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Microbial Profiles of Cirrhosis in the Human Small Intestine

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Abstract

Purpose of Review: The aim of this review is to summarize the recent literature on associations of small intestinal microbial and bile acid profiles with liver cirrhosis and its complications.

Recent Findings: Recent studies into the duodenal microbiome of patients with cirrhosis have linked the microbiome to certain etiologies of chronic liver disease as well as complications of cirrhosis. In particular, microbial differences in the duodenum of patients with cirrhosis have been linked to the presence of hepatic encephalopathy and varices.

Summary: While the fecal microbiome of patients with liver cirrhosis is well characterized, the small intestinal microbiome of cirrhotic patients is an active area of research. This review focuses on the current understanding of the small intestinal microbiome in human cirrhosis as well as future directions of the field.

Keywords

Microbiome; bile acids; cirrhosis; hepatic encephalopathy; portal hypertension; ascites

Introduction

Over the last several years, there has been a growing body of research examining the role of the microbiome in a variety of different diseases, including liver cirrhosis and related complications. Originally, it was believed that the major factor that connected the microbiome to complications of cirrhosis was bacterial translocation [1]. While this is still a major contributor, new research within the microbiome field has shown us that is likely a more complicated relationship that involves several pathways including bile acid synthesis, cell signaling, and host immune response [2-4]. However, the majority of studies on the gut

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microbiome and liver disease have focused on the microbiome of feces due to the ready accessibility of this sample type. However, this may not capture relevant host-microbiome interactions in the small intestine, which is a critical site of nutrient and bacterial product delivery to the liver via the portal circulation. Microbial profiles of the small intestine have the potential to be equally or more important to our understanding of the relationship between the liver and the microbiome.

Several studies have already shown that the microbiome of the small intestine is very distinct from the microbiome of the colon or feces [5-7]. In patients with ileostomies, the small intestinal microbiome was less diverse than that of feces and varied according to levels of carbohydrate uptake [7]. Similarly, in mouse models examining the differences between the small intestine and the large intestine, Onishi *et al* found that the small intestine had lower levels of Verrucomicrobia and distinct levels of Firmicutes and Bacteroidetes compared to the colon [6]. They also demonstrated that a high fat diet altered the microbiome of the small intestine differently from the microbiome of either the cecum or the colon [6]. Therefore, studies that only examine the microbial profiles of stool may miss important genera and phyla changes in the small intestine.

The duodenal microbiome has also been associated with other disease processes such as obesity and celiac disease. The duodenal microbiota of obese patients had a higher proportion of aerobic genera as compared to healthy controls [8]. In patients with refractory celiac disease, duodenal microbiome analysis showed a decrease in microbial diversity associated with a lower abundance of Bacteroidetes and Firmicutes and a higher abundance of Proteobacteria [9]. In a similar fashion, studies of the small intestinal microbiome have led to a better understanding of cirrhosis and its complications. This review will summarize the most recent research examining the association between the small intestinal microbiome and human cirrhosis.

Small Intestinal Microbial Profile of Cirrhotic Patients

Due to the increased difficulty of collecting small intestinal samples as compared to stool, few papers have examined the small intestine microbial composition of cirrhotic patients. There is a literature dating back to 1984 describing small intestinal bacterial overgrowth in cirrhosis using culture of jejunal aspirates or breath-testing, but the first paper utilizing culture-independent methods to assess the small intestinal microbiome of cirrhotics was published only in 2011 [10, 11]. Steed *et al* collected duodenal biopsies from 23 cirrhosis patients and 10 non-cirrhosis controls then performed quantitative polymerase chain reaction to measure Enterobacteriaceae, *Enterococcus*, *Staphylococcus*, *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Escherichia coli*, and *Helicobacter pylori* [10]. The only significant difference was increased *Enterococcus* in cirrhotics, which disappeared after stratifying patients by proton pump inhibitor usage. This study was limited by the use of targeted methods for assessing the microbiome as well as the lack of advanced cirrhosis in the cohort (18 out of 23 were Child-Pugh class A). It wasn't until 2016 that the first paper appeared utilizing 16S ribosomal RNA gene sequencing to perform an untargeted sequence-independent analysis of small intestinal microbiome composition [12]. In that study, Chen *et al* examined the duodenal mucosal microbiome (obtained via duodenal biopsy) of 30

cirrhotic patients undergoing upper endoscopy for esophageal surveillance or screening and compared it to 28 healthy controls. Twenty-seven of the 30 cirrhotic patients were Child-Pugh class A. None had hepatic encephalopathy and all 30 had some level of either esophageal or gastric varices on upper endoscopy. The cohort was also predominantly comprised of hepatitis B virus (HBV) patients (80%) with the remainder having primary biliary cirrhosis (PBC) as their cause for cirrhosis. At a phylum level, they saw an overabundance of Firmicutes and an underrepresentation of Proteobacteria and SR1 in patients with cirrhosis as compared to controls. On a genus level, cirrhotic patients had elevated *Veillonella*, *Megasphera*, *Dialister*, and *Prevotella*. They also saw differences in microbial profiles in patients using proton pump inhibitor, patients requiring endoscopic treatment for varices, and in HBV patients as compared to PBC. However, the generalizability of the study is its main limitation as the vast majority of patients were well-compensated Child-Pugh class A hepatitis B cirrhotic patients of Asian descent.

A second paper compared the duodenal mucosal microbiome of 20 patients with alcoholic cirrhosis, 28 patients with cirrhosis and alcohol-abstinence, and 18 healthy controls [13]. On a family level, alcoholic cirrhosis was associated with increased Pasteurellaceae and decreased Alcaligenaceae and Cryomorphaceae compared to controls. Alcohol-abstinent cirrhotics had lower Verrucomicrobiaceae, Aquificaceae, and Xanthomonadaceae compared to controls. There was increased Comamonadaceae, Acidaminococcaceae, and Hyphomonodaceae and decreased Xanthomonadaceae in alcoholic cirrhotics compared to alcohol-abstinent cirrhotics. This study supported the concept that microbial signatures of cirrhosis can vary by cirrhosis etiology. However, the study did not further investigate cirrhosis etiologies other than alcoholic cirrhosis and included predominantly Caucasians, precluding assessment of effects of race/ethnicity.

These limitations were addressed in a recent paper by Jacobs *et al* [14]. As part of an ongoing prospective cohort study in cirrhotic patients: The Microbiome, Microbial Markers, and Liver Disease (M_3LD) Study. We examined the duodenal aspirate of 30 patients of varying race/ethnicity and etiology of cirrhosis. Thirteen of the 30 patients had a history of encephalopathy. In our paper, we found that microbial differences occurred between patients with alcohol related disease as compared to patients with hepatitis C virus (HCV), in patients with hepatic encephalopathy, and in Hispanic patients as compared to non-Hispanic white patients. Specifically, patients with alcoholic cirrhosis had a significant decrease in *Prevotella* as compared to patients with HCV and patients with encephalopathy had a significant increase in *Mycobacterium* and *Granulicatella* and a decrease in *Clostridium*, *Ruminococcus*, and *Faecalibacterium*. The finding in HCV versus alcoholic cirrhotics is similar to other studies that have linked HCV cirrhosis to an increase in relative abundance of *Prevotella* in the stool [15].

Hepatic Encephalopathy, Portal Hypertension, and the Microbiome

While the existing literature on the duodenal microbiome in cirrhotic patients is relatively sparse, the data that is published to date indicates that there may be a relationship between the small intestinal microbiome and clinical complications of cirrhosis including hepatic

encephalopathy and portal hypertension [12, 14]. These findings in the small intestine mirror several studies of the fecal microbiome.

Hepatic encephalopathy, for example, is a complication of cirrhosis that is often linked to the microbiome. Ammonia has been long considered a key element in the pathogenesis of hepatic encephalopathy. The main producers of circulating ammonia arise from the breakdown of ingested amino acids and urea by the gut microbiome. Therefore, the two main treatments of hepatic encephalopathy are lactulose and rifaximin. Lactulose is a non-absorbable disaccharide that acidifies the gut lumen which inhibits urease-producing bacteria and limits the diffusion of ammonia into the bloodstream [16]. Rifaximin is a minimally absorbed broad-spectrum antibiotic that has been shown to greatly reduce the burden of hepatic encephalopathy [17]. The exact mechanism by which rifaximin does this is unknown but it has been implicated in altering the levels of toxic metabolites and secondary bile acids produced by the microbial dysbiosis of cirrhotic patients [18]. Prior studies that have examined the small intestinal microbiome in hepatic encephalopathy have often linked it to small intestinal bacterial overgrowth [19, 20]. In our recent study, we show that patients with a history of hepatic encephalopathy had decreased microbial diversity and richness, reduction of Bacteroidetes, and an overrepresentation of Proteobacteria [14]. In line with the idea of dysbiosis as a cause for hepatic encephalopathy, a recent small randomized trial showed that fecal microbial transplant may be of benefit in treating recurrent encephalopathy [21].

Portal hypertension is the other major complication of cirrhosis that often manifests itself as either ascites or varices. Chen *et al* showed that the duodenal microbiome differed significantly between patients that have underwent endoscopic treatment for varices versus those patients that did not [12]. In patients who had varices and endoscopic treatment, there was an increase in *SR1 genera incertae sedis* and a decrease in *Staphylococcus*. Endoscopic treatment of varices often leads to an increase in the severity of portal gastropathy [22]. Therefore, the changes seen in the duodenal microbiome after endoscopic treatment of varices may be related to worsening hypertensive portal gastropathy. To date, there has not been any published data demonstrating a relationship between the small intestinal microbiome and ascites. However, many papers have shown that patients with cirrhosis exhibit increased intestinal permeability [23-26]. Expression of tight junction proteins such as occludin and claudin-1 in the duodenum was found to be reduced in cirrhotic patients with ascites and was correlated with worsened endotoxemia and Child-Pugh score [27]. This increased permeability is one of the several proposed mechanisms for relating cirrhosis to spontaneous bacterial peritonitis, systemic inflammation, and endotoxemia [23, 25].

Bile Acids

Another mechanism by which the liver can directly communicate with the enteric system and vice versa is through the production and secretion of bile acids. Primary bile acids are produced and conjugated in the liver and eventually excreted into the first part of the small intestine (duodenum) where they undergo deconjugation and dihydroxylation by intestinal microbes to become secondary bile acids, the majority of which are reabsorbed into the portal circulation at the terminal ileum. Bile acids are important for the absorption of fat and

fat soluble vitamins, but over the last several years they have also been shown to be important in cell signaling, inflammation, steatosis, and hepatocarcinogenesis [28-30] . The composition of bile acids pools can directly affect microbiome composition/function and vice versa [31]. For example, Inagaki *et al* proposed that activation of farnesoid X receptor by bile acids can induce genes that can prevent bacterial proliferation in the small intestine.

In recent years, we have seen several papers that have begun to elucidate potential mechanisms whereby bile acids may be influencing liver cancer development. Yoshimoto *et al* reported a novel pathway whereby the secondary bile acid deoxycholic acid provokes hepatic stellate cells to secrete pro-inflammatory and pro-tumorigenic factors in what is known as the senescence-associated secretory phenotype (SASP), which was necessary for cancer development in a mouse model of NASH [32]. Additionally, Ma *et al* reported on a mechanism whereby microbiome-mediated primary-to-secondary bile acid conversion was key to antitumor immunity in the liver through recruitment of NKT cells through the interaction of CXCL16 secreted by endothelial cells with CXCR6 on NKT cells [30]. These investigators are currently translating these findings into the clinic where they will conduct a phase II study of combined nivolumab, oral vancomycin and tadalafil treatment in patients with HCC to examine tumor progression (NCT03785210).

However, the majority of studies in patients with liver cirrhosis have examined bile acids either in the serum or in the stool even though those areas account for less than 15% of the circulating pool of bile acids [31]. One prior study characterized bile acid levels in duodenal aspirates, identifying elevated levels of conjugated primary and secondary bile acids in alcoholic cirrhotic patients compared to alcohol-abstaining cirrhotic patients and healthy controls [13]. However, no analysis was performed to relate bile acid profiles to other subgroups within the cirrhosis cohort. By examining the duodenal aspirate of cirrhotic patients, we were able to show that Hispanic patients with cirrhosis as compared to non-Hispanic Caucasian patients had decreased levels of two conjugated forms of ursodeoxycholic acid (UDCA), a secondary bile acid with anti-inflammatory and oncogenic suppressive properties [14]. Other studies have shown that UDCA can suppress hepatocellular carcinoma *in vivo* and *in vitro*, which may contribute to the higher rates of liver cancer in Hispanics [33-35].

Future Directions

The work over the last several years has greatly expanded our knowledge about the microbiome and its interaction with the host. But despite those advances, there is still much that we still do not completely understand. One major limitation in the field so far is the lack of mechanistic and causal studies. The majority of the studies published so far in the field of the human microbiome and liver disease have been cross-sectional association studies. They are unable to clearly state if the dysbiosis seen in liver disease is potentially causal or merely a byproduct of the disease state. Presently, there is a shift away from these studies and more towards studies that define mechanism or causality by either using germ-free animals or a multi'omics approach [36]. By expressing the disease phenotype through fecal transplantation of human microbiota into germ-free animal models, a direct causal relationship can be established between the microbiome and disease development.

Additionally, prospective epidemiological studies with longitudinal specimen collection can help to elucidate the direction of association between the microbiome and disease states.

Furthermore, as sequencing technology continues to improve, there will likely be more studies that used shotgun metagenomics instead of 16S rRNA sequencing when examining the relationship between the microbiome and cirrhosis. Shotgun metagenomics provides a higher level of taxonomic resolution than 16S rRNA sequencing and has the added benefit of providing functional data that can elucidate microbial-specific metabolic pathways in the pathogenesis of disease.

Conclusions

In summary, while the fecal microbiome of cirrhotic patients has been extensively studied, very few papers have profiled and examined the microbiome of the small intestine. Even though the small intestine has a lower abundance of bacteria as compared to the colon, its contribution to the portal venous supply and the enterohepatic circulation makes the small intestine a potential key site to understand the relationship between the microbiome and the liver. Based on relatively small sample sizes, the microbiome of the small intestine of cirrhotic patients appears to be distinct from healthy controls. Furthermore, the microbiome of these patients varies by their etiology of cirrhosis and by their manifestation of either hepatic encephalopathy or varices. While there was a less distinct profile for bile acids by etiology or complications of cirrhosis, duodenal bile acids do appear to vary by ethnicity in cirrhotic patients. Whether these changes seen in either the microbiome or in the bile acid pool are merely associations or rather vital to the pathogenesis of disease remains unanswered. Future studies should utilize prospective study designs, recruit larger sample of well characterized patients, and integrate other modalities to assess the microbiome such as proteomics, metabolomics, or transcriptomics. Doing so will potentially elucidate novel microbial pathways in cirrhosis pathogenesis and open new avenues for developing therapeutics and biomarkers for cirrhosis and its complications.

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