ORIGINAL ARTICLE

Gut Microbiota Dysbiosis and Its Correlation with Viral Load in Chronic Hepatitis B Patients from Chennai, Tamil Nadu

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ABSTRACT

Background

Chronic hepatitis B virus (HBV) infection continues to pose a significant global health challenge, with viral load recognized as a crucial determinant of disease progression and prognosis. Growing evidence indicates that the gut microbiota plays a pivotal role in modulating immune responses and liver pathology through the gut–liver axis. Understanding microbial alterations associated with HBV viral load may offer insights into disease mechanisms and therapeutic opportunities.

Materials and Methods

A total of 104 participants were enrolled and categorized into three groups: healthy controls, low viral load HBV patients, and high viral load HBV patients. Clinical and biochemical parameters were recorded. Fecal samples were subjected to 16S rRNA gene sequencing to assess microbial diversity, taxonomic composition, and predicted functional pathways. Comparative analyses were performed across the groups to identify viral load–associated microbial changes.

Results

HBV-infected participants showed a marked reduction in gut microbial diversity compared to healthy controls. Notable taxonomic alterations included depletion of beneficial genera such as Faecalibacterium and Bacteroides, along with enrichment of potentially pathogenic Proteobacteria and Escherichia–Shigella. These changes were more pronounced in the high viral load group. Functional prediction analyses indicated significant disruptions in lipid metabolism pathways and an increase in bacterial cell wall biosynthesis processes, suggesting altered microbial functionality associated with disease severity.

Conclusion

The study demonstrates viral load–dependent gut dysbiosis in HBV-infected individuals, characterized by loss of beneficial commensals, expansion of pathogenic taxa, and functional metabolic disruption. These findings highlight the gut microbiota as a potential modifiable factor in HBV pathogenesis and support the exploration of microbiota-targeted therapies to enhance disease management.

Keywords

Hepatitis B virus, Gut microbiota, Viral load, Dysbiosis, 16S rRNA sequencing, Gut–liver axis, Functional prediction, Tamil Nadu population

INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains one of the most significant global public health challenges, affecting nearly 296 million individuals worldwide and causing over 820,000 deaths annually due to cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC). India is among the countries carrying the highest absolute burden, with approximately 40 million chronic carriers, representing about 11% of the global total. Although vaccination programs have improved coverage, adult vaccination remains inadequate, and many infections are only diagnosed at advanced stages when complications such as fibrosis or cirrhosis have already developed [1,2].

HBV is a small, hepatotropic DNA virus of the Hepadnaviridae family. Its persistence is largely attributed to the stability of covalently closed

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circular DNA (cccDNA) within hepatocyte nuclei, which serves as a template for viral transcription [3]. A central determinant of disease progression is the viral load, measured as HBV DNA concentration in serum. High viral load (HVL) is strongly associated with increased necroinflammatory activity, accelerated fibrosis, and elevated risk of HCC [4]. Conversely, low viral load (LVL) patients may remain in an inactive carrier state for years. However, significant inter-individual variability remains, with some patients progressing despite low viral replication, suggesting that host-related factors may influence disease course [5,6].

The gut and liver are anatomically and functionally linked via the portal circulation, forming the "gut–liver axis." In healthy states, the microbiota promotes immune tolerance, bile acid metabolism, and intestinal barrier integrity [7]. However, dysbiosis, characterized by reduced diversity and expansion of pro-inflammatory taxa, leads to barrier dysfunction ("leaky gut"), microbial translocation, and chronic immune activation [8]. In HBV infection, such dysbiosis may exacerbate hepatic inflammation, contribute to T cell exhaustion, and promote persistence of viral replication [9]. Importantly, microbial profiles differ between high and low viral load patients, suggesting that viral replication itself may shape gut ecology, while microbiota-derived metabolites can, in turn, affect HBV transcription and immune clearance [10]. Thus, the relationship between HBV viral load and the gut microbiota is increasingly recognized as bidirectional.

Tamil Nadu has unique dietary patterns characterized by high consumption of rice, millets, pulses, fermented foods such as dosa, idli, and buttermilk, and widespread use of spices like turmeric and cumin, which have antimicrobial and anti-inflammatory properties [11]. These diets typically enrich SCFA producers and lactic acid bacteria, potentially buffering against severe dysbiosis. This study therefore aimed to explore gut microbiota alterations in chronic HBV patients stratified by viral load, in comparison with healthy controls, within the Tamil Nadu population. These interactions can provide insights into the role of the gut–liver axis in HBV persistence, help identify microbial biomarkers of disease activity, and inform future microbiota-targeted adjunctive strategies in regions with constrained healthcare resources [12].

METHODOLOGY

This cross-sectional study enrolled 104 participants (aged 18–65 years) from a tertiary care hospital in Tamil Nadu. They were divided into three groups: healthy controls (Group A, n=52), low viral load CHB patients (Group B, HBV DNA <2000 IU/mL, n=22), and high viral load CHB patients (Group C, HBV DNA ≥2000 IU/mL, n=30). Exclusion criteria included prior antiviral therapy, antibiotic or probiotic intake within 3 months, diabetes, cirrhosis, or HBV-related cancer. Written informed consent was obtained from all participants.

Clinical and biochemical assessment

Medical history, medication use, and anthropometric data were collected. Complete blood counts were performed using the Sysmex XE-2100 analyzer. Serum ALT, AST, bilirubin, and lipid profile (total cholesterol, LDL-C, HDL-C) were quantified using the Roche Cobas c702 automated analyzer, while HbA1c was assessed by

immunoturbidimetric assay (Cobas c513). HBV DNA quantification and serological markers (HBeAg, HBeAb) were analyzed using electrochemiluminescence immunoassays (Cobas e801).

Fecal DNA extraction and sequencing

Stool samples were collected and processed for microbial DNA extraction using the QIAamp Fast DNA Stool Mini Kit. DNA purity was verified by NanoDrop spectrophotometry, and concentrations measured with Qubit fluorometry. Libraries were prepared targeting the V4 region of the 16S rRNA gene (~560 bp fragments), pooled, and sequenced on an Illumina MiSeq platform [13,14,15].

Bioinformatics and statistical analysis

Raw reads were processed in QIIME2 (v2023.2). Denoising, quality filtering, and chimera removal were performed using DADA2. Alpha diversity (Observed ASVs, Shannon index, Pielou's evenness, Faith's PD) and beta diversity (weighted/unweighted UniFrac, Bray–Curtis, Jaccard) were calculated. PERMANOVA (999 permutations) tested group differences. Differential abundance was assessed using ANCOM and LEfSe. Functional metagenomic predictions were carried out with PICRUSt2 and pathway analysis visualized in STAMP with Benjamini–Hochberg correction ($q < 0.05$).

RESULTS AND DISCUSSION

Demographic and Clinical Characteristics

Table 1. Demographic and Clinical Profile of Study Participant

Characteristics	Healthy Control (Group A)	Chronic Hepatitis B (CHB)	*p*-value[A,B]	HBV DNA IU/mL		p-value[A,B]	p-value (A, C)	p-value (B, C)
				Group B (HBV DNA <2000)	Group C (HBV DNA ≥2000)			
Number	52	52		22	30			
Age (years)	48±6	53±8	<0.001	51±5	54±7	<0.001	0.039	0.132
Male (%)	35 (38.4%)	37 (62.9%)	0.009	22 (65.5%)	15(56.8%)	0.008	0.179	0.530
BMI (kg/m²)	20.93 (1.11)	21.49 (1.05)	0.326	21.74 (0.96)	20.91 (1.27)	0.196	0.983	0.372
Lab Outcomes (mean, SD)								
WBC (cells/μL)	4116 (1207)	3389 (1241)	0.007	3443 (1566)	3261 (1125)	0.029	0.025	0.667
Platelets (cells/μL)	271,000 (57,060)	212,000 (49,740)	<0.001	212,000 (52,330)	214,000 (44,600)	<0.001	<0.001	0.913
Total Bilirubin (mg/dl)	0.48 (0.25)	0.71 (0.39)	<0.001	0.72 (0.39)	0.69 (0.40)	<0.001	0.052	0.814
AST (U/L)	19.18 (6.30)	21.86 (8.96)	0.067	20.98 (8.3)	23.94 (10.33)	0.228	0.086	0.304
ALT (U/L)	20.93 (14.02)	25.51 (24.13)	0.218	23.85 (25.11)	29.41 (21.84)	0.466	0.146	0.407
Lipid Profile (mean, SD)								
Total Cholesterol (mg/dl)	188.18 (32.14)	180.19 (30.64)	0.205	180.48 (30.62)	179.53 (31.67)	0.268	0.361	0.923
LDL-Cholesterol (mg/dl)	126.39 (31.83)	115.79 (26.50)	0.075	118.46 (26.52)	109.65 (26.34)	0.233	0.068	0.294
HDL-Cholesterol (mg/dl)	61.28 (13.83)	55.41 (15.35)	0.063	51.23 (11.83)	65.00 (18.97)	0.002	0.434	0.021

A total of 104 participants were studied: healthy controls (Group A, n=52), low viral load CHB patients (Group B, n=22), and high viral load patients (Group C, n=30). CHB groups had significantly higher mean age (53 ± 8 years) compared to controls (48 ± 6 years, p<0.001). Male proportion was higher in CHB (62.9%) than controls (38.4%, p=0.009). BMI showed no significant differences (Table 1).

Haematological and Biochemical Profiles

WBC counts were lowest in Group C (3261/μL) versus Group A (4116/μL, p=0.007). Platelets were reduced in CHB (~212,000/μL) compared to controls (271,000/μL, p<0.001). Bilirubin was elevated in Groups B and C (~0.70 mg/dL vs 0.48 mg/dL in controls, p<0.001). While AST and ALT levels were higher in CHB, differences were not statistically significant. HDL cholesterol was lowest in Group B (51.23 mg/dL) and highest in Group C (65.0 mg/dL, p=0.021), with no significant changes in LDL or total cholesterol [16]. These results suggest viral replication impacts hematological and metabolic markers independent of conventional liver enzymes.

Microbial DNA Quality and Sequencing

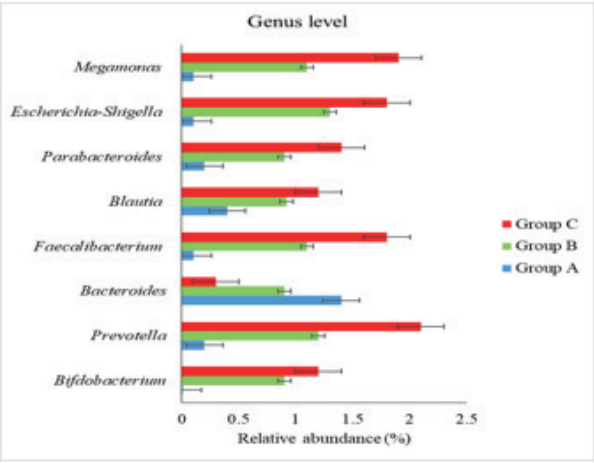
All stool DNA extractions yielded high-quality nucleic acids (A260/280 ratios 1.91–1.99). Libraries showed expected fragment sizes (560–595 bp) and sufficient concentrations, confirming suitability for Illumina sequencing.

Microbial Diversity

Fig. 1. Stated that Alpha diversity indicated highest ASVs in controls (230–345), while Group B showed reduced richness (123–323). Shannon diversity was greater in controls (3–5) compared to CHB (2.4–4.8). Faith's phylogenetic diversity peaked in Group C (18–25), suggesting expansion of phylogenetically distinct but potentially pathogenic taxa. Pielou's evenness was highest in Group B (0.7–1.0), reflecting even but less diverse communities. Beta diversity (PCoA using Bray–Curtis, Jaccard, and UniFrac) revealed significant compositional differences between controls and CHB ($p<0.01$) [17,18].

Taxonomic Shifts

At the phylum level, HBV patients exhibited increased *Firmicutes* and *Proteobacteria* compared to controls ($p<0.05$). At the genus level, Bacteroides predominated in controls, while CHB groups showed enrichment of *Bifidobacterium, Prevotella, Faecalibacterium, Blautia*, and *Escherichia–Shigella* (Fig. 2). Notably, *Faecalibacterium* abundance decreased with disease progression, consistent with loss of anti-inflammatory SCFA producers. In contrast, *Escherichia–Shigella* expansion reflects pro-inflammatory potential and compromised gut barrier function [19,20, 21].



Predicted Functional Pathways

PICRUSt2 and STAMP analyses identified several pathways differing across groups (Table 2). Saturated fatty acid elongation was significantly reduced in CHB, especially Group C (0.42 vs 0.70 in controls, $p<0.001$). Conversely, peptidoglycan maturation (0.48 vs 0.35, $p<0.001$), methylophosphonate degradation (0.64 vs 0.25, $p<0.001$), and thiamine diphosphate biosynthesis II (3.2 vs 2.2, $p<0.001$) were enriched in CHB, particularly in high viral load patients. These changes suggest impaired lipid metabolism but enhanced bacterial cell wall turnover and cofactor biosynthesis in dysbiotic communities [22].

Table2. Predicted microbial metabolic pathways with nominal significance across groups.

Pathway	Group A (Control)	Group B (Low Load)	Group C (High Load)	Statistical Significance
Saturated fatty acid elongation	0.70%	0.55%	0.42%	p < 0.001 (vs. A), p < 0.05 (vs. C)
Peptidoglycan maturation	0.35%	0.42%	0.48%	p < 0.001 (vs. A), p < 0.05 (vs. C)
Methylophosphonate degradation I	0.25%	0.55%	0.64%	p < 0.001 (vs. A), p < 0.05 (vs. C)
Super pathway of thiamine diphosphate biosynthesis II	2.2%	2.9%	3.2%	p < 0.001 (vs. A), p < 0.05 (vs. C)

Interpretation

Together, these results demonstrate that chronic HBV infection reshapes gut microbiota composition and predicted function in a viral load–dependent manner. Reduced microbial richness and loss of protective taxa (*Faecalibacterium, Bacteroides*) may impair SCFA production and gut–liver immune tolerance. The enrichment of *Proteobacteria* and *Escherichia–Shigella* indicates a pro-inflammatory milieu, consistent with microbial translocation and hepatic immune activation. Functional predictions further highlight disruption of host lipid metabolism and enrichment of microbial pathways linked to immune modulation. Importantly, liver enzyme levels (ALT/AST) did not correlate strongly with viral load, underscoring the added diagnostic value of microbiota profiling. These results align with the gut–liver axis model, suggesting that HBV pathogenesis is not purely hepatocentric but influenced by systemic microbial-immune interactions [23,24].

CONCLUSION

This study demonstrates that CHB is linked to distinct gut microbial and functional alterations, with viral load influencing the extent of dysbiosis. Reduced beneficial taxa and expansion of pro-inflammatory genera support a role for the gut–liver axis in disease progression. Microbiota-targeted interventions may offer promising adjuncts to current HBV therapies, warranting further longitudinal and interventional research

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