

Liver Diseases: The Role of Gut Microbiota and Probiotics

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Abstract

There is unique co-ordination between gut and liver; portal venous blood derived from the mesenteric venous circulation constitutes approximately 75% of total hepatic blood flow and its content activates multiple liver functions. Intestinal microbiota plays an important role in health and disease. The gut-liver axis provides interaction between microbial components and liver causing liver damage. Bacterial overgrowth, dysfunctional immunity, altered intestinal permeability are implicated in pathogenesis of infections, hepatic encephalopathy, spontaneous bacterial peritonitis and hepatorenal syndrome etc. Probiotics may prevent these abnormal interactions between gut and liver by improving intestinal barrier function and prevention of bacterial translocation. This review addresses gut-liver axis, gut microbiota in liver disease and probiotics in management of liver diseases.

Keywords: Liver diseases; Microbiota; Probiotics; Hepatic encephalopathy; Hepatic

Abbreviations: HE: Hepatic Encephalopathy; PAMPs: Pathogen associated molecular patterns; TLRs: Toll-like Receptors; NLRs: Nod-like Receptors; LPS: Lipopolysaccharide; HCV: Hepatitis C virus; CHB: Chronic Hepatitis B; HBV: Hepatitis B virus; ALD: Alcoholic Liver Disease; BM: Bone Marrow; NAFLD: Non-alcoholic Fatty Liver Disease; NASH: Non-alcoholic steatohepatitis; NO: Nitric Oxide; SIRS: Systemic Inflammatory Response Syndrome; CDR: Cirrhosis Dysbiosis Ratio; HCC: Hepatocellular Carcinoma; CTP: Child-Turcotte-Pugh; MELD: Model For End-Stage Liver Disease.

Introduction

The gut microbiota is a very diverse ecosystem in that it is comprised of over 2,000 distinct species and has a collective genome of 150 fold more genes than the human genome [1]. It performs vital functions related to not only nutrition, metabolism but also secretes a number of biologically active compounds which perform various functions like inhibition of pathogens and detoxification of toxic compounds. There is unique co-ordination between gut and liver. The gut-liver axis plays important role in the pathogenesis of various liver diseases [2]. The altered gut microbiota, intestinal permeability and bacterial translocation plays a relevant role in the development of bacterial infections and other complications of cirrhosis such as variceal bleed, ascites, hepatic encephalopathy (HE) and acute on chronic liver failure. Probiotics may modulate gut microbiota and alter pathogenic interactions in chronic liver disease [3,4].

Functions of gut microbiota in health and disease

Gut microbiota perform various functions which includes immunological, digestive and metabolic. A specialised digestive processes involving gut microbiota produces energy. Energy harvesting is believed to vary with variations in gut microbiota and excessive energy harvesting has been implicated in the causation of obesity. Gastrointestinal tract is exposed daily to a large number of pathogens; however immune system is able to handle it without any adverse events [5]. It involves complex interactions between pathogen associated molecular patterns (PAMPs) with pattern recognition receptors of the host like Toll-like receptors (TLRs) and Nod-like receptors (NLRs) [6]. Intestinal barrier includes physical, chemical and immunologic components. Altered intestinal barrier function can lead to leaky gut and causing systemic endotoxemia [7]. Gut microbiota is also involved

in metabolic functions such as synthesis of vitamins, biotransformation of drugs and toxins [8].

Microbiota in liver disease

Altered gut microbiota produces multiple PAMPs such as bacterial lipopolysaccharide (LPS), peptidoglycan, lipoproteins etc resulting into endotoxemia. Endotoxemia leads to initiation of liver injury via interaction with TLRs; [6] although TLR independent mechanisms by which gut microbiota produces liver injury has been described [9].

Viral hepatitis

A significant correlation is seen between systemic endotoxemia with viral replication and degree of the clinical and laboratory signs in patient with chronic viral hepatitis B [10]. LPS was undetectable in responders, while it was detectable in 42% non-responders in patients with chronic hepatitis C after interferon and ribavirin based therapy. Dolganic et al. demonstrated endotoxin and HCV core protein act in tandem to induce and maintain inflammation, resulting in persistent inflammation in patients with chronic hepatitis C [11]. The composition of intestinal *Bifidobacterium* has been shown to be altered in CHB and HBV cirrhotic patients with a shift from beneficial species to opportunistic pathogens, providing further insights into the dysbiosis of the intestinal microbiota in patients with hepatitis B virus induced chronic liver disease [12]. Another study revealed a progressive decrease in the ratio of *Bifidobacterium* to *Enterobacteriaceae* from healthy controls, asymptomatic HBV carrier, and chronic hepatitis B to decompensated HBV cirrhosis [13]. At least, in part altered gut microbiota plays some role in pathogenesis of chronic hepatitis B and C [14].

Alcoholic liver disease

The main mechanism of alcoholic liver disease (ALD) is its

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metabolism in hepatocytes. However, alcohol also can also induce liver injury via alter gut microbiota. In patients who consume alcohol, there is reduced levels of *Bacteroidetes* and increased levels of *Enterobacteriaceae* and *Proteobacteria* [15]. Alcohol promoted increased TLR activation and increased *Proteobacteria* leads to an increased tyrosine phosphorylation of tight junction and adherent junction proteins; which eventually leads to increased intestinal permeability to PAMPs [16]. In animal study, absence of IRAK-M which is negative regulator of TLR signalling showed increased alcohol-induced TLR4 signaling and microbiota alteration [17], also absent TLR4 gene in both BM-derived and non-BM-derived liver cells reduces the extent of alcohol induced liver injury [18]. Alcohol also increases susceptibility to bacterial infections by suppressing natural killer cell activity [19], antibody-dependent cell-mediated cytotoxicity [20] and the T cell-dependent antibody responses [21].

Non-alcoholic fatty liver disease

Patients with NASH have a lower percentage of *Bacteroidetes* compared to healthy controls. Western diet containing high fat and simple carbohydrates contributes to endotoxemia by causing alternation in intestinal barrier and gut microbiota [22]. In mice, shifting diet from high fat to high carbohydrate effectively improved low-grade inflammation, reduced endotoxemia [23]. Loss of function mutation in TLR4 prevents diet-induced obesity and insulin resistance [24]. Response to high fat diet is determined by gut microbiota and it also contributes to development of NAFLD independently of obesity [25]. There is a significant increase in *Bacteroidetes* with decreased levels of *Firmicutes* among obese and NASH; also significantly decreased *Actinobacteria* in NASH [26]. *Proteobacteria* showed gradual increase from healthy to obese and NASH. However, Ref. [27] and other studies by Ref. [22,28] has not confirmed increased *bacteroidetes/firmicutes* ratio.

Cirrhosis

Hyper-dynamic circulation in cirrhosis is mainly mediated by over production of NO which is mainly produced by LPS induced activation of iNOS, eNOS and nNOS. Bacterial translocation is responsible for increased LPS concentration in portal blood [29]. Bacterial translocation in cirrhosis occurs due to bacterial overgrowth, immune dysfunction and altered intestinal permeability [30]. In animal model, altered gut microbiota is associated with the development of pro-inflammatory state, progression of fibrosis and increased bacterial translocation [31]. Endotoxin in a TLR4/CD14-dependent manner exacerbates hepatic fibrogenesis [32]. Bacterial infections in cirrhosis have increased production of pro-inflammatory cytokines leading to systemic inflammatory response syndrome (SIRS) and multi-organ dysfunction [33]. Ammonia is a key factor in the pathogenesis of hepatic encephalopathy (HE), which is derived mainly by gut microbiota. Gut microbiota in patients with cirrhosis have been shown to alter with development of covert to overt HE [34]. Cirrhosis dysbiosis ratio (CDR), the ratio of autochthonous to non- autochthonous bacteria is significantly higher in controls than cirrhosis. Dysbiosis, lower CDR and relative abundance of gram negative bacteria is present despite of lactulose therapy in patients who developed HE [35]. Rifaximin is associated with improved cognitive function and endotoxemia in HE [36], which is accompanied by alteration of gut bacterial linkages with metabolites without significant change in microbial abundance [37].

Hepatocellular carcinoma

HCC initiation is not dependent on TLR4 or gut microbiota; but it

plays major role in HCC promotion involving hepatocyte proliferation and evasion of apoptosis [38]. Another study showed reduction of LPS induced signalling in mice prevented excessive tumor growth [39]. Thus LPS/TLR4 signalling pathways plays important role in HCC.

Therapeutic modulation of gut microbiota

Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts. Prebiotics are dietary substances that nurture specific changes in the composition and/or activity of the gastrointestinal microbiota (favouring beneficial bacteria), thus conferring benefit(s) upon host health and synbiotics are products that contain both probiotics and prebiotics [40]. The ability to modify gut microbiota, to improve the intestinal barrier and to modulate inflammatory response are the important properties of probiotics. The major limitation in development of probiotics as effective therapeutic option is due to its own variety and heterogeneity of their effects.

Several studies in animal models of ALD, NAFLD as well as HE has reported beneficial effects of certain probiotics on liver injury. In animal models of NAFLD, benefits of probiotics have been shown by Li et al. [41], Chen et al. [42] and Velayudham et al. [43] In the systemic review of randomised control trials evaluating metabolic benefits of dietary prebiotics in human found convincing evidence from short-term high quality human trials supporting the use of dietary prebiotics as a potential therapeutic intervention for NAFLD, however further studies are needed to correlate these findings with changes in gut microbiota [44]. VSL#3 for three months showed improvement in markers of lipid peroxidation in NAFLD and ALD [45]. A Spanish study evaluated effects of the mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in NAFLD showed a reduction in ALT and AST activity, however cardiometabolic risk factors were unchanged [46].

In the animal studies of ALD, benefits of probiotics have been demonstrated by Nanji et al. [47] and Forsyth et al. [48] In the pilot study by Kirpich et al. [49] short-term oral supplementation with *B. bifidum* and *L. plantarum* was associated with restoration of the gut microbiota and greater improvement in alcohol induced liver injury than standard therapy alone. In patients with compensated alcoholic cirrhosis, probiotics restored neutrophil phagocytic capacity, possibly by changing IL10 secretion and TLR4 expression [50]. The effectiveness of probiotic in cirrhosis can be divided into two scenarios either improvement of hepatic function or prevention and treatment of complications such as infection and hepatic encephalopathy. VSL#3 for 6 months significantly reduced the risk of hospitalization for HE as well as improved CTP and MELD scores in patients with cirrhosis [51]. Another study showed improvement in the hepatic and systemic haemodynamics in the decompensated cirrhosis [52]. Gupta et al. [53] evaluated VSL#3 as adjunctive probiotics to propranolol therapy showed improved response rate in relation with HVPG also greater mean fall in HVPG than propranolol alone. Rayes et al. [54] demonstrated a decrease in infection rate after liver transplantation using symbiotic therapy in addition to antibiotic therapy. However, addition of probiotics to norfloxacin does not improve efficacy in the primary or secondary prophylaxis of spontaneous bacterial peritonitis or in reducing the mortality in cirrhotic patients with ascites [55]. The ability to reduce ammonia and modulation of gut microbiota is basis for using probiotics in HE. Lunia et al. [56] in randomised controlled trial showed that probiotics are effective in preventing HE in patients with cirrhosis. In open label study, lactulose and probiotics are found to be effective for secondary prevention of HE [57]. Another study evaluated lactulose, probiotics and L-ornithine L-aspartate in treatment

of minimal hepatic encephalopathy showed significant improvement in MHE and quality of life in patients with chronic liver disease [58]. Probiotics as therapeutic modality has shown consistent benefits in chronic liver disease, even if variety of preparations used and different study protocols.

Conclusion

Gut microbiota has important role in pathogenesis of liver diseases. As knowledge regarding microbiota is improving; its role in health and diseases is being understood better. Gut microbiota has strong relationship with liver in health and its variations have significant role in pathogenesis of chronic liver diseases, including NAFLD, progression of fibrosis and HE etc.

The ability of probiotics to modulate gut microbiota is having therapeutic potential. There is some evidence regarding therapeutic use of probiotics in management of SBP and HE. Further research in evaluation of gut microbiota and appropriately selected useful bacterial strains as treatment modality should be undertaken.

Author Contributions

Jagtap N conceptualized the manuscript, critically reviewed relevant literature and drafted the paper; Sharma M, Rao PN, Reddy DN critically reviewed the manuscript and provided intellectual inputs.

Guarantor of the Article

Jagtap N is guarantor of the article.

Specific Author Contributions

Jagtap N conceptualized the manuscript, critically reviewed relevant literature and drafted the paper; Sharma M, Rao PN, Reddy DN critically reviewed the manuscript and provided intellectual inputs.

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Potential Competing Interests

None.

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