Observational Study

Medicine

OPEN

Changes in intestinal microbiota of HBV-associated liver cirrhosis with/without hepatic encephalopathy

Xiaoli Hua, PhD[a], Hao Feng, MD[a,b,*]

Abstract

The compositional balance of intestinal microbiota plays an important role in maintaining homeostasis. This study aimed to investigate the intestinal flora of hepatitis B virus-associated liver cirrhosis (HBV-LC) with or without hepatic encephalopathy (HE) and how it relates to the disease. A total of 20 patients with HBV-LC were enrolled in this study, along with 10 healthy adults. The participants were divided into HE group, non-HE group, and control group. Fecal samples were collected under the condition of patients' daily diet, and the 16S rRNA test was performed for each fecal sample. The relative abundance of *Bacteroidia*, *Streptococcaceae*, *Streptococcus*, *Veillonella*, *Bacteroidales*, *Lactobacillales*, *Pasteurellales,* and *Veillonella parvula* increased in the HBV-LC group. Meanwhile, the relative weights of *Pasteurellales*, *Pasteurellaceae*, *Haemophilus*, and *Selenomonas* significantly increased in the HE group. Furthermore, in the non-HE group, the relative abundance of *Veillonella* increased. Intestinal microbiota was significantly different from controls with respect to a lack of potentially beneficial autochthonous bacteria and overgrowth of potentially pathogenic genera in patients with HBV-LC. Moreover, there was a greater change in the relative abundance of intestinal flora when complicated with HE.

Abbreviations: HBV = hepatitis B virus, HBV-LC = hepatitis B virus-associated liver cirrhosis, HE = hepatic encephalopathy, nHE = nonhepatic encephalopathy, OTU = operational taxonomic unit, NAFLD = nonalcoholic fatty liver disease

Keywords: *Bacteroidia*, hepatic encephalopathy, hepatitis B virus, hepatitis B virus-associated liver cirrhosis, intestinal microbiota, nonhepatic encephalopathy, *Pasteurellaceae*, *Streptococcus*, *Streptococcaceae*, *Veillonella*

1. Introduction

It has been proved that the compositional balance of intestinal microbiota plays an important role in maintaining homeostasis. In patients with hepatitis B virus (HBV)–associated liver cirrhosis (HBV-LC), this balance is disturbed; when complicated with hepatic encephalopathy (HE), this imbalance is more overt.[1,2] However, the mechanism underlying this change in the intestinal flora affects the progression of HBV infection remains unclear, and there is still some conflicting data. Therefore, to further investigate the intestinal flora of patients with different grades of HBV-LC and how it relates to the disease, we designed the following study.

2. Materials and Methods

2.1. Patients

A total of 20 inpatients in Nanjing Jiangbei Hospital from January 2019 to June 2020 with HBV-LC were enrolled in this study (HBV-LC group). These patients had a mean age of 60.65 ± 12.42 years (range: 36–89 years) and included 5 females (25%) and 15 males (75%). Among them, 5 patients matching the definition of HE were included in the HE group and 15 in the non-HE (nHE) group. The diagnosis was based on the guidelines for chronic hepatitis B diagnosis of the American Association for the Study of Liver Diseases.[3] All patients tested positive for serum HBsAg for >24 weeks. Ten healthy individuals were also enrolled as normal controls (control group), as shown in Table 1.

The exclusion criteria included the following: a positive pregnancy test in females, received immunomodulator treatment in the previous 6 months, coinfection with human immunodeficiency virus, presence of thyroid dysfunction, alcoholic hepatitis, autoimmune diseases, and psychological issues. The study protocol was approved and monitored by the Ethics Committee of Nanjing Jiangbei Hospital, and written informed consent was obtained from all participants. All data could be accessed after data collection.

The project supported by the Medical Technology Development of Nanjing (YKK18243); Nanjing Medical Science and Technique Development Foundation (QRX17098).

Written informed consent was obtained from the patient for publication of this article and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The study protocol was approved and monitored by the ethics committee of Nanjing Jiangbei Hospital, and written informed consent was obtained from the patients.

Supplemental Digital Content is available for this article.

[a] *Infectious Diseases Section, Nanjing Jiangbei Hospital, Nanjing, China,* [b] *Infectious Diseases Section, Nanjing Pukou Central Hospital, Pukou Branch Hospital of Jiangsu Province Hospital, Nanjing, China.*

**Correspondence: Hao Feng, Infectious Diseases Section Nanjing Pukou Central Hospital, Pukou Branch Hospital of Jiangsu Province Hospital, No.166, Shanghe Street, Nanjing 211800, China (e-mail: drill2004@sina.com).*

How to cite this article: Hua X, Feng H. Changes in intestinal microbiota of HBV-associated liver cirrhosis with/without hepatic encephalopathy. Medicine 2022;101:33(e29935).

Received: 21 October 2021 / Received in final form: 7 June 2022 / Accepted: 16 June 2022

http://dx.doi.org/10.1097/MD.0000000000029935

Table 1

Baseline characteristics of the groups.

	Control	HBV-LC	nHE	HE
Age (yr)	59.21 ± 11.09	60.65 ± 12.42	61.73 ± 13.55	60.11 ± 9.54
M/F	7/3	15/5	11/4	4/1
ALT (U/L)	13.24 ± 16.64	101.35 ± 67.52**	140.13 ± 65.02**	95.16 ± 107.04**
AST (U/L)	19.58 ± 13.02	112.07 ± 133.45**	151.04 ± 39.41**	89.15 ± 118.25**
PT (s)	13.12 ± 3.04	18.21 ± 11.12*	16.46 ± 4.52*	20.16 ± 8.21**
TB (μmol/L)	18.02 ± 11.36	48.22 ± 71.62**	41.25 ± 39.41**	68.02 ± 70.16**
WBC ($\times 10^9$/L)	5.40 ± 1.68	4.14 ± 2.27*	4.45 ± 2.11*	2.40 ± 2.14**
ALB (g/L)	41.74 ± 7.6	31.17 ± 10.3**	34.11 ± 9.5**	25.75 ± 11.25**
HBV-DNA (Log10 copies/mL)	None	3.74 ± 3.07	3.98 ± 3.75	3.14 ± 2.97

ALB = albumin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, HBV = hepatitis B virus, HBV-LC = hepatitis B virus-associated liver cirrhosis, HE = hepatic encephalopathy, nHE = nonhepatic encephalopathy, PT = prothrombin time, TB = total bilirubin, WBC = white blood cell.

*$P < .05$;

**$P < .01$.

2.2. Methods

Fecal samples were collected under the condition of patients' daily diet, and the 16S rRNA test was performed for each fecal sample. 16S rRNA-targeted sequencing was performed according to the Earth Microbiome Project standard protocols (http://www.earthmicrobiome.org). PCR amplification of the extracted DNA, along with water controls, was conducted with barcoded primers targeting the V4 region of the 16S rDNA gene 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′).

2.3. Statistical analysis

Results of sequences are reported as mean ± standard deviation. Independent samples *t* test was performed for statistical comparison between the 2 groups, whereas the 1-way ANOVA method was performed for multiple comparisons. Data analysis and sample size calculation were conducted using the SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL). Comparisons of bacterial relative abundance between groups were performed using nonparametric *t* tests on nontransformed data. All statistical analyses involving 16S sequencing data were performed using QIIME 1.9. The confidence interval was 95%.

3. Results

3.1. Baseline characteristics of the groups

There was no significant difference in the average age and gender between the groups, and the liver function indexes of the cirrhosis group were significantly abnormal compared with the control group (Table 1).

A total of 1898,796 sequences were obtained, and these samples contained 57,042 to 72,107 sequences. These data were clustered into 655 operational taxonomic units (OTUs). The HBV-LC group had more OTUs than the control group (597 vs 532), whereas a total of 474 OTUs were present in both groups (Fig. 1A). Subgroup analysis showed that there were 568 OTUs in the nHE group, 443 OTUs in the HE group, and only 364 OTUs in all 3 groups (Fig. 1B).

3.2. Difference in bacterial relative abundance between HBV-LC and control groups

In the HBV-LC group, the abundance of the following significantly increased: *Bacteroidia* (37.95 ± 20.19 vs 23.52 ± 16.31, $P = .049$) at the class level; *Streptococcaceae* (5.60 ± 9.07 vs 0.81 ± 1.30, $P = .035$) at the family level; *Streptococcus* (5.60 ± 9.07 vs 0.81 ± 1.30, $P = .039$) and *Veillonella* (4.34 ± 5.96 vs 0.01 ± 0.01, $P < .001$) at the genus level; *Bacteroidales* (37.95 ± 20.20 vs 23.52 ± 16.31, $P = .049$), *Lactobacillales* (8.29 ± 12.75 vs 1.07 ± 1.61, $P = .024$), and *Pasteurellales* (2.00 ± 3.85 vs 0.48 ± 1.53, $P = .002$) at the order level; and *Veillonella parvula* (2.32 ± 3.485 vs 0.01 ± 0.01, $P < .001$) at the species level.

In contrast, in the control group, the relative abundance of the following significantly increased: *Lachnospiraceae* (9.69 ± 6.34 vs 29.67 ± 16.74, $P < .001$) at the family level; *Adlercreutzia* (0.004 ± 0.013 vs 0.06 ± 0.11, $P = .012$) and *Blautia* (1.31 ± 1.72 vs 13.48 ± 11.82, $P < .001$) at the genus level; *Turicibacterales* (0.01 ± 0.03 vs 1.65 ± 4.35, $P = .044$) at the order level; and *Blautia obeum* (0.01 ± 0.14 vs 0.95 ± 1.30, $P = .028$) at the species level (Table 2, Fig. 1C, Supplement material, http://links.lww.com/MD/H238).

3.3. Difference in bacterial relative abundance among nHE, HE, and control groups

Although there was no significant difference among the nHE, HE, and control groups at the class level, the relative abundance of the following significantly increased in both nHE and HE groups: *Pasteurellaceae* (1.86 ± 3.92 and 2.39 ± 4.04 vs 0.49 ± 1.53, $P = .007$) at the family level; *Haemophilus* (1.74 ± 3.64 and 2.34 ± 3.96 vs 0.43 ± 1.35, $P = .011$), *Selenomonas* (0.02 ± 0.04 and 0.01 ± 0.02 vs 0 ± 0, $P < .001$), and *Veillonella* (4.41 ± 6.13 and 4.13 ± 6.09 vs 0.01 ± 0.01, $P < .001$) at the genus level; *Pasteurellales* (1.86 ± 3.92 and 2.39 ± 4.05 vs 0.49 ± 1.53, $P = .007$) at the order level; and *Ruminococcus torques* (0.34 ± 0.07 and 0.42 ± 0.91 vs 0.22 ± 0.21, $P = .028$), *V. dispar* (0.41 ± 0.80 and 1.19 ± 2.17 vs 0 ± 0, $P < .001$), and *V. parvula* (2.78 ± 3.86 and 0.92 ± 1.40 vs 0.01 ± 0.01, $P < .001$) at the species level.

At the same time, in the control group, there was a significant increase in the relative abundance of *Lachnospiraceae* (9.09 ± 6.39 and 11.48 ± 6.53 vs 29.67 ± 16.73, $P = .004$) at the family level; *Adlercreutzia* (0.01 ± 0.02 and 0 ± 0 vs 0.059 ± 0.108, $P = .039$), *Blautia* (1.36 ± 1.88 and 1.13 ± 1.27 vs 13.48 ± 11.82, $P < .001$), *Coprococcus* (0.31 ± 0.43 and 0.31 ± 0.29 vs 4.50 ± 5.39, $P = .007$), and *Dorea* (0.33 ± 0.42 and 0.86 ± 0.72 vs 1.28 ± 1.2, $P = .024$) at the genus level; *Turicibacterales* (0.13 ± 0.04 and 0 ± 0 vs 1.65 ± 4.35, $P = .044$) at the order level; and *B. producta* (0.07 ± 0.16 and 0.11 ± 0.13 vs 0.65 ± 0.88, $P = .011$), *Clostridium clostridioforme* (0.11 ± 0.17 and 0.18 ± 0.21 vs 1.26 ± 1.878, $P = .011$), *Collinsella stercoris* (0 ± 0 and 0 ± 0 vs 0.14 ± 0.26, $P = .004$), and *Enterococcus casseliflavus* (0 ± 0 and 0 ± 0 vs 0.101 ± 0.02, $P = .036$) at the species level (Table 3, Fig. 1D, Supplement material, http://links.lww.com/MD/H238).

4. Discussion

It has been proved that the balance and type species of microorganisms inhabiting the intestine are crucial for maintaining the normal function of the host. Some diseases, such as obesity,

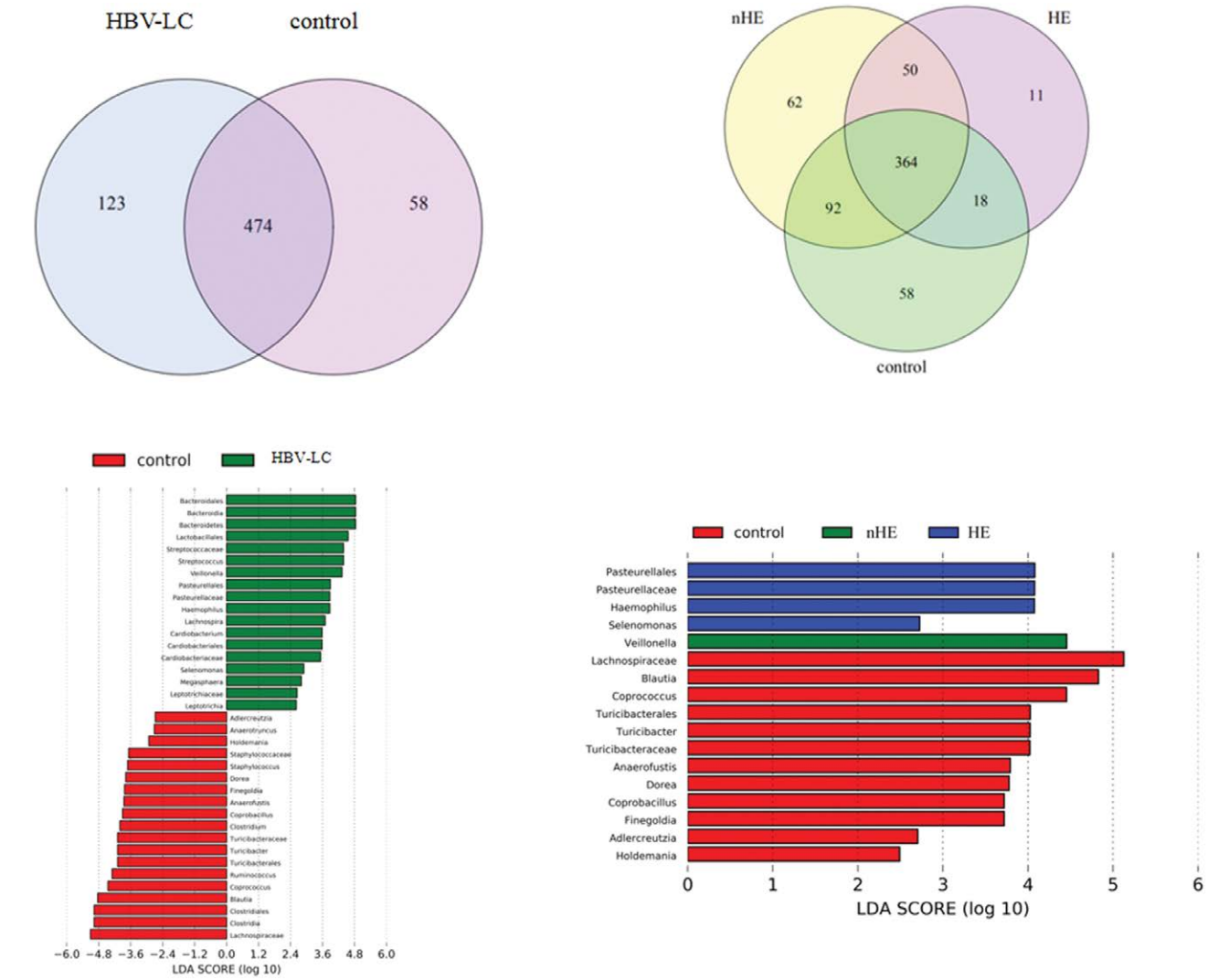

Figure 1. There were 655 OTUs in both groups. The HBV-LC group had 597 OTUs, with a total of 474 OTUs present in both HBV-LC and control groups (A). Subgroup analysis found that there were 568 OTUs in the nHE group, 443 OTUs in the HE group, and only 364 OTUs in all 3 groups (B). The relative abundance of *Bacteroidia*, *Streptococcaceae*, *Streptococcus*, *Veillonella*, *Bacteroidales*, *Lactobacillales*, *Pasteurellales*, and *Veillonella parvula* increased in the HBV-LC group (C). Meanwhile, the relative weight of *Pasteurellales*, *Pasteurellaceae*, *Haemophilus*, and *Selenomonas* significantly increased in the HE group. Further, *Veillonella* was abundant in the nHE group (D). HBV-LC = hepatitis B virus-associated liver cirrhosis, HE = hepatic encephalopathy, nHE = nonhepatic encephalopathy, OUT = operational taxonomic unit.

fatty liver, and inflammatory bowel disease, are associated with their imbalance.[4–6] Recently, growing evidence has shown that changes in the intestinal flora play an important role in the pathogenesis of liver disease; however, the conclusions of various studies are inconsistent.

In the present study, we observed and compared the community- and metabolism-wide changes in fecal microbiota in different groups. The results showed that there were significant differences in the abundance of bacterial flora between the HBV-LC group and the control group. Subgroup analysis of HBV-LC showed that the distribution of gut microbiota was also significantly altered between the 2 groups with or without HE, and the imbalance was more pronounced in the nHE group.

Our study findings show that the relative abundance of *Bacteroidia*, *Streptococcaceae*, *Cardiobacteriaceae*, *Veillonella*, *Bacteroidales*, and *Pasteurellales* was significantly higher in the HBV-LC group than in the control group, but the relative abundance of *Lachnospiraceae*, *Adlercreutzia*, *Blautia*, and *Turicibacterales* significantly reduced ($P < .05$). This suggests that the intestinal flora in patients with HBV-LC is significantly different from that in healthy people, which is consistent with previous findings in the literature.

Further subpopulation analysis revealed that the relative abundance of *Pasteurellales*, *Pasteurellaceae*, *Haemophilus*, and *Selenomonas* was much larger in the HE group, whereas in the nHE group, the relative abundance of *Veillonella* increased significantly. However, the relative abundance of *Lachnospiraceae*, *Blautia*, *Coprococcus*, *Turicibacterales*, *Anaerofustis*, *Dorea*, and *Finegoldia* was significantly higher in the control group.

Our results show that the relative abundance of *Pasteurellaceae* and *Pasteurellales* in the stool of patients with HBV-LC increased significantly. Through the study of model rats with liver cirrhosis, Feng et al[7] found that prebiotic supplements can increase the relative abundance of *Pasteurellaceae*, thereby reducing the degree of liver cirrhosis. Rao et al[8] found that the relative abundance of *Pasteurellales* was significantly higher in people who consumed alcohol, which was believed to be related to alcohol. Our study found that the relative abundance of *Pasteurellales* also increased in patients with HE caused by HBV-LC, indicating that *Pasteurellales* is related not only to alcohol but also to other factors, such as abnormal blood ammonia level and lower immunity in HE.

Table 2

The difference in bacterial relative abundance between HBV-LC and healthy groups.

	Control		HBV-LC		
	Mean	SD	Mean	SD	*P* value
Bacteroidales	23.523764	16.306013	37.954261	20.19444	.049031
Bacteroidia	23.523764	16.306013	37.954261	20.19444	.049031
Lactobacillales	1.073462	1.606202	8.288108	12.754144	.024424
Streptococcaceae	0.812192	1.299856	5.600653	9.068546	.034989
Streptococcus	0.810095	1.295156	5.595575	9.070227	.039247
Veillonella	0.005331	0.009151	2.318274	3.475544	2.90E-05
Pasteurellales	0.486285	1.528185	1.995783	3.847864	.002181
Pasteurellaceae	0.486285	1.528185	1.995783	3.847864	.002181
Haemophilus	0.427946	1.345397	1.887965	3.621697	.003491
Lachnospira	0.083902	0.117386	0.931776	1.386918	.032788
Cardiobacterium	0	0	0.00093	0.001366	.040647
Cardiobacteriales	0	0	0.00093	0.001366	.040647
Cardiobacteriaceae	0	0	0.00093	0.001366	.040647
Selenomonas	0	0	0.01597	0.037348	.000904
Megasphaera	0.047733	0.141477	0.150901	0.322845	.027435
Leptotrichiaceae	0	0	0.020583	0.06715	.025595
Leptotrichia	0	0	0.020583	0.06715	.025595
Adlercreutzia	0.059837	0.107748	0.004138	0.01272	.011633
Anaerotruncus	0.021005	0.022656	0.004771	0.016548	.019643
Holdemania	0.014102	0.009798	0.00733	0.014407	.027506
Staphylococcaceae	0.001626	0.002193	0.00029	0.000756	.027521
Staphylococcus	0.001626	0.002193	0.00029	0.000756	.027521
Dorea	1.283049	1.200551	0.458738	0.540815	.036559
Finegoldia	0.001934	0.004017	8.50E-05	0.00038	.019407
Anaerofustis	0.002649	0.005855	0	0	.012696
Coprobacillus	0.002395	0.005455	0	0	.003278
Turicibacteraceae	1.652491	4.353618	0.009481	0.034521	.043581
Turicibacter	1.652491	4.353618	0.009481	0.034521	.043581
Turicibacterales	1.652491	4.353618	0.009481	0.034521	.043581
Ruminococcus	0.22098	0.21495	0.130245	0.455451	.01229
Coprococcus	4.495605	5.387019	0.306876	0.393368	.001916
Blautia	13.478221	11.822472	1.306473	1.720395	3.20E-05
Lachnospiraceae	29.674821	16.738667	9.68757	6.336	5.00E-04

HBV-LC = hepatitis B virus-associated liver cirrhosis, SD = standard deviation.

Table 3

The difference in bacterial relative abundance among nHE, HE, and healthy groups.

	control		nHE		HE		
	Mean	SD	Mean	SD	Mean	SD	*P* value
Pasteurellales	0.486285	1.528185	1.863846	3.91694	2.391594	4.045885	.006597
Pasteurellaceae	0.486285	1.528185	1.863846	3.91694	2.391594	4.045885	.006597
Haemophilus	0.427946	1.345397	1.737249	3.635837	2.340111	3.961812	.011022
Selenomonas	0	0	0.016098	0.042187	0.015588	0.019909	.000855
Veillonella	0.008254	0.013243	4.406683	6.131336	4.128437	6.093566	8.60E-05
Lachnospiraceae	29.674821	16.738667	9.089692	6.383913	11.481204	6.533466	.003732
Blautia	13.478221	11.822472	1.36457	1.882414	1.132183	1.267295	.000161
Coprococcus	4.495605	5.387019	0.30539	0.430813	0.311334	0.292189	.007344
Turicibacterales	1.652491	4.353618	0.012642	0.039681	0	0	.044909
Turicibacter	1.652491	4.353618	0.012642	0.039681	0	0	.044909
Turicibacteraceae	1.652491	4.353618	0.012642	0.039681	0	0	.044909
Anaerofustis	0.002649	0.005855	0	0	0	0	.04029
Dorea	1.283049	1.200551	0.326409	0.418381	0.855722	0.71692	.023988
Coprobacillus	0.002395	0.005455	0	0	0	0	.011877
Adlercreutzia	0.059837	0.107748	0.005318	0.014598	0.000597	0.001334	.038697
Holdemania	0.014102	0.009798	0.009306	0.016191	0.001402	0.003136	.04656

HE = hepatic encephalopathy, nHE = no hepatic encephalopathy, SD = standard deviation.

Chen et al[9] proved that HBV-infected patients had higher *Haemophilus* abundance. On the other hand, Lv et al[10] found that the abundance of *Haemophilus* significantly reduced after rifaximin treatment while studying the effects of rifaximin in the treatment of refractory ascites. Through analysis of duodenal microbiota in patients with cirrhosis and healthy controls, Chen et al[11] found that *Haemophilus* was enriched in healthy controls. These conclusions are inconsistent; however, our study showed that the level of *Haemophilus* significantly increased in patients with HE.

It was proved that *Selenomonas* was a significantly abundant oral bacterium in HBV-LC.[12] Congruently, the present study

revealed that the relative abundance of *Selenomonas* in the feces of patients with HE increased. This confirmed that there was a translocation of *Selenomonas*, which may be related to the imbalance of normal intestinal flora, further suggesting that there was a major change in the intestinal microenvironment of patients with HE.

It was proved that severe patients with alcoholic hepatitis had high levels of *Veillonella*, which decreased after rifaximin treatment.[10] Wei et al[13] found that *Veillonella* was the most strongly disease-associated taxa, which was positively correlated with the serum level of aspartate aminotransferase and liver inflammation in patients with autoimmune hepatitis. Several studies confirmed that the abundance of *Veillonella* increases in patients with cirrhosis.[14,15] In the current study, we also found that the relative abundance of *Veillonella* increased in patients with liver cirrhosis, which is consistent with the foregoing conclusion. However, when complicated by HE, the relative abundance of *Veillonella* decreased. However, similar reports of this were not found.

Lachnospiraceae are found in the intestines of healthy people. It was revealed that the severity of liver cirrhosis in mouse models with higher abundance of *Lachnospiraceae* significantly reduced,[7] and compared with rats with CCl4-induced fibrotic liver, *Lachnospiraceae* was present in the feces of healthy mice at higher levels.[16] Through Bajaj successfully treated alcohol use disorder using fecal microbiota transplant via retention enema from a donor enriched with *Lachnospiraceae*,[17] which confirmed that *Lachnospiraceae* can protect liver cells. In the present study, the relative abundance of *Lachnospiraceae* was found to be significantly reduced in the HBV-LC group.

Benítez-Páez et al[18] studied the abundance of *Blautia* in the gut microbiota of obese children, and indicated that *Blautia* help reduce inflammation causally linked to obesity-related complications. Zhao et al[19] found that CHB can disrupt the balance of gut microbiota and that the change in its abundance is associated with liver injury. Our research results confirmed that the prevalence of *Blautia* was significantly lower in the stool of patients with HBV-LC than that of healthy adults. This suggests that the abundance of probiotic flora reduced and the intestinal protection ability was weakened when CHB developed with cirrhosis.

At present, related research on *Turicibacteraceae*, *Turicibacter*, and *Turicibacterales* focus on their association with alcoholic or nonalcoholic fatty liver disease (NAFLD) and diabetes. It is believed that their abundance is positively correlated with liver inflammation and can reduce when inflammation recovers.[20,21] Studies involving NAFLD have shown that a high-fat diet can induce a reduction in the relative abundance of *Turicibacter*.[22] Our research confirms that the abundance of *Turicibacteraceae*, *Turicibacter*, and *Turicibacterales* was significantly higher in the stool of healthy adults than in that of patients with HBV-LC, indicating the similarities of the intestinal flora between patients with HBV-LC and those with NAFLD.

Through a meta-analysis of 15 studies, Li et al[23] concluded that the abundance of *Coprococcus* in patients with NAFLD decreased. Yao et al[24] found that the abundance of *Coprococcus* was negatively correlated with serum bilirubin level and the international normalized ratio and was positively correlated with prothrombin time percent activity. Additionally, *Coprococcus* exists in the intestinal tract of adults. Lv et al[10] found that the abundance of *Coprobacillus* increased after rifaximin treatment with intravenous antibiotics. In the present study, it was found that the relative abundance of *Coprococcus* in the feces of healthy people was significantly higher than that of patients with HBV-LC.

5. Conclusions

This study demonstrated that the relative abundance of *Bacteroidia*, *Streptococcaceae*, *Streptococcus*, *Veillonella*, *Bacteroidales*, *Lactobacillales*, *Pasteurellales*, and *V. parvula* increased in patients with HBV-LC. In particular, *Pasteurellales*, *Pasteurellaceae*, *Haemophilus*, and *Selenomonas* were significantly abundant in patients with HE, whereas the abundance of *Veillonella* was high in the nHE group. We also found that intestinal microbiota of patients with HBV-LC was significantly different from that of controls with respect to a lack of potentially beneficial autochthonous bacteria and overgrowth of potentially pathogenic genera in patients with HBV-LC. Moreover, there was a higher change in the relative abundance of intestinal flora when complicated with HE.

These conclusions will help to further study the pathogenesis of patients with HBV-LC and HE.

The small sample population and short duration of follow-up are the limitations of this study. Moreover, the relative abundance of intestinal microbiota was preliminarily discussed, yet the exact mechanism of change remains unknown. Hence, we will further investigate the pathways related to the immune system and intestinal microbiota.

Author contributions

Xiaoli Hua analyzed and interpreted the patient data. Hao Feng was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

References

[1] Pimentel M, Saad RJ, Long MD, et al. ACG Clinical guideline: small intestinal bacterial overgrowth. Am J Gastroenterol. 2020;115:165–78.
[2] Le Roy T, Lécuyer E, Chassaing B, et al. The intestinal microbiota regulates host cholesterol homeostasis. BMC Biol. 2019;17:94.
[3] Chinese Society of Infectious Diseases, Chinese Medical Association; Chinese Society of Hepatology, Chinese Medical Association. The guidelines of prevention and treatment for chronic hepatitis B (2019 version). Zhonghua Gan Zang Bing Za Zhi. 2019;27:938–61.
[4] Yehualashet AS, Yikna BB. Microbial ecosystem in diabetes mellitus: consideration of the gastrointestinal system. Diabetes Metab Syndr Obes. 2021;14:1841–54.
[5] Hartmann P, Schnabl B. New developments in microbiome in alcohol-associated and nonalcoholic fatty liver disease. Semin Liver Dis. 2021;41:87–102.
[6] Marescotti D, Lo Sasso G, Guerrera D, et al. Development of an advanced multicellular intestinal model for assessing immunomodulatory properties of anti-inflammatory compounds. Front Pharmacol. 2021;12:639716.
[7] Feng Y, Gao SJ, Wei RD, et al. Effects of probiotics on intestinal flora, inflammation and degree of liver cirrhosis in rats with liver cirrhosis by regulating Wnt/β-catenin signaling pathway. J Biol Regul Homeost Agents. 2021;35:25–33.
[8] Rao W, Lin Z, Liu S, et al. Association between alcohol consumption and oesophageal microbiota in oesophageal squamous cell carcinoma. BMC Microbiol. 2021;21:73.
[9] Chen Z, Xie Y Zhou, F, et al. Featured gut microbiomes associated with the progression of chronic hepatitis B disease. Front Microbiol. 2020;11:383.
[10] Lv XY, Ding HG, Zheng JF, et al. Rifaximin improves survival in cirrhotic patients with refractory ascites: a real-world study. World J Gastroenterol. 2020;26:199–218.
[11] Chen Y, Ji F, Guo J, et al. Dysbiosis of small intestinal microbiota in liver cirrhosis and its association with etiology. Sci Rep. 2016;6:34055.
[12] Li D, Xi W, Zhang Z, et al. Oral microbial community analysis of the patients in the progression of liver cancer. Microb Pathog. 2020;149:104479.
[13] Wei Y, Li Y, Yan L, et al. Alterations of gut microbiome in autoimmune hepatitis. Gut. 2020;69:569–77.
[14] Deng YD, Peng XB, Zhao RR, et al. The intestinal microbial community dissimilarity in hepatitis B virus-related liver cirrhosis patients with and without at alcohol consumption. Gut Pathog. 2019;11:58.
[15] Sung CM, Lin YF, Chen KF, et al. Predicting clinical outcomes of cirrhosis patients with hepatic encephalopathy from the fecal microbiome. Cell Mol Gastroenterol Hepatol. 2019;8:301–318.e2.
[16] Yu L, Wang L, Wu X, et al. .RSPO4-CRISPR alleviates liver injury and restores gut microbiota in a rat model of liver fibrosis. Commun Biol. 2021;4:230.

[17] Bajaj JS, Gavis EA, Fagan A, et al. A randomized clinical trial of fecal microbiota transplant for alcohol use disorder. Hepatology. 2021;73:1688–700.
[18] Benítez-Páez A, Gómez Del Pugar EM, López-Almela I, et al. Depletion of blautia species in the microbiota of obese children relates to intestinal inflammation and metabolic phenotype worsening. mSystems. 2020;5:1–13.
[19] Zhao Z, Qin S, Wang L, et al. Correlation between gut microbiota and liver biochemical indicators in patients with chronic hepatitis B. Sheng Wu Gong Cheng Xue Bao. 2021;37:301–11.
[20] Ran B, Guo CE, Li W, et al. Sea buckthorn (Hippophae rhamnoides L.) fermentation liquid protects against alcoholic liver disease linked to regulation of liver metabolome and the abundance of gut microbiota. J Sci Food Agric. 2021;101:2846–54.
[21] Li C, Cui L, Wang X, et al. Using intestinal flora to distinguish non-alcoholic steatohepatitis from non-alcoholic fatty liver. J Int Med Res. 2020;48:300060520978122.
[22] Velázquez KT, Enos RT, Bader JE, et al. Prolonged high-fat-diet feeding promotes non-alcoholic fatty liver disease and alters gut microbiota in mice. World J Hepatol. 2019;11:619–37.
[23] Li F, Ye J, Shao C, et al. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and meta-analysis. Lipids Health Dis. 2021;20:22.
[24] Yao X, Yu H, Fan G, et al. Impact of the gut microbiome on the progression of hepatitis B virus related acute-on-chronic liver failure. Front Cell Infect Microbiol. 2021;11:573923.