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Review

Microbiome of Hepatobiliary Diseases

Yeseul Kim[1], **Dongho Choi**[2]

[1]Department of Pathology, Hanyang University College of Medicine, Seoul, Korea
[2]Division of HBP Surgery, Department of Surgery, Hanyang University College of Medicine, Seoul, Korea

The microbiome, which has been defined as 'the ecological community of commensal, symbiotic and pathogenic microorganisms that share our body space, may be distinguished from the microbiota as it includes the collective genomes. An increasing level of evidence reveals that the human microbiome plays a major role in health. For this reason, it is often referred to as the 'forgotten organ.' All surfaces of the human body that are exposed to the environment are colonized, including skin, respiratory system, urogenital tract and gastrointestinal (GI) tract, totaling at least 100 trillion microbial cells. The known roles of the GI microbiome include metabolic functions, synthesis functions, and immune roles. Recent studies indicate that the human gut microbiome plays a significant role in health and disease. Dysbiosis, defined as a pathological imbalance in a microbial community, is becoming increasingly appreciated as a 'central environmental factor' that is both associated with complex phenotypes and affected by host genetics, diet, and antibiotic use. More recently, a link has been established between the dysmetabolism of bile acids (BAs) in the gut and the gut-liver axis, and this relationship with the microbiome has been highlighted. This review summarizes the microbiome of the hepatobiliary system and how microbiome is related to diseases of the liver and biliary tract.

Key words: Microbiome; Liver; Gallbladder; Pancreas; Cancer

Corresponding Author: Dongho Choi
Division of HBP Surgery, Department of Surgery, Hanyang Medical Center, 222 Wangshimri-ro, Sungdong-gu, Seoul 04763, Korea
Tel: +82-2-2290-8449
Fax: +82-2-2281-0224
E-mail: crane87@hanyang.ac.kr

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INTRODUCTION

Hepatobiliary and pancreatic cancers are associated with poor prognosis owing to their high level of tumor invasiveness, recurrence, hematogenous and lymphatic metastasis, resistance to first-line chemotherapy, and lack of effective target therapy [1,2]. Evidence in the literature suggests that hepatobiliary and pancreatic cancers develop through the accumulation of genetic and epigenetic alterations, which is influenced by host immune state, food, and environmental and microbial exposures [1-4].

The human microbiota is the collection of microorganisms exists in the human being, and the relationships with microorganisms and host can be considered to maintain a wide range of the spectrum, from mutualism to pathogen [5]. Abrupt changes in the microbiota of various human body areas associate with diverse localized or systemic human diseases. The human gastrointestinal tract is one of the biggest storing spaces of microbes in the body and contains both commensal and pathogenic microbial species [6]. Research on intestinal microbiota has shown that inflammatory bowel disease is originated from the varied composition of microbial composition and abnormal and overflowing mucosal immune response [7]. Numerous pathogens can promote cancer through well-identified mechanisms [8]. Although most studies are confined to specific bacterial pathogens and viruses, the link between human cancer and bacterial microbiota has recently been studied actively by using next-generation sequencing technology for microbiome profiling [9]. There is an increasing interest in understanding the role of microbiome as a microenvironment for cancer development, particularly in the area of hepatobiliary and pancreatic cancers [10].

The liver, biliary tract, and pancreas are located in very close

proximity, and these three structures link up with the gastrointestinal tract. Therefore, the gut microbiome can easily reach the liver through the portal vein [11]. The incidence of hepatobiliary cancer is higher in east and southeast Asian countries, such as Japan, Korea, and Thailand [4]. The incidence rate of cholangiocarcinoma in South Korea correlates with the prevalence of liver fluke (Opisthorchi viverrini) infection in the region [12].

In the rest of this review, we will describe the role of the microbiome in the hepatobiliary and pancreatic diseases, including nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease, liver cirrhosis, hepatocellular carcinoma, and gallbladder cancer.

1. Microbiome of liver

The relationship between the gut and the liver is well understood [13]. The most prevalent type of hepatic disorder is NAFLD), and over 60 million Americans suffer from it [14]. The present understanding of the etiology of the spectrum of liver diseases is explained by proinflammatory changes in the host. Intestinal dysbiosis (anomalous or imbalanced gut microbial composition) and increased intestinal permeability lead to translocation of microorganisms and microbial products, including cell wall components and DNA, together referred to as microbial-associated molecular patterns (MAMPs) or pathogen-associated molecular patterns (PAMPs). These changes cause a basic spectrum of hepatic diseases with various bacterial species.

2. Microbiome of specific liver diseases

1) Nonalcoholic fatty liver disease (NAFLD)

NAFLD can be defined as a spectrum of liver diseases that can be generally classified into two categories: nonalcoholic fatty liver, the simple form of NAFLD, and nonalcoholic steatohepatitis (NASH), the aggressive form of NAFLD [15]. NASH is typically related to type 2 diabetes mellitus, heart and vascular risk factors, and obesity [16,17]. However, NAFLD has also been commonly found in nonobese patients, supporting that genetic parameters also contribute to disease development [18-21].

Some studies have focused on the effect of the gut microbiota in NAFLD, but the cause-effect relationship has not been verified [22]. Individuals with NAFLD have a higher incidence of microbial dysbiosis [23]. Using 16S amplicon sequencing, the bacterial genera *Bacteroides* and *Ruminococcus* were profoundly increased, and *Prevotella* was decreased in persons with NASH compared with those without NASH [23]. Whole genome metagenomics in patients with NAFLD showed an increased prevalence of *Escherichia coli* and *Bacteroides vulgatus* in patients with advanced fibrosis [24].

2) Alcoholic liver disease (ALD)

Similar to NAFLD, the benign form of ALD is characterized by the accumulation of fat inside the liver (fatty liver or steatosis), whereas the progressive form is marked by inflammation and liver injury (alcoholic steatohepatitis (ASH).

Our knowledge of contributions of the gut microbiota in ALD is increased. As in NAFLD, SIBO has been demonstrated as an important hallmark of alcohol-associated liver disease in humans [25] and mouse models [26,27]. Intestinal dysbiosis in individuals who abuse alcohol is characterized by marked enrichment of Enterobacteriaceae (family) and reduction in abundance of Bacteroidetes and *Lactobacillus* [26,28-30]. It has also been demonstrated that alcohol-induced dysbiosis is only partially reversible by alcohol withdrawal or probiotic supplement [31,32]. Interestingly, patients dependent on alcohol also displayed reduced fungal diversity and *Candida* overgrowth, presenting the first evidence of the role of the gut mycobiome in the pathogenesis of liver diseases [33].

3) Cirrhosis

Alterations in the gut microbiota, including dysbiosis and SIBO, have been associated with cirrhosis and its complications [34-37]. Gut microbiome alterations were observed in patients with alcohol-associated and hepatitis-associated cirrhosis in a Chinese cohort [38], with an invasion of the lower intestinal tract by microorganisms associated with the oral cavity, such as *Veillonella* and *Streptococcus*. Concordant with these findings, Chen and colleagues also found an over-representation of genera, including *Veillonella*, *Megasphaera*, *Dialister*, *Atopobium* and *Prevotella* in the duodenum of patients with cirrhosis. The genera *Neisseria* and *Gemella* were discriminative between HBV-related and PBC- related cirrhosis [37]. In 2017, Bajaj and colleagues observed a significantly high incidence of fungal dysbiosis in patients with cirrhosis and showed that the Bacteroidetes: *Ascomycota* ratio could independently predict hospitalization in these patients [39].

4) Hepatocellular carcinoma (HCC)

The etiology of HCC follows a so-called multiple step pathway, whereby liver steatosis, followed by oxidative and ER stress, together with intestinal dysbiosis and inflammation, contributes to the final cause of cancer. The gut microbiota definitely changes in composition in human bodies with HCC. Clostridium species have been found to be enriched in obesity-induced mouse models of HCC [40,41], but clinical studies of patients with HCC detected an overgrowth of intestinal *Escherichia coli* [42]. Mouse models and human studies have reported migration of *Helicobacter* species into HCC

tumor tissues [43-46].

3. Microbiome and gallbladder cancer

Gallbladder cancer (GBC) is a relatively uncommon primary malignancy. However, it is the most common malignant neoplasm of the biliary system and a lethal cancer with fatal outcome [47-49]. Globally, GBC rates exhibit marked regional variability, reaching epidemic levels for some regions and ethnicities, especially in countries in Asia such as India, Korea, Japan, and in South America [50]. The basis for this difference likely related to differences in environmental exposures interacting with genetic factors. The previous epidemiologic studies have revealed several risk factors associated with GBC, including gallstones, chronic cholecystitis, and infection, especially Salmonella [51].

Although the role of microbiota in gallbladder carcinogenesis is still not well known, the previous epidemiologic studies have revealed that the risk of GBC increases with chronic infection by *Salmonella* species [52]. A meta-analysis from 11 different epidemiologic studies revealed that overall odd ratio of GBC in chronic *Salmonella typhi* carrier patients is over 4. Importantly, recent experimental studies revealed morphologic evidence and molecular mechanism for *Salmonella*-induced GB cancer or premalignant lesion [53,54]. *Salmonella* infection of gallbladder organoids induces loss of polarity, nuclear atypia with prominent nucleoli, and discohesiveness with loss of epithelial marker, E-cadherin [53]. This malignant transformation is also observed in mouse embryonic fibroblasts, and Akt and MAP kinase pathways, which are well-known cancer pathways, are activated during Salmonella infection [53]. Chronic cholecystitis is commonly observed in the gallbladder of mice with gallstones. However, atypical hyperplasia that is a premalignant condition, is only associated with chronic *Salmonella* infection regardless of the presence of gallstones [54]. The biologic effect of *Helicobacter bilis* has been investigated on a cell line of human bile duct cancer and showed activation of transcript factors, such as NF-κB, E2F and CRE that stimulate the production of VEGF and lead to enhancement of angiogenesis [55]. These epidemiologic and experimental studies support the role of infection on GBC carcinogenesis.

CONCLUSION AND PERSPECTIVES

Various research activities for microbiome suggests that unknown pathophysiology of diseases of the hepatobiliary system can be solved and explained by the microbiome research in some part. The field is slowly moving from observation to real clinical practice. Also, cutting-edge techniques in this field will widen basic understating of hepatobiliary diseases and hopefully improve the cure rate of these fatal diseases in the near future.

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