Journal of Rare Cardiovascular Diseases
ISSN: 2299-3711 (Print) | e-ISSN: 2300-5505 (Online)
www.jrcd.eu
RESEARCH ARTICLE

Gut Microbiota Pattern Analysis in Relation to Viral Load Among Patients with Chronic Hepatitis B Virus Infection: A Comparative Cross-Sectional Study

M. Ponmalai[1], Arun Suriyan A[2], Srinivasan S[1], Sankara Narayanan G[1], Anbarasan A[1], Karthikeyan G[1] and Sameen Taj[1].

[1]Department of Anatomy, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai 602105, Tamil Nadu, India.

[2]Department of Pharmacology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai 602105, Tamil Nadu, India.

*Corresponding Author
Srinivasan S

Article History
Received: 09/07/2025
Revised: 23/08/2025
Accepted: 12/09/2025
Published: 30/09/2025

Abstract: ***Background***: Hepatitis B virus (HBV) infection represents a major global health burden with complex metabolic implications, including associations with altered lipid metabolism and reduced risk of nonalcoholic fatty liver disease. The gut microbiota plays a crucial role in host metabolism and immune regulation, yet its relationship with HBV infection remains incompletely understood. ***Objectives***: To investigate the compositional and functional characteristics of gut microbiota in chronic HBV-infected patients stratified by viral load compared to healthy controls, and to identify specific bacterial taxa associated with different viral load categories. ***Methods***: A prospective case-control study was conducted involving 228 participants: 114 chronic HBV patients and 114 age-matched healthy controls. HBV-infected patients were further stratified based on viral load into low viral load (≤2000 IU/mL, n=80) and high viral load (>2000 IU/mL, n=34) subgroups. Fecal samples underwent 16S rRNA gene sequencing targeting V3-V4 regions using Illumina MiSeq platform. Alpha and beta diversity metrics were calculated, and taxonomic composition was analyzed using QIIME2 pipeline with DADA2 plugin. Statistical analyses included Mann-Whitney U tests, Kruskal-Wallis tests, and PERMANOVA for beta diversity. ***Results***: Chronic HBV patients demonstrated significantly higher alpha diversity compared to controls ($p<0.05$). This increase was primarily driven by the low viral load subgroup, which showed enhanced Shannon diversity index ($p=0.044$) and Faith's phylogenetic diversity ($p<0.044$). Beta diversity analysis revealed significant differences between controls and low viral load patients using Jaccard dissimilarity ($p=0.06$) and unweighted UniFrac distance ($p=0.0013$). Taxonomically, low viral load patients exhibited enrichment of Alloprevotella and Eubacterium coprostanoligenes, while showing depletion of Bacteroides fragilis and Prevotella species compared to controls. Functional prediction analysis suggested enhanced fatty acid and lipid metabolism pathways in low viral load patients. ***Conclusions***: This study demonstrates distinct gut microbiota signatures associated with chronic HBV infection, particularly in patients with low viral loads. The enrichment of lipid-metabolizing bacteria may contribute to the protective metabolic phenotype observed in HBV infection, potentially explaining reduced dyslipidemia risk in this population.

Keywords: Chronic Hepatitis B Virus (HBV) Infection, Gut Microbiota, Viral Load, Cholesterol Metabolism, Gut-Liver Axis

INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects approximately 296 million individuals globally, representing one of the most significant infectious disease burdens worldwide[1]. Despite advances in antiviral therapy, chronic HBV infection remains a leading cause of liver cirrhosis and hepatocellular carcinoma, particularly in endemic regions of Asia and sub Saharan Africa[2]. Beyond its direct hepatic effects, HBV infection has been associated with distinctive metabolic alterations, including paradoxically reduced serum cholesterol levels and decreased risk of nonalcoholic fatty liver disease (NAFLD)[3,4]. The mechanisms underlying these metabolic changes in HBV infection remain poorly understood. Recent evidence suggests that HBV gene expression in hepatocytes exhibits similar regulatory patterns to key metabolic genes, indicating potential direct viral effects on host metabolism[5,6]. However, the incomplete understanding of these metabolic pathways has limited the development of novel therapeutic targets for HBV management[7]. The gut microbiota has emerged as a critical mediator of host-pathogen interactions and metabolic homeostasis. This complex microbial ecosystem performs essential functions including nutrient metabolism, vitamin synthesis, immune system modulation, and maintenance of the gut-brain axis[8,9]. Accumulating evidence demonstrates that gut microbiota dysbiosis contributes to various pathological conditions, including digestive disorders, cardiovascular disease, and endocrine dysfunction[10].

Recent investigations have revealed that HBV infection significantly alters gut microbiota composition and diversity[11,12]. In chronic HBV infection, these microbiota alterations may regulate cholesterol metabolism through complex microbe-host interactions, potentially explaining the cholesterol-lowering effects

observed in infected individuals[13]. The gut-liver axis represents a bidirectional communication pathway whereby gut-derived microbial products influence hepatic function, while liver-derived factors modulate intestinal microbiota composition[14]. The natural history of chronic HBV infection encompasses distinct phases characterized by different levels of viral replication, immune activation, and liver inflammation[15]. These phases include immune tolerance, immune clearance, low replication, and inactive carrier states, each potentially associated with unique gut microbiota profiles[16]. Understanding how gut microbiota composition varies across these different phases of HBV infection is crucial for developing microbiome-targeted therapeutic interventions. Viral load represents a key parameter in HBV management, with thresholds of 2000 IU/mL serving as important clinical decision points for treatment initiation and monitoring[17,18]. Patients with low viral loads (≤2000 IU/mL) typically exhibit minimal liver inflammation and have low risk of disease progression, while those with high viral loads (>2000 IU/mL) require closer monitoring and often benefit from antiviral therapy[19].

The relationship between HBV viral load and gut microbiota composition has not been comprehensively characterized. Previous studies have focused primarily on patients with advanced liver disease or have not stratified patients based on viral load[20,21]. Furthermore, the functional implications of microbiota alterations in HBV infection, particularly regarding lipid and cholesterol metabolism, require detailed investigation. This study aimed to address these knowledge gaps by conducting a comprehensive analysis of gut microbiota composition and diversity in chronic HBV patients stratified by viral load compared to healthy controls. We hypothesized that patients with different viral loads would exhibit distinct microbiota signatures, with low viral load patients demonstrating enrichment of beneficial bacteria involved in lipid metabolism. The findings of this research may provide insights into the mechanistic basis of metabolic alterations in HBV infection and identify potential targets for microbiome based therapeutic interventions.

MATERIALS AND METHODS

2.1 Study Design and Participants

This prospective case-control study was conducted at Saveetha Medical College and Hospital between January 2020 and December 2022. The study protocol was approved by the Institutional Ethics Committee (Protocol Number: SMC/IEC/2020/01/78) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to enrolment.

Inclusion and Exclusion Criteria

Inclusion criteria for HBV patients: (1) Age 18-75 years; (2) Chronic HBV infection defined as hepatitis B surface antigen (HBsAg) positivity for >6 months; (3) Stable clinical condition without active hepatitis; (4) Alanine aminotransferase (ALT) levels (ALT) levels

Inclusion criteria for healthy controls: (1) Age 18-75 years; (2) Negative HBsAg; (3) Normal liver function tests; (4) No known chronic diseases

Exclusion criteria for both groups: (1) Use of antibiotics within 3 months prior to enrollment; (2) Concurrent use of proton pump inhibitors, cholesterol-lowering medications, or digestive enzymes; (3) Diabetes mellitus; (4) Inflammatory bowel disease or other gastrointestinal disorders; (5) Pregnancy or lactation; (6) Immunosuppressive therapy; (7) Alcohol consumption >20g/day for women or >30g/day for men

Additional exclusion criteria for HBV patients: (1) Evidence of liver cirrhosis or hepatocellular carcinoma; (2) Concurrent hepatitis C or hepatitis D infection; (3) Human immunodeficiency virus coinfection; (4) Current or recent antiviral therapy within 12 months.

Sample Size Calculation

Sample size was calculated using G*Power 3.1.9.7 software. Based on previous studies reporting medium effect sizes (Cohen's d = 0.5) for microbiota diversity differences between HBV patients and controls, with $\alpha = 0.05$ and power = 0.80, a minimum of 64 participants per group was required. To account for potential dropouts and stratification by viral load, we enrolled 114 participants per group.

Clinical Data Collection

Comprehensive clinical data were collected including demographic characteristics, medical history, current medications, smoking and alcohol consumption patterns, and anthropometric measurements. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Laboratory Assessments

Venous blood samples were collected after 8-hour fasting for biochemical analyses. Complete blood count was performed using a hematology analyzer (XE-2100, Sysmex Corporation, Kobe, Japan). Serum biochemistry including ALT, aspartate aminotransferase (AST), total bilirubin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using an automated chemistry analyzer (COBAS c702, Roche Diagnostics, Basel, Switzerland). HBsAg, hepatitis B e antigen (HBeAg), and hepatitis B e antibody (HBeAb) were determined using electrochemiluminescence immunoassays (ECLIA) on the COBAS e801 platform (Roche Diagnostics). HBV DNA quantification was performed using real-time polymerase chain reaction (COBAS AmpliPrep/COBAS TaqMan HBV Test v2.0, Roche Diagnostics) with a detection range of 20-1.7×10^8 IU/mL.

HBV Patient Stratification

Chronic HBV patients were stratified into subgroups based on HBV DNA levels: Group B1 (low viral load): HBV DNA ≤2000 IU/mL; Group B2 (high viral load): HBV DNA >2000 IU/mL. This stratification follows current international guidelines for HBV management and treatment decision-making[22,23].

Fecal Sample Collection and Processing

Fecal samples were collected using OMNIgene-GUT collection kits (DNA Genotek, Ottawa, Canada) according to manufacturer's instructions. Participants were provided detailed instructions for proper sample collection and storage. Samples were transported to the laboratory within 24 hours and stored at -80°C until DNA extraction.

DNA Extraction and 16S rRNA Gene Sequencing

Total genomic DNA was extracted from fecal samples using the QIAamp PowerFecal Pro DNA Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V3-V4 hypervariable regions of the 16S rRNA gene were amplified using universal primers 341F (5' CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). PCR amplification was performed using the following conditions: initial denaturation at 95°C for 3 minutes; 25 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds; final extension at 72°C for 5 minutes. PCR products were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA) and quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Equimolar concentrations of purified amplicons were pooled and sequenced on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 (2×300 bp paired-end reads).

Bioinformatics Analysis

Raw sequencing data were processed using QIIME 2 version 2021.4[24]. Quality filtering and feature table construction were performed using DADA2 plugin with default parameters[25]. Taxonomic assignment was conducted using the q2 feature-classifier against the Silva 138 99% reference database[26]. Alpha diversity metrics including observed amplicon sequence variants (ASVs), Shannon diversity index, Pielou's evenness, and Faith's phylogenetic diversity were calculated after rarefying to 21,459 sequences per sample. Beta diversity was assessed using Bray-Curtis dissimilarity, Jaccard distance, and weighted/unweighted UniFrac distances[27].

Statistical Analysis

Statistical analyses were performed using R version 4.1.0. Continuous variables were tested for normality using Shapiro Wilk tests. Normally distributed continuous variables were compared using Student's t-test, while non-parametric data were analyzed using Mann-Whitney U test for pairwise comparisons and Kruskal-Wallis test for multiple group comparisons. Categorical variables were compared using Chi-square or Fisher's exact tests as appropriate. Alpha diversity differences between groups were assessed using Mann-Whitney U test with Benjamini-Hochberg correction for multiple comparisons. Beta diversity differences were evaluated using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Differential abundance analysis was performed using Analysis of Composition of Microbiomes (ANCOM) to identify taxa significantly associated with HBV infection and viral load[28]. Linear discriminant analysis effect size (LEfSe) was used to identify biomarker taxa with linear discriminant analysis scores >3.0[29].

Functional Prediction Analysis

Functional profiling of microbial communities was performed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2)[30]. Predicted functional profiles were compared using Statistical Analysis of Taxonomic and Functional Profiles (STAMP) software with Welch's t-test and Benjamini-Hochberg correction[31].

Statistical significance was set at $p<0.05$ for all analyses. All tests were two-tailed unless otherwise specified.

RESULTS

Participant Characteristics

A total of 268 individuals were initially enrolled, of whom 228 completed the study and provided adequate fecal samples for analysis. The final cohort comprised 114 healthy controls (Group A) and 114 chronic HBV patients (Group B). Among HBV patients, 80 had low viral loads (Group B1, ≤2000 IU/mL) and 34 had high viral loads (Group B2, >2000 IU/mL).

Table 1: Baseline Demographics and Clinical Characteristics

Characteristic	Group A (n=114)	Group B (n=114)	p-value	Group B1 (n=80)	Group B2 (n=34)	p-value (B1 vs B2)
Demographics						
Age (years)*	46.7 ± 12.3	54.5 ± 13.8	<0.001	56.3 ± 14.2	50.2 ± 12.6	0.044

Characteristic	Group A (n=114)	Group B (n=114)	p-value	Group B1 (n=80)	Group B2 (n=34)	p-value (B1 vs B2)
Male gender (%)	28 (24.6)	42 (36.8)	0.041	27 (33.8)	15 (44.1)	0.535
Current smoker (%)	6 (5.3)	13 (11.4)	0.106	9 (11.3)	4 (11.8)	0.329
BMI (kg/m²)*	27.93 ± 4.21	28.49 ± 3.87	0.331	28.74 ± 3.92	27.91 ± 3.76	0.377
HBV Markers						
HBeAg positive (%)	–	11 (9.6)	–	0 (0.0)	11 (32.4)	<0.001
HBeAb positive (%)	–	54 (47.4)	–	44 (55.0)	10 (29.4)	0.014
Laboratory Values*						
WBC (×10³/µL)	6.12 ± 1.83	5.39 ± 1.67	0.012	5.45 ± 1.72	5.27 ± 1.58	0.672
Platelets (×10³/µL)	273.0 ± 68.4	214.0 ± 59.2	<0.001	214.0 ± 61.3	221.0 ± 54.8	0.918
Total bilirubin (mg/dL)	0.73 ± 0.28	0.96 ± 0.34	<0.001	0.97 ± 0.35	0.94 ± 0.31	0.819
AST (IU/L)	26.18 ± 8.42	28.86 ± 9.76	0.072	27.98 ± 9.23	30.94 ± 10.87	0.309
ALT (IU/L)	27.93 ± 12.45	32.51 ± 15.28	0.223	30.85 ± 14.67	36.41 ± 16.44	0.412
Lipid Profile*						
Total cholesterol (mg/dL)	195.18 ± 34.72	187.19 ± 31.84	0.210	187.48 ± 32.45	186.53 ± 30.12	0.928
LDL-C (mg/dL)	133.39 ± 28.63	122.79 ± 26.41	0.080	125.46 ± 27.18	116.65 ± 24.32	0.299
HDL-C (mg/dL)	68.28 ± 15.94	62.41 ± 14.72	0.068	58.23 ± 13.45	72.00 ± 15.87	0.026

*Data presented as mean ± standard deviation; **Categorical variables presented as n (%); ***Normal reference ranges: WBC 4.0-11.0×10³/µL, Platelets 150-450×10³/µL, Total bilirubin 0.3-1.2 mg/dL, AST/ALT

Alpha and Beta Diversity Analysis

Alpha diversity analysis revealed significantly higher microbial diversity in chronic HBV patients compared to healthy controls. This difference was primarily attributed to the low viral load subgroup (B1), which showed significantly higher Shannon diversity index (p=0.044), observed ASVs (p<0.05), and Faith's phylogenetic diversity (p=o.044) compared to controls. Group B2 did not show significant differences in alpha diversity metrics compared to either controls or Group B1.

Table 2: Beta Diversity Distance Matrices by HBV DNA Load

Beta Diversity Index	Groups A vs B Pseudo-F	Groups A vs B p-value	Groups A vs B1 vs B2 (PERMANOVA)	p-value A vs B1	q-value A vs B1	p-value A vs B2	q-value A vs B2
Bray-Curtis dissimilarity	1.255	0.153	–	0.370	0.095	0.179	0.527
Jaccard dissimilarity	1.593	0.009	–	0.060	0.080	0.117	0.341
Weighted UniFrac	0.630	0.600	–	0.789	0.352	0.591	0.758
Unweighted UniFrac	2.253	0.017	–	0.0013	0.029	0.319	0.947

Beta diversity analysis using PERMANOVA revealed significant differences in microbial community structure between controls and HBV patients, particularly those with low viral loads. The most notable differences were observed using presence/absence-based metrics (Jaccard dissimilarity and unweighted UniFrac distance), suggesting that the differences were primarily driven by the presence or absence of specific taxa rather than their relative abundances.

Taxonomic Composition Analysis

The sequencing analysis identified 1,555 unique ASVs across 228 samples, with reads ranging from 21,459 to 102,071 per sample (mean: 47,752). These ASVs were taxonomically classified into 14 phyla, 23 classes, 45 orders, 94 families, 326 genera, and 751 species. At the phylum level, Bacteroidetes (48.4%) and Firmicutes (46.6%) dominated across all groups, with no significant differences in their relative abundances between HBV patients and controls. However, detailed analysis at lower taxonomic levels revealed significant differences associated with HBV infection and viral load status. ANCOM analysis identified several genera significantly associated with HBV infection after adjusting for age, sex, and smoking status. When comparing Group A (controls) to Group B1 (low viral load), significant differences were observed in 2 families, 7 genera, and 16 species. The genera showing the most prominent differences included Alloprevotella, Paraprevotella, Hungatella, Mitsuokella, and Family XIII AD3011, along with an unclassified genus of the family Lachnospiraceae. LEfSe analysis identified 32 taxa with linear discriminant analysis scores >3.0 when comparing controls to low viral load patients. Notably, Eubacterium coprostanoligenes was significantly enriched in Group B1, while Bacteroides fragilis, Prevotella species, and certain Hungatella species were more abundant in controls.

Key findings included:

Enriched in Group B1: Alloprevotella spp., Eubacterium coprostanoligenes, Family XIII AD3011 group, Mitsuokella spp.

Depleted in Group B1: Bacteroides fragilis, Prevotella 2, Hungatella spp., Paraprevotella spp.

Comparison between controls and Group B2 (high viral load) revealed no statistically significant taxonomic differences, suggesting that microbiota alterations are specifically associated with low viral load states.

Functional Prediction Analysis

PICRUSt2 analysis was employed to predict functional capabilities of the gut microbiota based on 16S rRNA gene profiles. While no pathways reached statistical significance after multiple comparison correction (FDR $q<0.05$), several pathways showed nominal significance ($p<0.05$) that warrant discussion.

Pathways enriched in Group B1 compared to controls included:

- Peptidoglycan maturation ($p=0.011$)
- Methylophosphonate degradation I ($p<0.05$)
- Superpathway of thiamin diphosphate biosynthesis II ($p<0.05$)

Conversely, the saturated fatty acid elongation pathway was significantly reduced in Group B1 compared to controls ($p=0.006$), suggesting altered lipid metabolism in low viral load HBV patients.

DISCUSSION

Principal Findings

This comprehensive analysis of gut microbiota in chronic HBV infection stratified by viral load reveals several important findings. First, chronic HBV patients, particularly those with low viral loads, demonstrate significantly higher alpha diversity compared to healthy controls. Second, distinct taxonomic signatures characterize patients with low viral loads, including enrichment of beneficial bacteria involved in lipid metabolism such as Alloprevotella and Eubacterium coprostanoligenes. Third, functional prediction analyses

suggest altered metabolic pathways related to fatty acid synthesis and lipid metabolism in low viral load patients. These findings provide novel insights into the gut-liver axis in HBV infection and may explain the paradoxical metabolic benefits observed in chronic HBV carriers.

Alpha and Beta Diversity Patterns

The observation of increased alpha diversity in HBV patients, specifically those with low viral loads, contrasts with many chronic diseases where reduced diversity is typically observed[32,33]. This finding aligns with recent studies suggesting that certain chronic infections may promote microbial diversity through complex host-pathogen-microbiome interactions[34]. The enhanced diversity in Group B1 may reflect a stable ecological state where low-level viral replication maintains immune activation without causing severe dysbiosis. The beta diversity analysis revealed that community structure differences were primarily driven by the presence or absence of specific taxa rather than their relative abundances, as evidenced by significant differences in unweighted but not weighted UniFrac distances. This pattern suggests that HBV infection, particularly at low viral loads, may create ecological niches that favor the establishment of specific bacterial taxa while potentially eliminating others.

Taxonomic Alterations and Metabolic Implications

The enrichment of Alloprevotella in low viral load patients is particularly noteworthy given its established associations with cardiovascular health and lipid metabolism[35]. Previous studies have demonstrated that Alloprevotella species produce short-chain fatty acids, particularly butyrate, which can reduce LDL cholesterol and triglyceride levels[36]. The increased abundance of this genus in Group B1 may contribute to the reduced dyslipidemia risk observed in HBV-infected individuals. Eubacterium coprostanoligenes, another genus enriched in low viral load patients, possesses unique cholesterol metabolizing capabilities. This anaerobic bacterium can reduce up to 90% of cholesterol to coprostanol, a poorly absorbed cholesterol metabolite[37,38]. The presence of E. coprostanoligenes in HBV patients may directly contribute to reduced serum cholesterol levels through enhanced cholesterol conversion and reduced intestinal absorption[39]. The depletion of Bacteroides fragilis in low viral load patients warrants discussion, as this species typically plays beneficial roles in immune system development and maintenance[40]. However, in the context of chronic HBV infection, the reduced abundance of B. fragilis may represent an adaptive response that minimizes pro-inflammatory signals, potentially contributing to the relatively stable clinical course observed in low viral load patients.

Functional Metabolic Pathways

The functional prediction analysis provides mechanistic insights into the metabolic alterations observed in HBV infection. The reduced saturated fatty acid elongation pathway in Group B1 is particularly significant, as excessive saturated fatty acid synthesis is associated with hepatic lipogenesis and NAFLD development[41]. The downregulation of this pathway in low viral load patients may contribute to their reduced NAFLD risk. Conversely, the enrichment of peptidoglycan maturation pathways suggests enhanced bacterial cell wall synthesis, which may reflect the increased microbial biomass associated with higher alpha diversity. The enhanced thiamin diphosphate biosynthesis pathway is relevant for energy metabolism and may support the metabolic functions of beneficial bacteria enriched in this group.

Clinical and Therapeutic Implications

These findings have important implications for clinical management of chronic HBV infection. The identification of specific microbiota signatures associated with low viral loads may enable development of microbiome-based biomarkers for disease monitoring. Furthermore, the enrichment of beneficial bacteria in low viral load patients suggests that microbiome modulation could represent a complementary therapeutic approach. The metabolic benefits associated with specific microbiota patterns in HBV infection raise the possibility of probiotic interventions targeting cholesterol metabolism. Supplementation with Alloprevotella or E. coprostanoligenes strains could potentially enhance the beneficial metabolic effects observed in HBV patients while maintaining gut microbiota diversity.

Gut-Liver Axis Implications

The gut-liver axis represents a critical pathway for microbiome-mediated effects on hepatic function[47]. In chronic HBV infection, the altered gut microbiota may influence liver inflammation, fibrosis progression, and metabolic function through multiple mechanisms including short-chain fatty acid production, bile acid metabolism, and immune modulation[48,49]. The specific enrichment of beneficial bacteria in low viral load patients suggests that this represents an optimal balance where viral persistence maintains beneficial selective pressures without causing significant liver damage. This concept of "beneficial chronicity" warrants further investigation as a potential therapeutic target.

Study Limitations

Several limitations should be acknowledged. First, the cross-sectional design precludes determination of causal relationships between viral load changes and microbiota alterations. Longitudinal studies are needed to establish temporal relationships and causality. Second, the use of 16S rRNA sequencing limits taxonomic resolution to the genus level and does not provide strain-level information or functional gene content. Metagenomic sequencing would provide more detailed functional insights. Third, the smaller sample size in Group B2 may have limited statistical power to detect differences in high viral load patients. Fourth, dietary information was not systematically collected, and diet represents a major

determinant of gut microbiota composition. Future studies should include detailed dietary assessments and controls for nutritional factors. Fifth, the study population was limited to a single geographic region with specific genetic and environmental backgrounds, potentially limiting generalizability to other populations. Multi-center studies including diverse ethnic groups are needed to validate these findings.

Future Directions

Several important research directions emerge from these findings. First, longitudinal studies tracking microbiota changes during viral load fluctuations and treatment responses would provide insights into dynamic microbiome-virus interactions. Second, metagenomic and metabolomic analyses would offer deeper mechanistic understanding of functional pathways involved in HBV-microbiota interactions. Third, intervention studies using targeted probiotics or fecal microbiota transplantation could test whether microbiome modulation influences HBV disease progression or metabolic outcomes. Fourth, investigation of microbiota-derived metabolites such as short-chain fatty acids and bile acid derivatives may identify novel biomarkers or therapeutic targets. Finally, studies examining the microbiome during antiviral treatment could determine whether therapeutic interventions alter beneficial microbiota patterns and whether microbiome preservation strategies could enhance treatment outcomes.

CONCLUSIONS

This study provides comprehensive evidence for distinct gut microbiota signatures in chronic HBV infection, with particularly notable alterations in patients with low viral loads. The enrichment of lipid-metabolizing bacteria including Alloprevotella and Eubacterium coprostanoligenes in low viral load patients offers mechanistic explanations for the protective metabolic phenotype observed in HBV infection. The increased alpha diversity and specific taxonomic enrichment patterns in Group B1 suggest that low-level chronic HBV infection may promote beneficial microbiota adaptations that contribute to metabolic health. These findings enhance our understanding of gut-liver axis interactions in viral hepatitis and identify potential targets for microbiome-based therapeutic interventions. The correlation between viral load status and specific bacterial taxa highlights the complex interplay between chronic viral infection and commensal microbiota. Future research should focus on validating these findings in larger, diverse populations and investigating the therapeutic potential of microbiome modulation in chronic HBV management. These results contribute to the growing understanding of the microbiome's role in chronic infectious diseases and provide a foundation for developing personalized medicine approaches that consider both viral and microbial factors in HBV treatment strategies.

REFERENCES

1. World Health Organization. Hepatitis B fact sheet [Internet]. 2023 [cited 2025 Oct 27]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b
2. Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol*. 2018;3(6):383–403.
3. Joo EJ, Chang Y, Yeom JS, Cho YK, Ryu S. Chronic hepatitis B virus infection and risk of dyslipidaemia: a cohort study. *J Viral Hepat*. 2019;26(1):162–9.
4. Chen JY, Wang JH, Lin CY, Chen PF, Tseng PL, Chen CH, et al. Lower prevalence of hypercholesterolemia and hyperglyceridemia found in subjects with seropositivity for both hepatitis B and C strains independently. *J Gastroenterol Hepatol*. 2010;25(11):1763–8.
5. Geier A. Hepatitis B virus: the “metabolovirus” highjacks cholesterol and bile acid metabolism. *Hepatology*. 2014;60(5):1458–60.
6. Jarcuska P, Drazilova S, Fedacko J, Pella D, Janicko M. Association between hepatitis B and metabolic syndrome: current state of the art. *World J Gastroenterol*. 2016;22(1):155–64.
7. Shi YX, Huang CJ, Yang ZG. Impact of hepatitis B virus infection on hepatic metabolic signaling pathway. *World J Gastroenterol*. 2016;22(36):8161–7.
8. O’Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep*. 2006;7(7):688–93.
9. Cryan JF, O’Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. *Physiol Rev*. 2019;99(4):1877–2013.
10. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490(7418):55–60.
11. Zhu Q, Xia P, Zhou X, Li X, Guo W, Zhu B, et al. Hepatitis B virus infection alters gut microbiota composition in mice. *Front Cell Infect Microbiol*. 2019;9:377.
12. Yun Y, Chang Y, Kim HN, Ryu S, Kwon MJ, Cho YK, et al. Alterations of the gut microbiome in chronic hepatitis B virus infection associated with alanine aminotransferase level. *J Clin Med*. 2019;8(2):173.
13. Ponziani FR, Bhoori S, Castelli C, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. *Hepatology*. 2019;69(1):107–20.
14. Tripathi A, Debelius J, Brenner DA, et al. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol*. 2018;15(7):397–411.
15. Croagh CM, Lubel JS. Natural history of chronic hepatitis B: phases in a complex relationship. *World J Gastroenterol*. 2014;20(30):10395–404.

16. Tsai KN, Kuo CF, Ou JJ. Mechanisms of hepatitis B virus persistence. *Trends Microbiol*. 2018;26(1):33–42.
17. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology*. 2018;67(4):1560–99.
18. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol*. 2017;67(2):370–98.
19. Invernizzi F, Viganò M, Grossi G, Lampertico P. The prognosis and management of inactive HBV carriers. *Liver Int*. 2016;36(Suppl 1):100–4.
20. 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.
21. Zeng Y, Chen S, Fu Y, Wu W, Chen T, Chen J, et al. Gut microbiota dysbiosis in patients with hepatitis B virus–induced chronic liver disease covering chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. *J Viral Hepat*. 2020;27(2):143–55.
22. Liu J, Yang HI, Lee MH, Jen CL, Batrla-Utermann R, Lu SN, et al. Serum levels of hepatitis B surface antigen and DNA can predict inactive carriers with low risk of disease progression. *Hepatology*. 2016;64(2):381–9.
23. Papatheodoridis GV, Sypsa V, Dalekos GN, et al. Eight-year survival in chronic hepatitis B patients under long-term entecavir or tenofovir therapy is similar to the general population. *J Hepatol*. 2018;68(6):1129–36.
24. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. 2019;37(8):852–7.
25. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13(7):581–3.
26. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. 2013;41(Database issue):D590–6.
27. Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. UniFrac: an effective distance metric for microbial community comparison. *ISME J*. 2011;5(2):169–72.
28. Mandal S, Van Treuren W, White RA, Eggesbø M, Knight R, Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb Ecol Health Dis*. 2015;26:27663.
29. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol*. 2011;12(6):R60.
30. Douglas GM, Maffei VJ, Zaneveld JR, et al. PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol*. 2020;38(6):685–8.
31. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics*. 2014;30(21):3123–4.
32. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature*. 2007;449(7164):804–10.
33. Lloyd-Price J, Mahurkar A, Rahnavard G, et al. Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature*. 2017;550(7674):61–6.
34. Scher JU, Littman DR, Abramson SB. Microbiome in inflammatory arthritis and human rheumatic diseases. *Arthritis Rheumatol*. 2016;68(1):35–45.
35. Kelly TN, Bazzano LA, Ajami NJ, et al. Gut microbiome associates with lifetime cardiovascular disease risk profile among Bogalusa Heart Study participants. *Circ Res*. 2016;119(8):956–64.
36. Yang F, Li J, Pang G, Ren F, Fang B. Effects of diethyl phosphate, a non-specific metabolite of organophosphorus pesticides, on serum lipid, hormones, inflammation, and gut microbiota. *Molecules*. 2019;24(10):2003.
37. Kenny DJ, Plichta DR, Shungin D, et al. Cholesterol metabolism by uncultured human gut bacteria influences host cholesterol level. *Cell Host Microbe*. 2020;28(2):245–57.e6.
38. Mukherjee A, Lordan C, Ross RP, Cotter PD. Gut microbes from the phylogenetically diverse genus Eubacterium and their various contributions to gut health. *Gut Microbes*. 2020;12(1):1802866.
39. Gerard P. Metabolism of cholesterol and bile acids by the gut microbiota. *Pathogens*. 2013;3(1):14–24.
40. Wexler HM. Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev*. 2007;20(4):593–621.
41. van Dijk SJ, Feskens EJ, Bos MB, et al. A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. *Am J Clin Nutr*. 2009;90(6):1656–64.
42. Li YN, Huang F, Cheng HJ, et al. Gut microbiota of hepatitis B virus–infected patients in the immune-tolerant and immune-active phases. *Front Cell Infect Microbiol*. 2022;12:943889.
43. Shen F, Zheng RD, Sun XQ, Ding WJ, Wang XY, Fan JG. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int*. 2017;16(4):375–81.
44. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. 2014;157(1):121–41.
45. Joo EJ, Chang Y, Yeom JS, Ryu S. Hepatitis B virus infection and decreased risk of nonalcoholic fatty liver disease: a cohort study. *Hepatology*. 2017;65(3):828–35.

46. Huang J, Jing M, Wang X, et al. Hepatitis B virus infection status and risk of type 2 diabetes mellitus: a meta-analysis. *Dig Liver Dis*. 2017;49(4):403–10.
47. Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J Hepatol*. 2020;72(3):558–77.
48. Bajaj JS, Heuman DM, Hylemon PB, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol*. 2014;60(5):940–7.
49. Hartmann P, Hochrath K, Horvath A, et al. Modulation of the intestinal bile acid–FXR–FGF19 axis by microbes and host during liver disease. *J Hepatol*. 2018;68(2):287–95.
50. Vujkovic-Cvijin I, Dunham RM, Iwai S, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med*. 2013;5(193):193ra91.