal inflammation, immune system activation, altering intestinal permeability, bile acid metabolism, dietary choline metabolism, and generating endogenous ethanol[27,28]. The brain-gut axis likewise shares a bidirectional relationship and similar changes in the brain-gut axis are implicated in the pathogenesis of IBS. The hypothalamic-pituitary-adrenal (HPA) axis and serotonin (5-HT) signalling are some of the pathways affected by dysregulation in the brain-gut axis[28,29]. These alterations lead to abnormal gut motility and visceral hypersensitivity[30]. Emerging evidence suggests a link between NAFLD and IBS symptoms such as diarrhoea. Population-based data from the National Health and Nutrition Examination Surveys revealed that NAFLD and diabetes were independently associated with diarrhoea as opposed to constipation or normal bowel patterns even after adjusting for BMI[31]. This is consistent with our results showing a correlation between IBS and NAFLD with the common mechanism of gut dysbiosis.

The gut microbiota has a significant role in the regulation of the various mechanisms and dysregulation of the gut microbiome contributes to the development of both NAFLD and IBS[26,32]. NAFLD patients have been shown to have increased *Firmicutes, Proteobacteria, Actinobacteria, Escherichia*, *Clostridium*, and *Bacteroides*, and decreased *Bifdobacterium*, *Prevotella* and *Faecalibacterium*[27,33-35]. Somewhat similarly, IBS patients have shown increased abundance of *Ruminococcus*, *Streptococci, Firmicutes*, and decreased *Bifidobacterium*, *Faecalibacterium*, and *Lactobacillus*[18,36-38]. However, no specific microbial signature exists for IBS and NAFLD patients to distinguish them from healthy controls, in part due to the variability of sequencing techniques and population groups, as well as various confounding factors such as dietary habits[35,39].

Chronic inflammation and immune system activation

Activation of the innate and adaptive immune pathways has been implicated in the pathogenesis of IBS, both in the intestinal mucosa and neuroinflammation *via* the brain-gut axis. This involves an overall state of inflammatory overdrive, a dysregulated HPA axis and serotonergic signaling[40]. IBS patients display persistent signs of low-grade mucosal inflammation with increased counts of $CD3^+$, $CD4^+$ and $CD8^+$ T-lymphocytes, mast cells[41]. Enhanced expression of pro-inflammatory cytokines including interleukin (IL)-1β, tumour necrosis factor (TNF)-α, IL-6, IL-8 are observed in IBS patients[18,41,42]. Higher baseline TNF-α, lipopolysaccharide-induced TNF-α, IL-6 and IL-8 levels were significantly correlated with increased bowel frequency and severity of IBS symptoms[42,43].

NAFLD shares similar points of contact with IBS with a low-grade chronic inflammation as a main driver of disease progression in NAFLD[18,44]. Obesity triggers activation of innate and adaptive immune pathways and adipose tissue inflammation exacerbates NASH[18]. Liver metabolism is affected directly *via* circulating free fatty acids (FFA) from food,

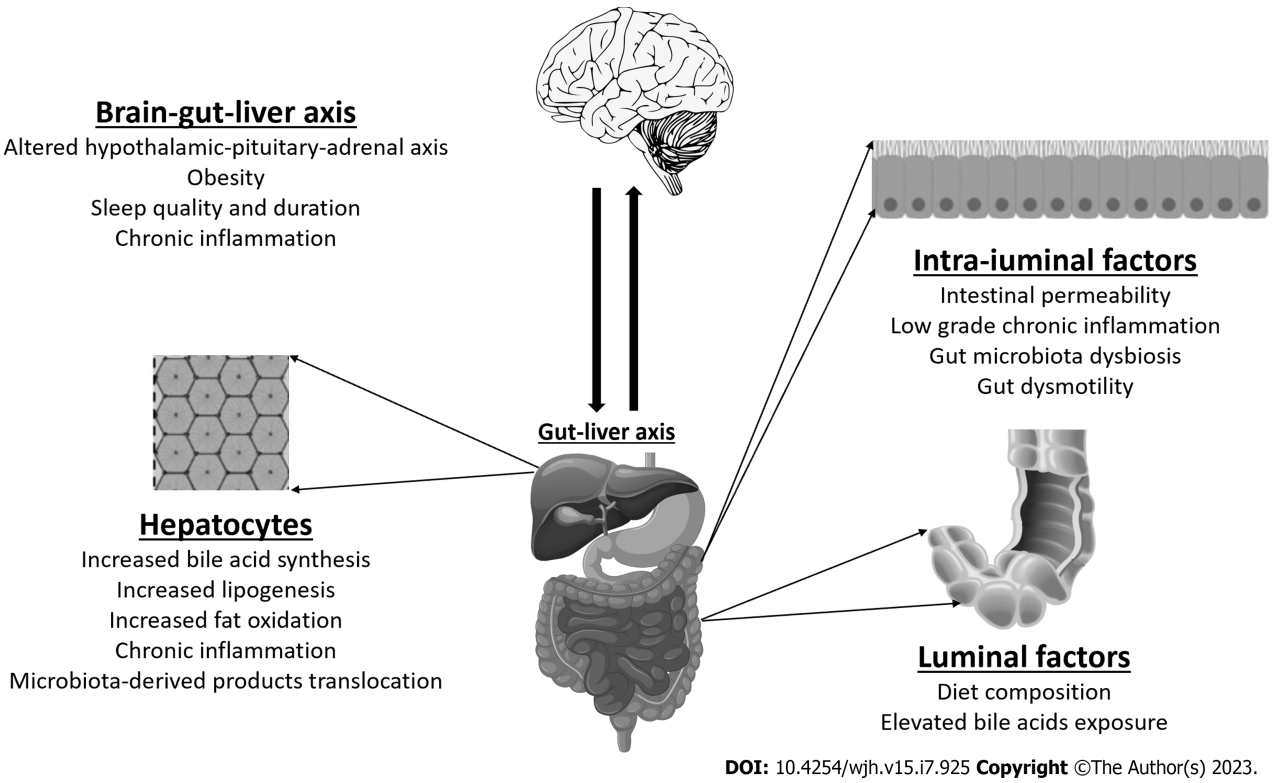

Figure 3 Postulated pathophysiology.

adipose tissue, intestinal bacteria, and indirectly *via* pro-inflammatory cytokines[18,45]. Higher levels of IL-6, C-reactive protein, TNF-α are detected in NAFLD subjects[18,46]. FFA binds toll-like receptors (TLR) on immune cells and in the liver, contributing to activity of the immune system. FFA are also involved in production of reactive oxygen species, mitochondrial dysfunction and endoplasmic reticulum stress in the liver[18,46]. Increased natural killer (NK) cell and NKT cell activity is linked with hepatic expression of inflammatory cytokines and activation of Kupffer cells[47]. All these mediators drive the inflammatory cascade and consequent fibrogenesis in NAFLD.

Bile acids

Bile acid-mediated mechanisms are involved in the pathophysiology of both NAFLD and IBS. There is bile acid signaling dysregulation resulting in increased bile acid production and bile acid malabsorption[48]. Altered bile acid metabolism with defective farnesoid X receptor (FXR) and fibroblast growth factor 19 (FGF19) contributes to abnormal hepatic lipid metabolism in NAFLD[48,49]. Patients with IBS have higher colonic bile acid exposure compared with healthy controls which affects bowel habits in IBS patients, predominantly in the IBS-D subgroup[50,51]. This stimulates colonic motility with acceleration of colonic transit, activation of visceral fluid sensation and fluid secretion[52].

In NAFLD, patients were found to have higher total faecal bile acid levels, increased rates of bile acid synthesis in the liver and a predominance of primary bile acids in the stool[53]. Primary unconjugated faecal bile acids correlated with the degree of hepatic steatosis, the presence of ballooning and severity of fibrosis in NASH subjects[53]. A retrospective study described an increased prevalence of NAFLD in individuals with bile acid diarrhoea[54]. These findings were further confirmed in a prospective study of 127 NAFLD patients that showed a correlation between increased hepatic bile acid production and diarrhoea with increased NAFLD fibrosis scores[49].

Small intestinal bacterial overgrowth

A higher prevalence of small intestinal bacterial overgrowth (SIBO) has been reported in patients with IBS[55]. Studies have demonstrated that IBS patients were more likely to develop SIBO compared with healthy controls, predominantly of the diarrhoea subtype[56,57]. SIBO is also found to be more prevalent in NAFLD patients attributed to proposed mechanisms including endotoxaemia and induction of TLR and pro-inflammatory cytokines[58,59]. A study by Sabaté *et al*[60] showed an association between SIBO and severity of hepatic steatosis in obese individuals[60].

Intestinal permeability

Impaired intestinal permeability is a key factor in the development of IBS[61]. Alterations in gut barrier function were observed in IBS patients which correlated with severity of symptoms[62,63]. A subgroup of IBS-D patients with increased intestinal permeability experienced more severe IBS symptoms and visceral hypersensitivity[62]. This is in part due to bacterial translocation and inflammatory agents through disruption of the epithelial tight junctions[7,18].

NAFLD is similarly associated with increased gut permeability which has an important role in the pathogenesis of NASH[64]. Several studies have described increased intestinal permeability in correlation with the degree of hepatic steatosis[65,66]. Impaired intestinal permeability allows for translocation of bacterial-derived products into the portal circulation and increasing hepatic exposure to harmful substances resulting in inflammation and fibrosis[66]. Conversely, a study by Luther *et al*[67] suggests that initial hepatic injury may contribute to impaired intestinal permeability although the mechanism is undetermined[67].

Obesity and metabolic syndrome

A multitude of studies denotes the association between IBS and obesity including the metabolic syndrome. Talley *et al*[68] demonstrated that older age, less early satiety, increased stool frequency and heartburn were all independently associated with increasing BMI[68]. Visceral abdominal obesity is correlated with an increased risk of developing IBS, primarily diarrhoea-predominant IBS. This is attributed to alteration in visceral fat metabolism which triggers production of adipokines and immunologic factors[69]. IBS patients have an augmented visceral perception of luminal stimuli, dysmotility and abdominal pain related to increased visceral adiposity[70]. Conversely, recent studies have shown that IBS subjects with morbid obesity achieved significant improvement in bowel symptoms after undergoing weight loss intervention[71].

The interplay between NAFLD, obesity and the metabolic syndrome with insulin resistance as a key pathogenic driver has been well-established. A meta-analysis by Li *et al*[72] showed that obese individuals had a 3.5-fold increased risk of developing NAFLD which has a dose-dependent relationship with BMI[72]. A high pooled prevalence of NAFLD was found in type 2 diabetes patients, with 60% of diabetes patients being diagnosed with NAFLD[73].

Sleep

Poor sleep quality and circadian misalignment have been implicated in the pathogenesis of IBS[74]. Exacerbation of symptoms have been observed after a night of poor sleep in IBS patients[74]. Likewise, impaired sleep quality, short sleep duration and daytime sleepiness are associated with NAFLD risk with correlation with insulin resistance[75,76]. The hypothalamus-pituitary-adrenal axis can also be affected with impaired cortisol metabolism leading to hepatic steatosis [75]. In addition, obstructive sleep apnoea could have deleterious effects on liver metabolism in the disease progression of NAFLD[76].

Clinical impact

In view of the postulated shared mechanisms underlying IBS and NAFLD, therapeutic strategies for IBS may also be beneficial for patients with NAFLD and vice versa. Lifestyle modification with diet and exercise leading to weight loss remains the cornerstone of NAFLD management[77]. Weight loss in IBS subjects showed marked improvement in bowel symptoms along with subjective well-being[71].

Rifaximin has been established as an effective drug in improving global IBS symptoms and bloating[78]. The administration of Rifaximin in NAFLD demonstrated effects including the reduction of serum endotoxin, pro-inflammatory cytokines, NAFLD-liver fat score and improvement in insulin resistance[79,80]. The postulated mechanism could be due to Rifaximin's effect on gram-negative bacteria leading to the inhibition of endotoxin proinflammatory cytokine production in NAFLD patients[79]. Several studies have shown that probiotics can lead to decrease in serum cytokine levels, oxidative stress markers, liver fat and biochemistry in NAFLD[81-83]. Similarly, studies have identified that probiotics can aid in alleviating IBS symptoms[84,85].

Obeticholic acid (OCA), a semi-synthetic FXR agonist, has been shown to stimulate FGF19 and decrease bile acid synthesis, improve stool form and diarrhoea in patients with IBS-D symptoms[86]. The promising impact of OCA on NASH and its associated metabolic features has been described with significant improvement in fibrosis and NASH histology[87]. Lubiprostone has been demonstrated to be effective in treating global IBS-C symptoms[88]. It has also been shown to decrease hepatic enzyme levels in NAFLD patients with constipation. Greater reduction in hepatic steatosis levels and levels of endotoxin were seen in those with improved intestinal permeability[89]. This may provide a basis for the role of Lubiprostone in a subset of NAFLD patients.

There are several limitations. The number of studies included was small given the lack of data on this topic to date. Different ultrasonographic diagnostic criteria was used for NAFLD. One study used codes from the International Disease Classification of diseases for identification of IBS while the other did not disclose how IBS patients were diagnosed, resulting in heterogeneity among the studies. Statistical analysis was unable to be carried out as not all studies assessed OR and 95%CI.

CONCLUSION

In conclusion, the evidence supports the association between IBS and NAFLD. IBS and NAFLD may co-exist and patients with IBS should be assessed for NAFLD and vice versa. Given the common postulated pathophysiology of both conditions, this may form the basis for further studies to assess suitability and benefits of utilising known therapeutics for IBS to treat NAFLD. This may guide future therapeutic strategies, especially in patients who suffer from both conditions. However, further prospective studies are required to confirm this association.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease with the rise of obesity and metabolic syndrome. Functional gastrointestinal disorders like irritable bowel syndrome (IBS) are increasing in prevalence.

Research motivation

At present, there is limited understanding regarding the links between the two conditions despite there being suggestions of possible overlap between IBS and NAFLD. We hope to explore literature to assess this overlap and also possible common pathophysiological links.

Research objectives

To review the current literature regarding the overlap of NAFLD and IBS and potentially identify common pathophysiological links which may show potential for utilizing common therapeutics to treat both conditions.

Research methods

A systematic search was done to assess current literature showing overlap between NAFLD and IBS in human subjects from PubMed, EMBASE and Cochrane.

Research results

We identified studies showing overlap between NAFLD and IBS. Both IBS and NAFLD patients demonstrated more metabolic risk factors like obesity, hypertension, dyslipidaemia and diabetes. IBS was seen to be more common in NAFLD patients and vice versa. Common pathophysiological links included the brain-gut-liver axis, intestinal permeability, gut microbiota dysbiosis, bile acid signalling dysregulation, obesity and metabolic syndrome.

Research conclusions

Our systematic review summarizes the current literature regarding IBS and NAFLD and demonstrates overlap between the two conditions. Common pathophysiological links were identified between both conditions.

Research perspectives

The evidence supports the association between IBS and NAFLD. With common postulated pathophysiology of both conditions discussed, further studies would be useful to further strengthen the association between both conditions and also look into possible common therapeutics.

FOOTNOTES

Author contributions: Siah KTH contributed to the design and implementation of the research and supervision; Ng JJJ and Loo WM performed the analysis of the results and writing of the manuscript.

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