Austin Journal of Gastroenterology

Open Access

Austin Publishing Group

Research Article

Fecal Microbiota Transplantation Slows the Progression of HBV-Related Liver Diseases and Induces Virologic Response in Patients with HBV Infection

Guo Q[1#], Huang S-S[1#], Li J[1], Tian Y[1], Zhou Y[1,2*] and Li X-A[1,2*]

[1]The Gastroenterology Tumor and Microenvironment Laboratory, Department Of Gastroenterology, The First Affiliated Hospital of Chengdu Medical College, Chengdu Medical College, Chengdu, Sichuan, P.R. China

[2]Department of Gastroenterology, Mianyang central hospital, Mianyang, Sichuan, P.R. China

[#]Contributed Equally to this Work

***Corresponding author:** Xiao-An Li, Department of Gastroenterology, Mianyang central hospital, Mianyang, Sichuan, P.R. China

Yan Zhou, Department of Gastroenterology, Mianyang central hospital, Mianyang, Sichuan, P.R. China

Received: September 16, 2021; **Accepted:** October 14, 2021; **Published:** October 21, 2021

Abstract

Aims: Healthy gut microbiome plays a crucial role in the treatment of Hepatitis B Virus (HBV) infection and chronic liver disease. Based on existing studies, we aimed to explore the difference in the efficacy of Fecal Microbiota Transplantation (FMT) in patients with different stages of HBV-related chronic liver disease.

Methods: In this study, 10 HBV patients with HBeAg-negative infection, 8 patients with Chronic Hepatitis B (CHB) infection, and 8 patients with hepatitis B cirrhosis Child-Pugh score A, 9 patients with hepatitis B cirrhosis Child-Pugh score B/C were treated with FMT.

Results: Our results demonstrated that continuous FMT treatment improved liver function, controlled the replication of HBV-DNA, enhanced the intestinal mucosal barrier function, relieve the degree of liver fibrosis and postponed the progression of HBV-related chronic liver disease. The result of 16S ribosomal RNA (rRNA) sequencing indicated that the individual genus and composition of the bacteria in the feces of patients gradually approached the structure seen in case of the feces of healthy donors; the number of specific Operational Taxonomic Units (OTUs) in the stool samples of patients gradually decreased.

Conclusion: Our study further confirmed that FMT could be a novel and effective treatment strategy for patients with chronic HBV infection.

Keywords: Hepatitis B virus; Fecal microbiota transplantation; Chronic hepatitis B; Cirrhosis

Introduction

Continuous HBV infection and repeated inflammation of the liver could easily progress to end-stage liver diseases, such as liver failure, cirrhosis, and primary liver cancer, having a great impact on the quality of life and lifespan of patients [1,2]. In China, HBV infection is the most common cause of cirrhosis and primary liver cancer [3], with the proportion of patients with HBV infection-induced cirrhosis being as high as 60% [4], thereby posing a prominent public health problem in China. Therefore, it is particularly important to study HBV-related chronic liver diseases.

With the implementation of the Intestinal Microbial Genome Project, research on the microecology of the digestive tract has received extensive attention [5]. Animal studies have shown that pathways of HBV immune tolerance, particularly through the Toll-Like Receptor 4 (TLR4)-dependent pathway [6,7], exist in young mice before the establishment of intestinal bacteria, while the microbiota in the mature intestinal tract of adult mice have been reported to stimulate the immune response of the liver, leading to rapid HBV clearance [8]. A previous study has shown that the gut microbiome appears to play an important role in the pathophysiological process of chronic liver disease, and the severity of disorders in the flora was shown to be related to the severity of liver disease [9]. Another study has shown that the gut microbiome could regulate immune homeostasis, thereby protecting the liver from viral hepatitis infections [10]. Due to the importance of the intestinal microecological changes on the prognosis of liver diseases [11], effectively improving the intestinal microecological status of patients with chronic HBV infection, understanding the mechanisms governing its occurrence and development, and exploring appropriate interventions and treatment measures for the management of these diseases is urgently needed.

Fecal Microbiota Transplantation (FMT), which refers to the processing of fresh feces from selected healthy donors into a fecal suspension introduced into the gut of patients, has been studied as a method for the treatment of intestinal and extra-intestinal diseases [12,13]. In recent years, as the incidence of Clostridium Difficile Infection (CDI) has increased year by year, FMT has been used increasely in clinical practice. For this reason, the European Conference proposed a consensus on FMT clinical practice and published it in Gut magazine in 2017 [14]. FMT is widely used in intestinal diseases, but there are still relatively few clinical studies on chronic liver diseases, especially HBV-related chronic liver diseases. Therefore, this study will explore the efficacy of FMT in the treatment of HBV-related chronic liver disease and the changes in intestinal microbes.

Citation: Guo Q, Huang S-S, Li J, Tian Y, Zhou Y and Li X-A. Fecal Microbiota Transplantation Slows the Progression of HBV-Related Liver Diseases and Induces Virologic Response in Patients with HBV Infection. Austin J Gastroenterol. 2021; 8(2): 1117.

Materials and Methods

Diagnostic criteria of patients

Patients were diagnosed with chronic HBV infection or hepatitis B cirrhosis according to the Chinese Medical Association Liver Diseases Branch and the Chinese Medical Association Infectious Diseases Branch October 2015 Guidelines for Prevention and Treatment of Chronic Hepatitis B (updated version 2015).

Inclusion criteria

The following criteria needed to be met by patients to be included in our study:

- Patients who have been diagnosed with chronic HBV infection without taking any treatment measures.
- Patients meeting the diagnostic criteria for hepatitis B cirrhosis, with no improvement in the past 3 months through routine treatment of liver (Glutathione, Polyene phosphatidylcholine), diuretic (Furosemide, Spironolactone), or nutritional support.
- Patients who were 18–85 years old, without gender limitations, and a follow-up time greater than 3 months.
- All patients refused to use antiviral drugs due to their own reasons, and at the same time, it was fully evaluated that no antiviral drugs would not cause serious consequences. Therefore, no antiviral drugs were used during treatment and follow-up.
- Patients suitable for and undergoing gastroscopy and FMT treatment.
- Patients who agreed to participate in this clinical study and signed an informed consent form to receive follow-up, as well as consented to the retention of their specimens on time.

Exclusion criteria

Any of the following criteria met by patients, led to their exclusion from our study:

- Pregnant or lactating women; drug users; suspected or confirmed history of alcohol/drug abuse.
- Patients with a history of large abdominal surgery, gastrointestinal bleeding, genetic or metabolic liver diseases.
- Patients suffering from diseases that might cause gastrointestinal tract damage, such as diabetes, thyroid disease, intestinal obstruction, inflammatory bowel disease, etc.
- Patients taking antibiotics, non-steroidal anti-inflammatory drugs, microecological preparations, gastrointestinal motility drugs, acid suppression drugs, and other drugs affecting the intestinal flora 2 weeks before treatment.
- Patients with other serious diseases (including serious infections, severe heart, kidney, respiratory, blood, neuropsychiatric diseases, etc.) that might affect their survival or combined with other types of tumors.

Research design

Due to the complexity of clinical diagnosis and treatment, the clinical implementation process of FMT, and the poor acceptance of FMT in some patients, the random double-blind design could not be effectively applied in clinical practice. Therefore, we conducted a prospective study. According to the clinical diagnosis, the included patients were divided into four groups: Group A1 comprised HBeAg-negative infection. Group A2 included patients with CHB. The included HBV patients with HBeAg-negative infection and CHB had not took antiviral drugs for various reasons, and it is fully assessed that without antiviral drugs would not lead to serious consequences. Therefore, In order to understand the effect of FMT on hepatitis B virus, only a single FMT treatment (1 time/month, 3 times) was accepted during treatment. Group B1 included Child A patients with hepatitis B cirrhosis. Group B2 comprised Child B/C patients with hepatitis B cirrhosis. The included patients with liver cirrhosis who are ineffective in conventional hepatoprotection and diuresis, in order to fully benefit the patients, receive both conventional treatment (comprehensive treatment of routine liver protection, diuresis, nutritional support) and FMT treatment (1 time/month, 3 times). All participants had healthy diet during treatment and follow-up. All the Patients signed an informed consent form. The study was approved by the Ethics Committee of the First Affiliated Hospital of Chengdu Medical College and passed the American Clinical Trial Registration Certification on 01/09/2017 (ClinicalTrials.gov ID: NCT03014505).

Donor screening

We choose healthy men between the ages of 18 and 30 and have a body fat index between 18.5 and 23.9 as donors for FMT. Donors were healthy individuals without diseases or pathologic conditions potentially associated with changes in the gut microbiota. Donors were not use of antibiotics, immunosuppressive medications, systemic antineoplastic agents, and recent ingestion of a potential allergen in the preceding 3 months before screening. After the initial screening, they were subjected to strict examinations (serology and stool tests). Serological tests include blood routine, liver and kidney function, electrolytes, blood sugar, blood lipids, syphilis, HIV, HAV, HBV, HCV. Stool examination includes stool routine, human rotavirus, ova and parasites, Cryptosporidium, Cyclospora, Isospora, Clostridium difficile toxin, and Giardia, Blastocystis hominis and Dientamoeba fragilis. They are considered healthy blood donors only if all serological and stool tests are normal. Taking into account the incubation period of the disease, screening and inspection are repeated every 6 months. In addition, the fecal donors with healthy diet, regular exercise every day, no smoking, no alcohol, and no other unhealthy foods, such as preserved and canned food. In the end, 4 healthy people who met the requirements successfully donated their stools.

Preparation of fecal suspensions

In the morning, the fecal sample of the donor that was not contaminated by urine was placed in a sterile closed container. Immediately after receiving the specimen, the fecal suspension was prepared (completed within 2h). A 50g stool sample was added to 250ml of 0.9 % physiological saline, and a glass rod was used to homogenize it. The homogenized suspension was then filtered through a stainless-steel molecular sieve having a diameter of 2.0, 1.0, 0.5, and 0.25 mm, with the larger particulate matter being filtered out. Subsequently, the filtered slurry was centrifuged at 6000 g for 15 min, discard the supernatant and added 50 ml physiological saline solution to homogenize and resuspended the pellet.

FMT infusion

Patients were required to be fasting for at least 8 h before FMT treatment. For the FMT treatment, freshly prepared bacterial solution was used. All patients underwent gastroscope FMT treatment: the patient was placed in the left lateral position under propofol intravenous anesthesia, and after the gastroscope reached the duodenal level, a sterile catheter was inserted from the gastroscopic biopsy hole, and then, the prepared bacterial solution (50ml) was slowly injected from the catheter with a syringe. After completion of the injection of the solution, the catheter and the gastroscope were slowly withdrawn to avoid aspirating the air, and patients were observed for potential back flow of the fecal suspension. After FMT treatment, the patient was required to maintain a half-sitting position of 45 degrees for at least 4h, follow a light liquid diet after fasting for 2-4 h, and stay in the hospital for at least 8-10 h.

Assessments

Blood samples: 5ml of peripheral venous blood was collected from patients 2 days before FMT treatment and 3 months after the last FMT treatment. We evaluate liver function and HBV-DNA indicators before and after treatment. The amount of Diamine Oxidase (DAO), D-lactic acid, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), Collagen Type IV (COL IV), Procollagen III Propeptide (PIIIP), Hyaluronic Acid (HA) and Laminin (LN) were measured in the serum of patients using the human ELISA kit, according to the manufacturer's instructions.

Intestinal flora analysis: 3-5 g of early morning feces were collected 2 days before FMT treatment and one month after each treatment of FMT, and 2 and 3 months after the last treatment, placed in sterile EP tubes, and then stored in a refrigerator at -80°C. We used the fecal genomic DNA extraction kit to extract total bacterial DNA from the stool samples. The concentration and purity of DNA samples were verified by nanodrop 2000 instrument and sent to Tianjin novo Zhiyuan sequencing Co., Ltd. for 16S sequencing. The Illumina Hiseq-PE250 technology-sequencing platform was used to perform 16S V3-V4 region sequencing of the sample using the double-end sequencing method, and corresponding data were statistically analyzed according to the sequencing results.

Statistical analysis

Obtained data were expressed as the mean ± standard error (se). Mean values were compared using the *t*-test. The Mann-Whitney test was used to compare the measurement data between two or more groups. The Wilcoxon rank sum test was used for comparison between two or more groups, and the chi-square test was used for comparison of the count data. A P value <0.05 indicated a statistically significant difference.

Results

Baseline conditions of the subjects in this study

A total of 35 patients were enrolled in this study: 18 patients with chronic HBV infection, including 14 males and 4 females, aged between 24 to 71 y, with an average age of 44.83 ± 12.24 y (Among these, there were 10 cases of HBeAg-negative infection, and 8 cases of patients with CHB). 17 patients with hepatitis B cirrhosis, including 12 males and 5 females, aged between 41 to 82 y, with an average age of 54.59 ± 11.09 y (among these, there were 8 cases of Child-Pugh A grade, 8 cases of B grade, and 1 case of C grade) (Table 1).

FMT improved liver function in patients with HBV-related chronic liver disease

In the group of chronic HBV infection, the liver function indicators did not change much after FMT, but all indexes were within the clinical normal reference range before and after treatment in HBeAg-negative infection patients. In contrast, with the treatment of FMT, the levels of Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Gamma-Glutamyl Transferase (GGT) were significantly reduced in CHB patients (P <0.05, Table 2).

Table 1: Baseline Demographic of the enrolled Patients.

	Chronic HBV infection		Hepatitis B cirrhosis		
Group	**HBV patients with HbeAg negative**	**CHB**	**Child-pugh A**	**Child-pugh B**	**Child-pugh C**
Number	10	8	8	8	1
Age (y)	44.83±12.24		41-82 (54.59±11.09)		

Data are expressed as n, mean ± standard deviation.

Table 2: The level of liver function indexes in patients with chronic HBV infection.

Group (n) Time	Group A1 (10)			Group A2 (8)		
	Before FMT	**After FMT**	**P**	**Before FMT**	**After FMT**	**P**
ALT (U/L)	34.56±13.99	35.00±20.86	0.9072	129.1±79.56	52.38±37.78	0.0469*
AST (U/L)	36.67±17.32	34.11±17.52	0.6563	73.13±48.88	42.25±29.82	0.0395*
GGT (U/L)	21.11±12.87	20.11±11.29	0.7109	68.75±47.56	47.25±31.84	0.0424*
PA (mg/L)	192.0±61.70	197.0±40.22	0.6021	204.8±79.61	186.8±81.76	0.2614
ALB (g/L)	43.79±5.022	44.56±4.988	0.6631	43.83±3.764	42.76±3.504	0.3681
CHE (U/L)	310.9±119.0	325.2±88.61	0.2359	344.0±121.5	327.9±118.0	0.2653
ALP (U/L)	89.67±31.81	81.44±20.22	0.1549	111.8±80.30	88.75±39.38	0.2081
TBI (umol/L)	25.11±23.33	18.48±8.255	0.2391	15.06±4.905	15.61±4.409	0.745
TB (umol/L)	24.91±58.22	12.32±20.35	0.3515	3.513±2.039	3.325±1.757	0.6741

Group A1 was HBeAg-negative infection patients. Group A2 was CHB patients. Compared with before treatment. *P<0.05.

Table 3: The level of liver function in patients with Hepatitis B cirrhosis.

Group (n) Time	Group B1 (8)			Group B2 (9)		
	Before FMT	After FMT	P	Before FMT	After FMT	P
ALT (U/L)	45.88±22.67	57.75±27.19	0.5845	30.67±14.15	25.67±8.078	0.5162
AST (U/L)	51.63±21.37	54.63±44.86	0.8509	46.22±21.63	40.11±13.20	0.241
GGT (U/L)	78.75±39.49	66.38±45.14	0.556	90.11±86.39	69.11±79.40	0.0717
PA (mg/L)	141.1±44.51	135.5±38.97	0.7036	89.78±19.27	100.7±55.52	0.5116
ALB (g/L)	40.48±5.763	41.80±3.507	0.3963	33.62±3.118	34.68±3.492	0.4583
CHE (U/L)	209.9±64.58	223.8±50.72	0.4226	178.6±68.95	177.3±73.01	0.9262
ALP (U/L)	95.13±22.83	94±29.96	0.9336	145.7±52.51	158.4±47.85	0.1159
TBI (umol/L)	22.59±11.88	25.68±12.34	0.5037	33.08±15.47	30.73±17.85	0.0425*
TB (umol/L)	24.51±19.62	20.96±16.74	0.6983	60.12±48.33	57.08±41.08	0.6052
Score	5.375±0.5175	5.250±0.4629	0.5983	8.444±1.333	6.556±1.333	0.0013*

Group B1 was Child-pugh A patients. Group B2 is Child-Pugh B/C patients. Score was Child-pugh score. Compared with before treatment. *P<0.05.

Table 4: The level of HBeAg and HBV-DNA in patients with HBV-related chronic liver disease.

Group	Time	HbeAg(n)			HBV-DNA(n)		
		Positive(+)	Negative(-)	Response rate (%)	Positive(+)	Negative(-)	Response rate (%)
A	Before FMT	9	9	36.4	11	7	53.3
	After FMT	6	12		6	12	
B	Before FMT	2	15		4	13	
	After FMT	1	16		1	16	

Group A was HBeAg-negative infection and CHB patients. Group B was Child-pugh A and Child-Pugh B/C patients.

Table 5: The serum level of TNF-α, IL-6, DAO and D-lactate in patients with Hepatitis B cirrhosis.

Group	Time	TNF-α (ng/ml)	IL-6 (pg/ml)	DAO (mIU/ml)	D-lactate (uM/ml)
B1	Before FMT	16.87±3.940	170.0±21.50	321.3±81.10	215.8±34.10
	After FMT	16.05±3.760	167.6±40.53	258.9±53.48	194.9±55.52
	p	0.0447*	0.8391	0.0375*	0.0423*
B2	Before FMT	22.88±3.843	245.9±50.28	383.6±99.2	243.5±55.13
	After FMT	21.95±4.515	231.8±33.62	352.2±83.61	221.9±51.10
	P	0.2611	0.0489*	0.2066	0.0448*

Group B1 was Child-pugh A patients. Group B2 was Child-Pugh B/C patients. Compared with before treatment. *P<0.05.

In the group of hepatitis B cirrhosis, there was no significant difference in liver function after FMT treatment for Child-Pugh A patients. However, the levels of ALT, AST, GGT, total bilirubin (TBIL), TBA and Child-Pugh scores decreased in Child-Pugh B/C patients of hepatitis B liver cirrhosis, while the levels of PA and ALB increased. In particular, TBIL and Child-Pugh scores were significantly reduced (P <0.05, Table 3).

These results indicated that FMT could improve liver function in some patients with HBV-related chronic liver disease. And these results show a trend that the improvement is more obvious as the severity of the HBV-related chronic liver disease.

FMT controlled the replication of hepatitis B virus, and converted HBeAg-positive patients to HBeAg-negative

In order to evaluate the effect of FMT on hepatitis virus, all patients included did not take any antiviral drugs before and during the study. In the chronic HBV infection group, 3 of 9 HBeAg-positive patients achieved negative conversion, and in the liver cirrhosis group, 1 of 2 HBeAg-positive patients achieved negative conversion after FMT. Therefore, among the 11 HBeAg-positive patients, the converted negative ratio of E antigen was 36.4% (4/11) (Table 4). With the treatment of FMT, in the chronic HBV infection group, 5 of 11 HBV-DNA positive patients achieved negative conversion, and in the liver cirrhosis group, 3 of 4 HBV-DNA positive patients achieved negative conversion. The overall viral response rate among 15 HBV-DNA positive patients was 53.3% (8/15) (Table 4). Those results indicated that FMT treatment could control the replication of hepatitis B virus, decrease HBV-DNA positive to negative, and convert HBeAg-positive patients to the HBeAg-negative status. It showed that FMT could elicit antiviral effects.

FMT enhanced the intestinal mucosal barrier function in patients with hepatitis B cirrhosis

TNF-α and IL-6 are the initiating factors of the damage of the intestinal mucosal barrier [15]. And DAO and D-lactic acid are released into the blood when the intestinal mucosal barrier function is impaired. Therefore, determination of the levels of TNF-α, IL-6, DAO and D-lactic in peripheral blood can be used to indirectly reflect the intestinal mucosal barrier [16]. The levels of serum serum TNF-α,

Table 6: The serum level of COL IV, HA, PIIIP and LN in Patients with Chronic HBV Infection.

Group	Time	COL IV (ng/ml)	HA (ng/ml)	P III P (ng/ml)	LN (ng/ml)
A1	Before FMT	1722±792.9	36.73±24.00	29.33±17.55	14.33±13.40
	After FMT	1216±916.1	32.94±22.56	26.85±18.30	14.29±11.75
	p	0.0447*	0.0049*	0.2555	0.9743
A2	Before FMT	1624±935.6	21.18±19.33	24.57±15.52	23.30±11.07
	After FMT	1579±748.9	21.62±19.01	23.95±14.67	21.66±14.18
	P	0.8476	0.6359	0.8225	0.0374*

Group A1 was HBeAg-negative infection patients. Group A2 was CHB patients. Compared with before treatment. *P<0.05.

IL-6, DAO and D-lactic acid in patients with hepatitis B liver cirrhosis decreased after FMT. For Child A patients, the indicators of TNF-α, DAO and D-lactic acid showed a significant decrease, while for Child B/C patients, IL-6 and D-lactic acid were significantly reduced (P <0.05, Table 5). It suggested that the intestinal mucosal barrier of patients with cirrhosis was improved after FMT treatment.

FMT relieved the degree of liver fibrosis in patients with chronic HBV infection

Liver biopsy is the gold standard for testing liver fibrosis, but it was not used in this study due to its high risk, difficulty and cost. For patients with chronic liver disease, the levels of COL IV, HA, PIIIP, and LN could be used to evaluate the degree of liver fibrosis. In this study, the serum levels of COL IV, HA, PIIIP, LN decreased in patients with chronic HBV infection after FMT treatment. For HBeAg-negative infection patients, the levels of COL IV and HA decreased significantly, whereas the levels of LN decreased significantly (P <0.05, Table 6) in patients with CHB. Which indicated that FMT might improve liver fibrosis through a certain mechanism.

Differences in gut microbiota in patients with HBV-related chronic liver disease

In order to evaluate changes in the intestinal microbial community induced by FMT, we used 16srDNA gene amplification and sequencing technology to analyze the variable region V3-V4 of stool samples before and after FMT treatment. The results were evaluated using OTUs with a 97% homology cutoff. We found that the A (chronic HBV infection) and B (hepatitis B cirrhosis) group of the study yielded 4577262 and 4477089 high-quality sequences, respectively, resulting in 9692 and 9780 OTUs, respectively.

Next, we evaluated the relative abundances of the main taxonomic groups of the intestinal flora and analyzed the structures of the intestinal flora at the phylum and genus. In samples of chronic HBV infection patients, *Bacteroides* represented the most important bacterial category, accounting for more than 54.3% of all bacteria at the phylum (Figure 1A). In addition, at the genus level, bacteria with relatively high abundances included (in decreasing order) *Bacteroides*, *Prevotella* and *Rumenococcus* accounted for 26.4%, 21.3%, and 9.4% of the total sequence, respectively (Figure 1B). Meanwhile, we valuation of changes in fecal bacterial composition of donor and patient samples at different treatment stages. The composition of fecal bacteria in the samples of donors and patients at different treatment stages was dominated by Pachybacteria and *Bacteroides* (Figure 1C). At the genus level, *Bacteroides* (Bacteroidetes), *Prevotella* (Bacteroidetes), and *Rumenococcus* (Firmicutes) were the most important genera in all samples (Figure 1D). In the samples of patients with hepatitis B cirrhosis, Pachybacteria and *Bacteroides* form the two most important phyla, accounting for 43.0% and 49.3% of the total sequences, respectively (Figure 1E). At the genus level, *Bacteroides* was the most important genus in all stool specimens (Figure 1F). In the grouped samples of donors and patients at different treatment stages, the composition of fecal bacteria was dominated by Pachybacteria and *Bacteroides* (Figure 1G). At the genus level, the feces of donors and patients mainly comprised *Bacteroides*, *Prevotella*, and *Rumenococcus* (Figure 1H). In short, the individual bacterial phyla and genera were observed to gradually resemble the gut microbiota of the donor after FMT treatment.

Venn map analysis was performed to analyze the difference of intestinal flora before and after FMT treatment, as well as the difference of intestinal flora between the donors and the patients. The Venn diagram reflects total number of OTUs in each group, the overlap OTUs between all the group, and unique OTUs in each group. The number of specific OTUs before (D0) and after (D1, D2, D3) treatment in patients with chronic HBV infection was 383, 290, 563, and 49, respectively, showing a gradually decreasing trend (Figure 2A). The number of specific OTUs of fecal samples before (D0) and after (D1, D2, D3) treatment was 381, 287, 223, and 93, respectively, in patients with hepatitis B cirrhosis (Figure 2B). Accordingly, it was shown that the relative abundance of specific gut microbiota in the feces of patients after FMT treatment was gradually decreased.

Mild discomfort after FMT treatment

35 patients with HBV-related liver diseases had no serious adverse reactions during treatment and follow-up. 18 patients experienced mild discomfort, including 5 patients with mild abdominal pain and discomfort after treatment, 3 patient with abdominal distension. 4 patients with diarrhea, 2 patients with difficulty defecation, 4 patients with fever. Without medical treatment, all discomfort eased itself within 48-72 hours.

Discussion

FMT has been shown to improve liver function in patients with HBV-related chronic liver disease. In this study, the levels of ALT, AST, GGT, ALP and TBA were observed to be decreased after FMT treatment, indicating that the liver function in patients with CHB was restored. In Child-Pugh B/C patients, the synthesis and metabolism of the liver are poor. After FMT treatment, all indicators showed signs of improvement. Especially, the TBIL content and Child-Pugh score were significantly improved. Another study has clearly indicated that the metabolism of bilirubin was correlated to the gut microbiota, and cholestasis could lead to increased cytotoxicity [17]. In this study, the TBIL index of the patients was significantly restored, suggesting that the gut microbiota of the patients may changed following FMT. In

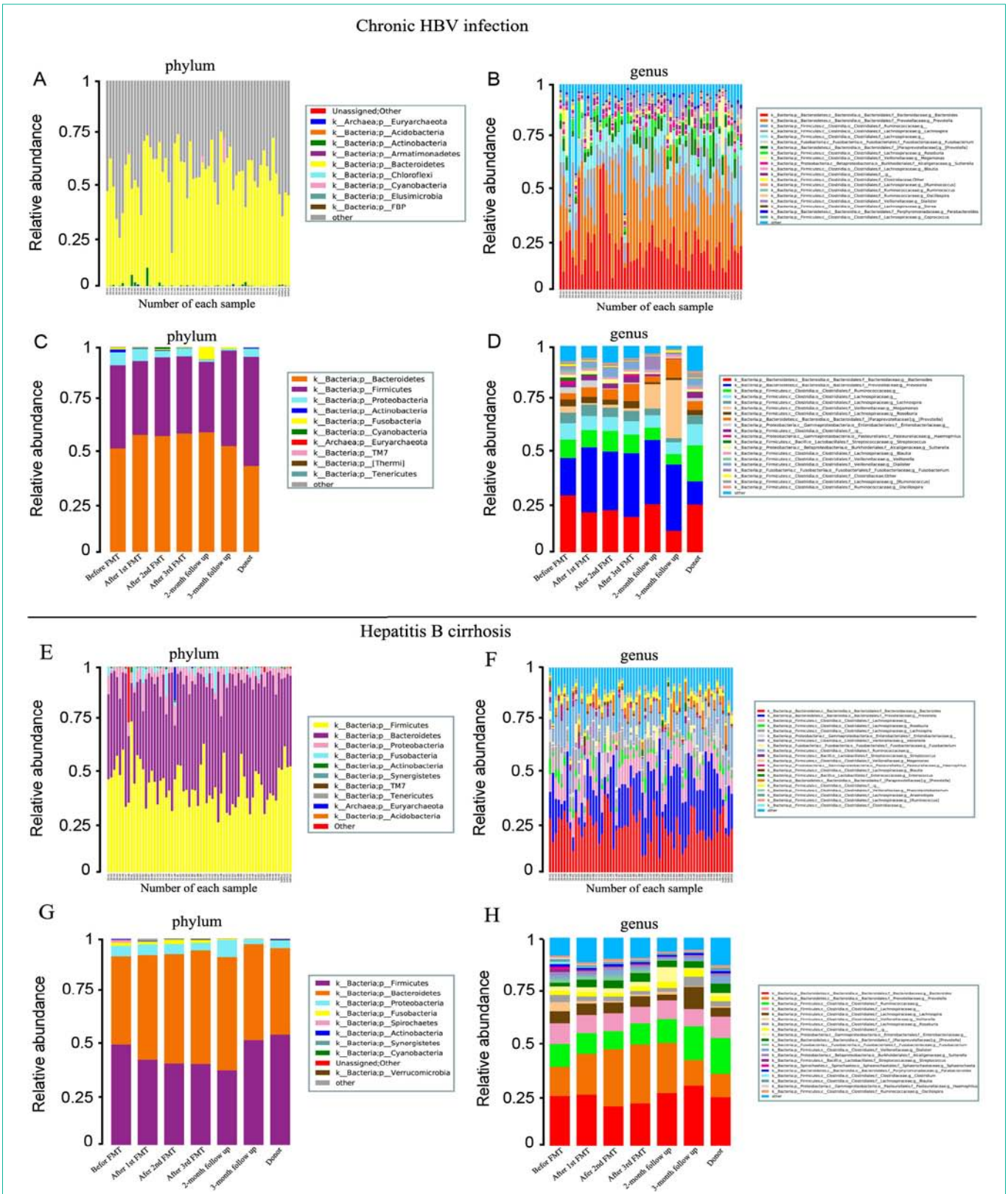

Figure 1: Community structure of gut microbiota (phylum and genus level) in patients with chronic HBV infection, hepatitis B cirrhosis and donors. The x-coordinate represents the sample group, while different colors represent different species. (A) Phylum level for each sample in patients with chronic HBV infection. (B) Genus level for each sample in patients with chronic HBV infection. (C) Relative abundance of species for each group at the phylum levels in patients with chronic HBV infection. (D) Relative abundance of species for each group at the genus levels in patients with chronic HBV infection. (E) Phylum level for each sample in patients with hepatitis B cirrhosis. (F) Genus level for each sample in patients with hepatitis B cirrhosis. (G) Relative abundance of species for each group at the phylum levels in patients with hepatitis B cirrhosis. (H) Relative abundance of species for each group at the genus levels in patients with hepatitis B cirrhosis.

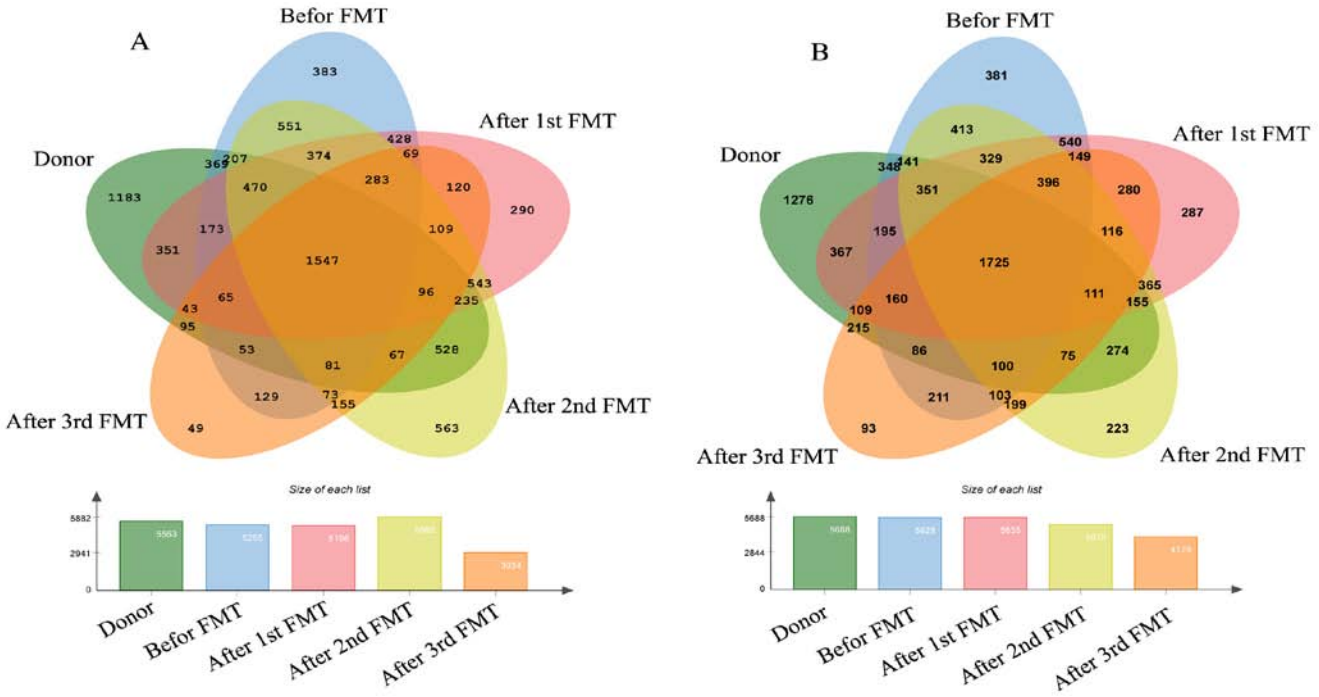

Figure 2: Venn map of the common and unique gut microbiota OTUs of donors and patients with chronic HBV infection (A) and hepatitis B cirrhosis (B). Each circle represents a sample group. The number of circles in overlapping areas represent the number of OTUs shared between the sample groups, whereas the number without overlap represents the number of OTUs unique to the sample group. The histogram showed the total OTUs in each group.

conclusion, in our study, FMT treatment improved liver function in patients with hepatitis B cirrhosis.

HBV DNA is a marker of HBV infection and can thus be used for risk stratification of liver cancer. Studies reported that the serum HBV DNA level (≥10,000 copies/mL) is a strong risk predictor of hepatocellular carcinoma independent of HBeAg, serum alanine aminotransferase level, and liver cirrhosis [18]. For patients with chronic HBV infection, it has been recommended that the amount of HBV DNA and HBsAg be reduced to low levels in order to reduce the risk of liver cancer [19]. To achieve this goal, the currently accepted method has been antiviral therapy, but antiviral drugs can only inhibit viral replication, and not completely eradicate the virus [20]. Moreover, a series of problems such as long use period, poor compliance and high price of antiviral drugs often prevent some patients from achieving the treatment goals. In our study, it was found that FMT treatment could control the replication of hepatitis B virus, decrease HBV-DNA positive to negative, and convert HBeAg-positive patients to the HBeAg-negative status. These results showed that FMT could elicit antiviral effects. FMT can achieve the efficacy of certain antiviral drugs, and this method may fundamentally improve the body's immunity to eliminate HBV, instead of inhibiting viral replication like antiviral drugs to achieve therapeutic goals. This result provides a certain basis for the use of FMT in the treatment of hepatitis B. However, in the experiment, not all HBV-DNA-positive patients were well controlled after FMT treatment. Therefore, in the next step, we need to design more rigorous experiments for in-depth research.

The gut microbiota plays an important role in maintaining the integrity of the intestinal barrier [21]. Our study showed that serum TNF-α, IL-6, DAO, and D-lactic acid levels decreased in patients with hepatitis B cirrhosis after FMT treatment, suggesting that the intestinal mucosal barrier of patients with liver cirrhosis improved after FMT treatment. The intervention of FMT on the intestinal flora of patients with hepatitis B liver cirrhosis can delay the progression of the disease by regulating the disturbance of intestinal flora, the imbalance of intestinal barrier function and related cytokines. At the same time, the results suggest that intestinal flora disorder, intestinal barrier function imbalance and related cytokine networks may be involved in the pathogenesis of chronic liver disease, and the intervention and regulation of intestinal flora may be the target to delay the occurrence and development of chronic liver disease and improve the prognosis.

The unique analysis of OTUs, which revealed the number of OTUs unique to the intestinal tract of the two types of patients, exhibited a significant downward trend with time delay, similar to other studies [22]. This finding indicated that unique flora are involved in the process of disease progression, and as the number of unique flora in patients was significantly reduced after FMT treatment, the condition of the patients could be controlled. However, specifying and identifying the flora that might be playing a role in this process might require a more in-depth statistical analysis or further clarification using metagenomic sequencing [23].

Our study further confirmed that FMT might serve as a novel and effective treatment for patients with chronic HBV infection. At present, the technology used by FMT is relatively mature, and the treatment mechanism is mainly to achieve the purpose of curing diseases by rebuilding a new intestinal flora and realizing a new flora-host interaction. However, there are still many problems in the

clinical application of FMT (e.g: How long is the best time for the effect of FMT? How strict is the donor screening? How to transplant FMT?), and further research and discussion are needed. Due to the limitations of study time, manpower, and patient FMT acceptance, the number of cases included in this study is small and the study period is short. This has a certain impact on the results of the study, and further large-scale clinical studies are needed. In short, FMT research needs to combine short-term efficacy and long-term follow-up to better evaluate clinical efficacy and safety.

Declaration

Authors' contributions: Yan Zhou, Xiao-An Li were involved in the conception and design of the study. Si-Si Huang, Qin Guo and Jun li acquired the data. Si-Si Huang and Qin Guo conducted data analysis. Qin Guo drafted the manuscript. Si-Si Huang, Qin Guo, Jun li, Yan Tian, Yan Zhou, Xiao-an Li critically revised the manuscript for intellectual content, approved the final version and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Funding: The present study was supported by the Key project of Sichuan provincial health commission (Grant No. 19ZD003).

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate: The trial was registered with American Clinical Trial Registration Certification (ClinicalTrials.gov ID: NCT03013712). All patients and donors gave written informed consent after counseling about the study and its potential risks and benefits.

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

References

1. Sarmati L, Malagnino V. HBV Infection in HIV-Driven Immune Suppression. Viruses. 2019; 11: 1077.
2. Jia L, Gao Y, He Y, et al. HBV induced hepatocellular carcinoma and related potential immunotherapy. Pharmacol Res. 2020; 159: 104992.
3. Liu Y, Corsa AC, Buti M, et al. No detectable resistance to tenofovir disoproxil fumarate in HBeAg+ and HBeAg- patients with chronic hepatitis B after 8 years of treatment. J Viral Hepat. 2017; 24: 68-74.
4. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of Hepatocellular Carcinoma. Hepatology. 2021; 73: 4-13.
5. Yang M, Yang Y, He Q, et al. Intestinal Microbiota-A Promising Target for Antiviral Therapy? Front Immunol. 2021; 12: 676232.
6. Zhang E, Lu M. Toll-like receptor (TLR)-mediated innate immune responses in the control of hepatitis B virus (HBV) infection. Med Microbiol Immunol. 2015; 204: 11-20.
7. Tsai KN, Kuo CF, Ou JJ. Mechanisms of Hepatitis B Virus Persistence. Trends Microbiol. 2018; 26: 33-42.
8. Terrault NA, Bzowej NH, Chang KM, et al. AASLD guidelines for treatment of chronic hepatitis B. Hepatology. American Association for the Study of Liver Diseases. 2016; 63: 261-283.
9. Chou HH, Chien WH, Wu LL, et al. Age-related immune clearance of hepatitis B virus infection requires the establishment of gut microbiota. Proc Natl Acad Sci USA. 2015; 112: 2175-2180.
10. Chauhan A, Kumar R, Sharma S, et al. Fecal Microbiota Transplantation in Hepatitis B e Antigen-Positive Chronic Hepatitis B Patients: A Pilot Study. Dig Dis Sci. 2021; 66: 873-880.
11. Wahlström A. Outside the liver box: The gut microbiota as pivotal modulator of liver diseases. Biochim Biophys Acta Mol Basis Dis. 2019; 1865: 912-919.
12. Choi HH, Cho YS. Fecal Microbiota Transplantation: Current Applications, Effectiveness, and Future Perspectives. Clin Endosc. 2016; 49: 257-265.
13. Brandt LJ, Aroniadis OC. An overview of fecal microbiota transplantation: techniques, indications, and outcomes. Gastrointest Endosc. 2013; 78: 240-249.
14. Cammarota G, Ianiro G, Tilg H, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. 2017; 66: 569-580.
15. Aller R, De Luis DA, Izaola O, et al. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci. 2011; 15: 1090-1095.
16. Malaguarnera M, Vacante M, Antic T, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci. 2012; 57: 545-553.
17. Li Y, Tang R, Leung PSC, et al. Bile acids and intestinal microbiota in autoimmune cholestatic liver diseases. Autoimmun Rev. 2017; 16: 885-896.
18. Chen CJ, Yang HI, Su J, et al. REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006; 295: 65-73.
19. Yang Y, Gao J, Tan YT, et al. Individual and combined effects of hepatitis B surface antigen level and viral load on liver cancer risk. J Gastroenterol Hepatol. 2018; 33: 1131-1137.
20. Yapali S, Talaat N, Lok AS. Management of hepatitis B: our practice and how it relates to the guidelines. Clin Gastroenterol Hepatol. 2014; 12: 16-26.
21. Caricilli AM, Castoldi A, Câmara NO. Intestinal barrier: A gentlemen's agreement between microbiota and immunity. World J Gastrointest Pathophysiol. 2014; 5: 18-32.
22. Wang J, Wang Y, Zhang X, et al. Gut Microbial Dysbiosis Is Associated with Altered Hepatic Functions and Serum Metabolites in Chronic Hepatitis B Patients. Front Microbiol. 2017; 8: 2222.
23. Wu ZW, Lu HF, Wu J, et al. Assessment of the fecal lactobacilli population in patients with hepatitis B virus-related decompensated cirrhosis and hepatitis B cirrhosis treated with liver transplant. Microb Ecol. 2012; 63: 929-937.