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Original Article

The salivary microbiome and oral health status in HBeAg-negative chronic hepatitis B

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KEYWORDS
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Abstract *Background/purpose:* Dysbiosis of oral microbiota has been reported in late stage of chronic hepatitis B (CHB) infection with cirrhosis. CHB is characterized by the constant virus-induced liver injury which may lead to liver cirrhosis and hepatocellular carcinoma (HCC). However, some patients show normal liver function without antiviral treatment, associating with favourable prognosis. The oral microbiota composition and oral health status in these patients is unidentified.
Materials and methods: The study focuses on the composition of oral microbiota and oral health status in individuals with CHB and HBV vaccinees as controls. The CHB patients were hepatitis B 'e' antigen (HBeAg)-negative, with or without elevated liver enzyme increase at time of sampling, The 16S rRNA high-throughput sequencing and bioinformatic analysis were applied to investigate oral bacterial diversity, and oral examination including decay-missing-filled teeth (DMFT) index, probing depth (PD) and mucosal status was performed, along with oral health questionnaire, to assess the oral health status in CHB patients and healthy controls.
Results: Our results indicate that their oral microbiome compositions are not significantly different though some have increased ALT/AST liver enzyme levels at the time of sampling, compared to the healthy control participants who are vaccinated e.g. protected from this viral

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disease. CHB patients here bore a good oral health status and life-style habits as comparing to healthy controls.
Conclusion: These findings suggest that a health-associated salivary microflora is present in CHB without severe liver injury. Continued regular dental health and lifestyle support in liver disease patients is therefore justified.

Introduction

The infection of hepatitis B virus (HBV) can cause a wide spectrum of liver diseases, including acute hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).[1] According to WHO, Chronic hepatitis B (CHB) affects more than 254 million people globally, killing approximately one million people every year. The deaths due to CHB have increased during last decade, with increased burden on the health care.[2] CHB has a variable and dynamic course resulted from the interactions between the virus and the immune system of the host. The gradual deterioration of liver function induced by HBV through abnormal immune response can lead to liver cirrhosis; and is responsible for making liver cancer the most common cancer in many parts of the world.[3] According to European Association for the Study of the Liver (EASL) guideline,[4] patients with low risk of progression to cirrhosis or HCC can remain in the phase of "HBeAg-negative chronic HBV infection", previously referred as "inactive carries". With a favourable prognosis, this phase is characterised by the loss of HBeAg, serum antibodies to HBeAg, undectable or low HBV DNA levels, and normal ALT (alanine aminotransferase). Despite many years of chronic infection, liver health in this phase is often preserved with minimal HBV-induced injury (no fibrosis or low inflammation). While specific immune control is believed to play a role in this phase, the complete mechanisms remain unclear.[1]

Gut-liver axis has been extensively studied in liver disease, showing linkage to the pathogenesis of different liver diseases, including viral hepatitis.[5] Gut microbiota is essential for the preservation of the integrity of the mucosal barrier function. Perturbed gut microbiota and gut barrier homeostasis can lead to increased hepatic injury due to inflammatory signalling and increased epithelial barrier permeability.[6] In advanced liver cirrhosis, an increase in dysbiosis has been shown. A study of Chinese patients with liver cirrhosis due to HBV or alcohol also showed a correlation between alterations in gut microbiota composition with the severity of the liver disease.[7]

While many studies have established associations between oral diseases and systemic diseases, the influence of oral disease and inflammation on HBV associated liver disease progression is still scarce.[8] It is a routine clinical practice to treat oral diseases including periodontitis prior to liver transplantation in order to reduce the risk of post-operative complications driven by potential oral pathogens.[9] It is possible that these pathogens and/or their products and inflammatory mediators could translocate via the gut and oral cavity through impaired mucosa tissue barrier, thus deteriorate the liver function. Changes in oral health and salivary microbiota were recently reported in patient groups with liver cirrhosis, hepatic encephalopathy and non-alcoholic fatty liver disease without considering the role of HBV.[10] However, the oral microbial community analysis of patients in the mild disease stage of CHB during an ongoing systemic immune control is still limited. Our study aims to investigate the microbial structure of oral flora in a CHB patient cohort without severe liver dysfunction to deepen the understanding of the relationship between oral health and liver diseases.

Material and methods

Subjects and clinical examination

The study focused on the composition analysis of oral microbiota and oral health status in individuals with CHB with no to limited liver damage, with HBV vaccinees as controls. The patients were recruited from the Department of Infectious Diseases at Karolinska University Hospital after oral and written patient consent between 2018 and 2020. The study was performed in accordance with the Declaration of Helsinki and the legislation in Sweden following approval from the Regional Ethics Board in Stockholm (EPN Dnr: 2018/454-31). All participants involved in the study were informed the intention of the study and sample collection, written informed consent was obtained from each participant. A total number of 65 subjects took part in the present study, of which 50 were diagnosed with viral hepatitis and 15 age- and gender-matched vaccinated donors. Four patients were excluded due to the hepatitis B 'e' antigen (HBeAg) positivity, which indicates presence of active HBV replication and high infectivity. The remaining CHB patients were divided into two groups based on the serological level of alanine aminotransferase (ALT) which assess the liver damage and malfunction.[4] Patients with normal ALT level were classified into group chronic infection (CI) and patients with elevated ALT level were classified into group chronic hepatitis (CH). The patient selection was performed based on CHB classification (European Association for the Study of the Liver (EASL) guideline)[4] and health assessment on virological and liver data obtained from their medical records. The data include test results on HBeAg, levels of HBV DNA, HBsAg, liver ALT and AST, APRI, as well as liver stiffness scores, ALP, GTT, creatinine, leucocyte count, neutrophil count. Besides, the following

exclusion criteria were applied: metastatic liver cancer; diagnose with other malignant diseases within five years; acute infection of the tonsil or salivary gland within six months; use of antibiotics, prebiotics and symbiotic in the previous month; diabetes, active coronary heart disease and kidney disease. There was no usage of antibiotics, prebiotics, or symbiotic within one month prior to sampling, and no known other chronic diseases in healthy individuals.

All the participants accepted oral examination to evaluate the oral health status. The clinical examination was performed by the same dentist, who measured endodontic and periodontal status including decay-missing-filled teeth (DMFT) index, pocket depth (PD), number of deep pockets, presenting of calculus and mucosal status.[11] Questionnaires focusing on cigarette and alcohol, yogurt consuming, and dental hygiene habits was completed in both the patient group and control group.

Sample collection and storage

Unstimulated saliva samples were collected in the morning (9—11 am). Participants were refrained from eating, drinking, smoking, or using oral hygiene products for at least 1 h prior to the sample collection. Sterile 50 ml Falcon collection tubes were kept on ice and used during the sample donation and the samples were transferred immediately to the lab. While in the lab, the samples were vortexed and aliquoted into sterile 1.5 ml sterile Eppendorf tubes with 1 ml saliva and store in −80 °C freezer within 4 h after collection.

DNA extraction for bacteria, amplification of 16S rRNA, library preparation, and sequencing

The bacterial DNA of saliva was extracted with Lysozymatic Lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) together with Mutanolysin (Sigma-aldrich, St. Louis, MO, USA). PCR amplification of 16S rRNA gene (hypervariable V3—V4 region) was performed in a thermal cycle by the primers pair (341F 5′CCTACGGGAGGCAGCAG-3'; 805R 5′GACTACHVGGGTATCTAATCC-3′). The construction of 16S rRNA gene library was performed in the following reaction components with 50 μL reactions: 25 μL of KAPA HiFi Kit (Roche, Basel, Switzerland), 22 μL of bacterial DNA, and 1.5 μL of each primer. The library preparation started with an initial denaturation of 5 min at 95 °C, then 35 cycles of a three-step temperature cycle including denaturation of 30 s at 98 °C, annealing of 30 s at 60 °C, and elongation of 30 s at 72 °C, and ends with a final extension of 5min at 72 °C.The quality of amplicons was then checked through gel electrophoresis on 2% agarose gel (Thermo Fisher Scientific). Thereafter, purified products by 1.8X Agencourt AMPure XP purification kit (Beckman Coulter Inc, Bear, CA, USA.) were evaluated on Qubit 2.0 fluorescence meter (Thermo Fisher Scientific) and adjusted to 4 nM concentration. The libraries were pooled and barcoded using dual indexing primers in a second PCR step. The 4 nM purified library was sequenced on the Illumina™MiSeq platform on 10 pM library and 10 % PhiX using 2 × 300 bp paired-end protocol (Miseq V3 reagents kit, Illumina Inc, San Diego, CA, USA). The experiments and sequencing process were quality-checked and were verified through non-template controls (NTCs), home-brew positive controls, and commercial control (mock bacterial community's standard) to trace any lab contaminations and user pitfalls.

Bioinformatic analysis

Raw sequencing data was applied to QIIME2™ microbiome bioinformatics analysis pipeline.[12] The raw reads were demultiplexed, denoised and QC-filtered to obtain high quality clean reads under specific filtration conditions. Two samples were excluded due to the shallow sequencing depth including one patient and one donor, leaving total 59 participants with 45 patients (31 in group CI and 14 in group CH) and 14 healthy controls. Amplicon Sequence Variants (ASVs) were generated for bacterial classification against the eHOMD 16srRNA database (version 15.22).[13] A phylogenetic tree was built for further analysis. Alpha diversity was calculated by adjusting the ASV table and diversity indices were applied to analyze the difference in richness and evenness of the microbial community. Mann—Whitney tests were used to test statistical significance. Beta-diversity was estimated by Bray—Curtis dissimilarity and Jaccard index. Principal coordinate analysis (PCoA) of Bray—Curtis dissimilarity was performed to compare the similarity among the microbiota of samples. Venn diagram was produced to illustrate the overlap of bacterial communities. Envfit analysis was performed to evaluate the environmental factors on the ordination of the microbiome structure.

Results

Clinical characteristics of chronic hepatitis B (CHB) patients

A total of 45 patients were diagnosed with chronic HBV Infection according to the EASL 2017 Clinical Practice Guidelines for management of HBV infection.[10] Table 1 shows the characteristics of the CHB patients and vaccinated saliva donors. The CHB participants were further subgrouped based on their liver enzyme ALT level, with 31 patients in group CI (chronic infection) and 14 in group CH (chronic hepatitis). 14 vaccinated donors were categorized into the control group. The groups do not show difference in age and sex, but the control group had lower body fatness. The virological and liver parameters indicated that CHB patients are in the immune-clearance phase with ALT low or moderately increased. All patients were HBeAg negative and their HBV DNA levels were controlled and low. FibroScan® (Echosens SA, Paris, France) scores of the patients ranged under 8 kPa, indicated low liver stiffness.[14,15] None of them requires antiviral treatment during this phase.

Salivary bacterial community in patients and healthy individuals

The salivary bacteria were annotated at the respective taxonomic level using the RDP (Ribosomal database

Table 1 Clinical characteristics of different participants.

Characteristics	CI	CH	Ctrl	*P* value
Subject (n)	31	14	14	
Age, years, mean ± SD	34.77 ± 9.94	38.93 ± 14.05	32.64 ± 7.03	0.275
Sex, men, n (%)	16 (51.61%)	11 (78.57%)	6 (42.86%)	0.137
BMI, kg/m^2, mean ± SD	24.91 ± 4.73	25.78 ± 4.18	21.48 ± 2.21	0.016*
HBV DNA level, log^{10} IU/ml, median (IQR)	2.51 (1.28)	2.91 (1.53)	n.d.	0.135
ALT, U/L, median (IQR)	21.76 (11.47)	49.12 (17.36)	n.d.	$P < 0.01$**
AST, U/L, median (IQR)	24.2 (5.01)	28.96 (18.39)	n.d.	0.216
FibroScan®,kPa, median (IQR)	4.9 (0.95)	5.75 (1.6)	n.d.	0.278

*$P < 0.05$; **$P < 0.01$.
Comparison of continuous variables by ANOVA test among three groups. Comparison of categorical variables by Chi-square test among three groups. Comparison of continuous variables by independent sample t-test between two groups. CH: chronic hepatitis; CI: chronic infection; Ctrl: control; HBV: hepatitis B virus; ALT: alanine aminotransferase; AST: aspartate aminotransferase; IU: international unit; IQR: interquartile range; SD: standard deviation.

project) taxonomic database. Shown in Fig. 1A, at the phylum level, the predominant saliva bacteriome phyla were *Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria*. And Fig. 1B illustrated a Venn diagram showing the overlap of ASVs for saliva from patient groups and healthy individuals. The compositional of saliva bacteriome in patients and controls appeared comparatively and similar across the participants.

To investigate the microbial community richness and diversity of the oral microbiome, Kruskal–Wallis non-parametric analysis of variance test was used to compare the richness and evenness based on alpha-diversity indices, the Observed, Chao 1, Ace, Shannon, and Simpson (Fig. 2). There were no significant differences in alpha-diversity of the microbial community noted between patients and controls.

Beta-diversity between the control group and CHB groups compared on the Bray–Curtis dissimilarity and Jaccard index were calculated and showed no significant differences in microbial clustering between the CHB patient groups and controls. Principal coordinates analysis (PCoA) based on Bray–Curtis distance as illustrated in Fig. 3, illustrates an ecological resemblance in the structure of microbiome community between CI and CH group. LEfSe analysis was also performed but no discriminative features were found among the groups.

Dental parameters in CHB patients and vaccinated donors

On the same day after the saliva sampling, participants were given a full-mouth dental examination and a questionnaire to evaluate their oral health and lifestyle. In Table 2, the dental parameters showed no significant difference between the CHB patients and vaccinated controls, except the periodontal indices, including number of deep pockets and manifestation of calculus, suggesting more periodontal inflammation in the patients especially the CH group. In CHB patients, dental treatment is regular and more frequent than the vaccinated controls. Besides, to evaluate the environmental factors on the shifting of the bacterial abundance, the envfit analysis was performed which showed that endodontic, periodontic and mucosal health have different ordination to the structure of the oral microbiome, reflecting the role of specific oral pathogens that are related with the oral status and microbiome community.

Lifestyle parameters in CHB patients and vaccinated donors

Questionnaires focusing on lifestyle were completed by all the participants, with questions including smoking, snus

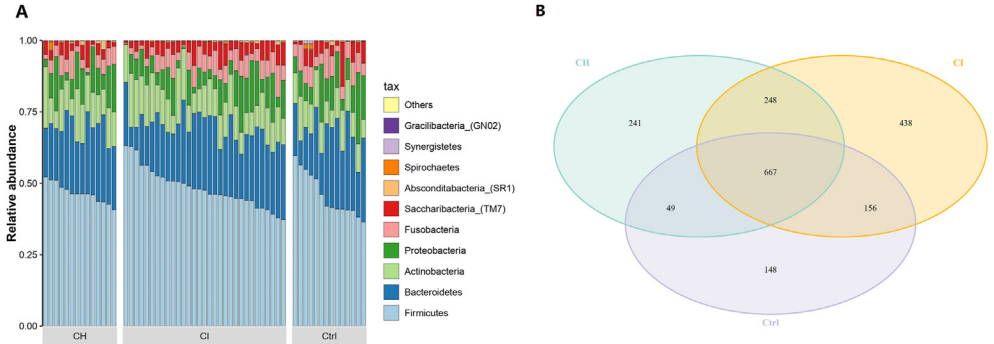

Figure 1 A) Bacterial composition of the salivary microbiota in the three groups (at Phylum level). B) Venn diagram of identified ASVs in respective group. CH: chronic hepatitis; CI: chronic infection; Ctrl: control.

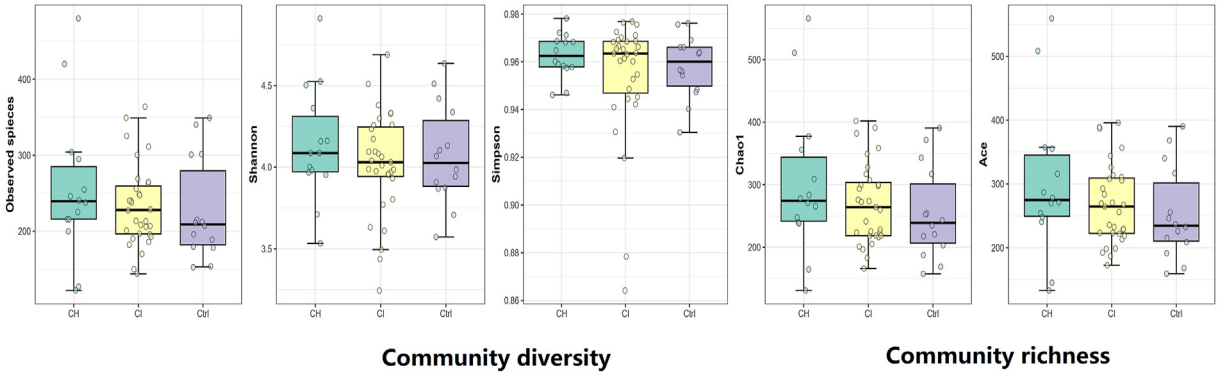

Figure 2 Comparative analysis of the diversity of the oral microbial community in each group using the indicated alpha-diversity indices (Y-axis). CH: chronic hepatitis; CI: chronic infection; Ctrl: control (X-axis).

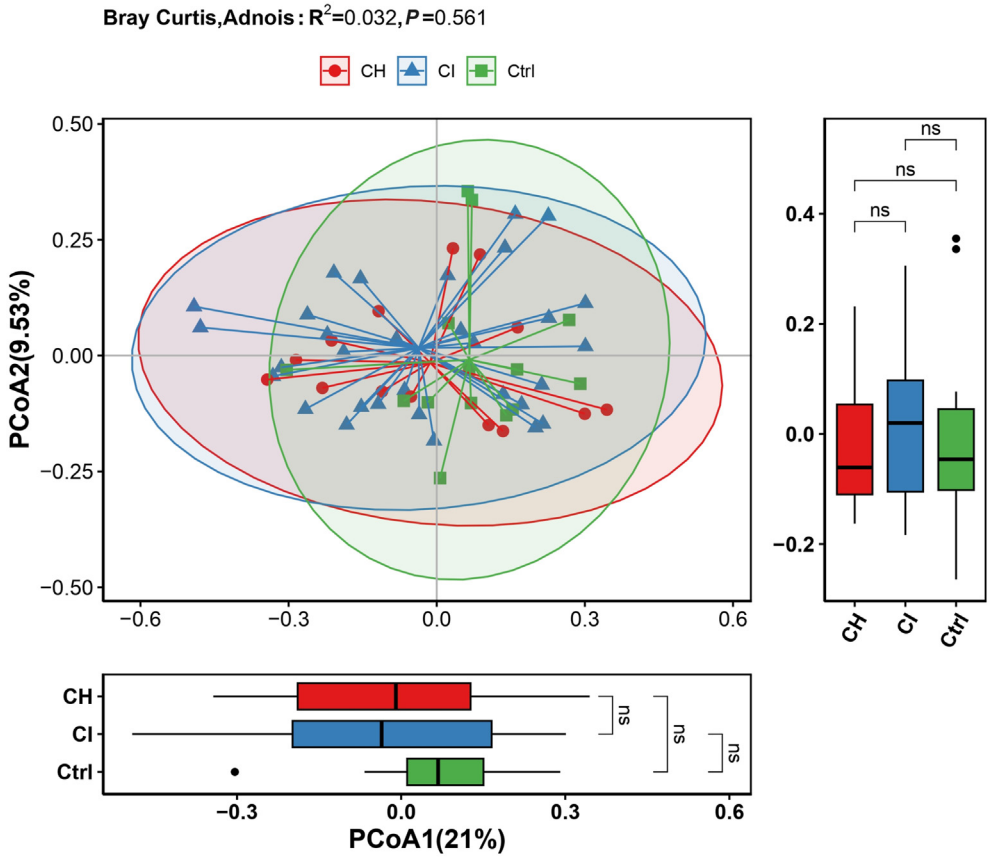

Figure 3 Principal coordinates analysis (PCoA) of oral bacterial community compositions among three groups. CH: chronic hepatitis; CI: chronic infection; Ctrl: control.

consumption, alcohol consumption, yoghurt with product name, food product based on fermentation process such as mozzarella, chorizo and other products, usage of vitamin supplement, and vegetarian or non-vegetarian. Questionnaire data collected from the participants shown in Table 3, indicated no major significant differences in the lifestyle and dietary habits between the groups. However, the snus and yoghurt consumption habits appeared lighter among vaccinated controls than CHB patients ($P < 0.05$). Shown in Fig. 4, the envfit analysis was performed to investigate the oral health status and oral hygiene habits on the ordination of the oral microbiome community among the three groups. It showed the indices of periodontal inflammation shared a relatively equivalent ordination on the shifting of the ecological community. Lifestyle factors on the ordination of the oral microbiome community among three groups on the other hand examined by envfit analysis (Fig. 5) showed that smoking and snus, yoghurt, and fermentation-based food have different ordination on the potential changing direction of the microbiome abundance. Alcohol consumption,

Table 2 Characteristics of CHB patients and vaccinated donners (oral health and oral habits).

Characteristics	CI	CH	Ctrl	*P* value
Subject (n)	31	14	14	
DMFT, mean ± SD	4.23 ± 5.87	5.50 ± 7.49	0.714 ± 1.44	0.07
PD, mean ± SD	1.24 ± 0.33	1.33 ± 0.64	1.18 ± 0.39	0.684
Number of deep pockets, mean ± SD	0	0.50 ± 1.16	0	0.019*
Calculus, n (%)	8 (25.8%)	5 (37.5%)	0 (0%)	0.040*
Ulcers, n (%)	6 (19.4%)	1 (7.1%)	0 (0%)	0.176
Tooth brush				0.177
None (edentulous)	0 (%)	1 (7.1%)	0 (0%)	
Once per day, n (%)	11 (35.5%)	3 (21.4%)	1 (7.1%)	
Twice per day, n (%)	18 (58.1%)	10 (71.4%)	12 (85.7%)	
More than twice per day, n (%)	2 (6.5%)	0 (0%)	1 (7.1%)	
Mouth wash, n (%)	13 (41.9%)	7 (50%)	2 (14.3%)	0.110
Dental treatment, n (%)	10 (32.3%)	2 (14.3%)	0 (0%)	0.026*

*$P < 0.05$.
Comparison of continuous variables by ANOVA test among three groups. Comparison of categorical variables by Chi-square test and Fisher Exact test among three groups. CH: chronic hepatitis; CI: chronic infection; Ctrl: control; DMFT: decayed, missing, and filled teeth; alanine aminotransferase; PD: periodontal disease; SD: standard deviation.

Table 3 Characteristics of CHB patients and vaccinated donors (lifestyle).

Characteristics	CI	CH	Ctrl	*P* value
Subject (n)	31	14	14	
Smoking, n (%)	4 (12.9%)	4 (28.6%)	0 (0%)	0.071
Snus consuming, n (%)	2 (6.5%)	5 (35.7%)	0 (0%)	0.015*
Alcohol consuming, n (%)	7 (50%)	11 (35.5%)	4 (28.6%)	0.481
Yoghurt consuming, n (%)	22 (71%)	10 (71.4%)	4 (28.6%)	0.017*
Fermentation, n (%)	13 (41.9%)	6 (42.9%)	4 (28.6%)	0.657
Vitamin supplements, n (%)	7 (50%)	8 (25.8%)	2 (14.3%)	0.098
Vegetarian, n (%)	1 (3.2%)	0 (0%)	0 (0%)	1

*$P < 0.05$.
Comparison of categorical variables by Chi-square test and Fisher Exact test among three groups. CH: chronic hepatitis; CI: chronic infection; Ctrl: control.

comparing to mucosal health, has the similar ordination on the microbiome community.

Discussion

Viral hepatitis is the seventh most common cause of death worldwide, among which at least half of the mortality is caused by the complications of infections with the hepatitis B virus (HBV).[2] Altered oral microbiome has been reported in several systemic diseases including bacteremia, diabetes, endocarditis, autoimmune disease, and cancer.[16–18] In the late stage of liver disease, especially in patients who suffer from liver cirrhosis and decompensation, the oral microbiome often is found to be altered as with the gut microbiome.[19–21] Thus, this "oral-gut-liver axis" is of clinical relevance.[22] A better insight will support the understanding of the impact of the oral health and host microbiome on the progression of liver diseases.

Our present study has investigated CHB patients with a relatively mild liver injury, with or without liver ALT/AST elevation at the time of sampling and dental examination, by assessing their oral microbiome composition and dental health. Our results indicate that their oral microbiome compositions are not significantly different though some had increased ALT/AST liver enzyme levels at the time of sampling, compared to the healthy control participants who are vaccinated e.g. protected from this viral disease. Of note, our CHB participants were mainly in their immune-clearance phase with low or moderately increased ALT levels. They were all HBeAg negative with low or no replicon of the virus in the blood.[23] Their HBV DNA were controlled and low, and the liver stiffness scores were normal in all CHB participants.[4] Apart from varying immunological control of HBV, the whole picture behind non-progressive viral hepatitis are currently not fully understood. Lifestyle factors are known to influence the immune system. Key lifestyle factors, including diet, alcohol consumption, smoking, and drug use, are also known to influence liver health and the progression of hepatitis B.[1] Poor oral hygiene and an imbalanced oral microbiome on the other hand can lead to an infection that may drive exacerbation of liver inflammation.[20,22] Maintaining good oral health and healthy lifestyle is therefore relevant in managing inflammation and preventing further liver complications. The oral microbial ecosystem, though sensitive to

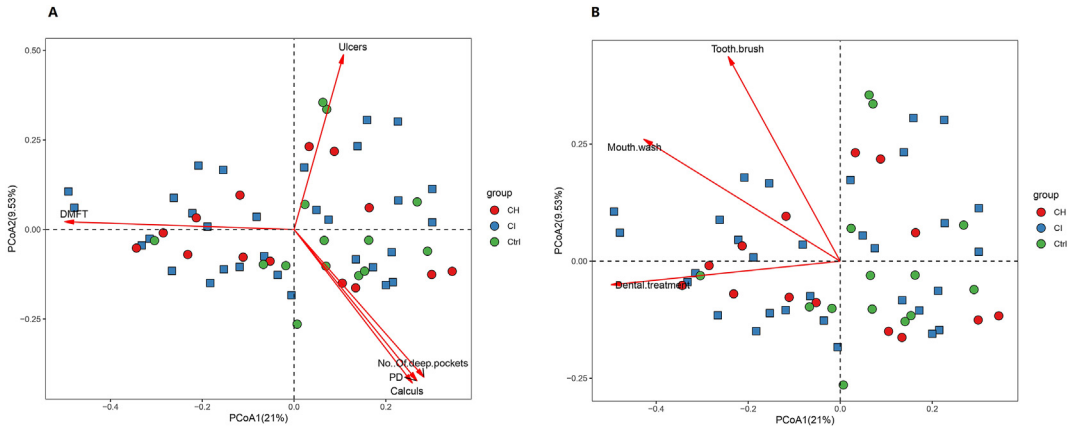

Figure 4 Envfit analysis fits oral health status and oral hygiene habits onto the ordination of the microbiome community. CH: chronic hepatitis; CI: chronic infection; Ctrl: control.

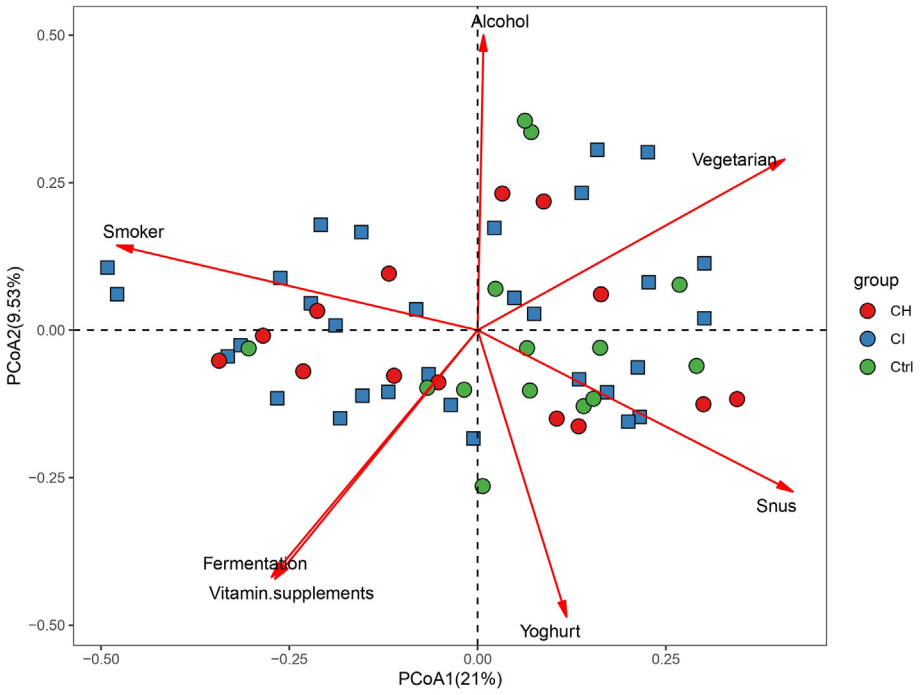

Figure 5 Envfit analysis fits lifestyle and dietary habits onto the ordination of the microbiome community. CH: chronic hepatitis; CI: chronic infection; Ctrl: control.

disruption by poor lifestyle factors and oral diseases, is known to be resilient and can recover quickly.[17,18,22,29] Relevant dental treatments that restore microbial balance and lifestyle changes including nutrient supplementation, have been shown to positively affect the oral microbiome and restore diversity in salivary microbiome profiles.[24,25] Based on the dental health and lifestyle data, we could also conclude that, apart from limited number of deep pockets and calculus, the degree of dental diseases noted in our patients did not substantially differ from the vaccinated controls. Besides, the CHB patients accepted curative care of the dental treatment. For the lifestyle, the yoghurt and snus consumption appeared higher. Overall, these findings suggest that our CHB patient cohort is relatively well-informed about dental hygiene and lifestyle routines and capable of maintaining good practices, as indicated by our collected data. This, in turn, could be reflected in their oral microbiome compositions, which showed no clear major differences as compared to the HBV-vaccinated control individuals.

Chronic hepatitis B(CHB) is a life-long infectious status due to the failure of clearance of infection with HBV, which has variable clinical manifestations and syndromes, from mild liver infection to active diseases that even lead to liver failure and carcinoma.[26] The different progress of CHB may be the result of the complex interactions between the virulence of the virus, the host immune responses, and other endogenous and exogenous factors.[27] Oral cavity and its colonized microbiome feature a complex relationship between the heredity and environment.[28–30] Dysbiosis of

oral microbiome can be found in many diseases, indicating its role in the maintain of homogeneous of the body.[31,32] Therefore, the results from our study could contribute to understanding the oral microbial community and its behavior under various disease conditions, such as mild, well-controlled viral hepatitis without significant liver injury that our patients present here. It suggested that the crosstalk between the systematic viral infection status and the oral microbiome could be kept in a relatively stable stage with the restraining of the viral replicon. In another study, oral microbial dysbiosis has been found in the progression of liver cancer, indicating that with the damage of liver function, the oral microbiota could be affected to lose the balance of the community. Thus, the initiation of the dysbiosis of the oral flora needs to be further investigated to help to provide possible diagnostic biomarkers for patients suffered from liver diseases.

There are several limitations in our study. First, there are no CHB groups with severe liver conditions in our comparison, this could provide more insights into disease development and the progression of this difficult-to-treat liver disease; longitudinal follow-up is lacking that could help to identify risk factors for potential progression of CHB to severe liver conditions; cohort sample size could have been larger and multicenter to allow better statistical power; definition of food items could have been more rigorous; microbiome data is limited to saliva and targeted 16S gene approach lacking the resolving power for identifying bacterial to species level or functional annotations of entire oral microbiome genes.

Overall, our study underscores the importance of the oral microbiome in the context of viral hepatitis, suggesting that oral health interventions could be a beneficial adjunctive strategy in managing liver diseases. Further studies are needed to establish causal relationships and develop effective microbiome-based therapies.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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References

1. Jeng WJ, Papatheodoridis GV, Lok ASF. Hepatitis B. *Lancet* 2023;401:1039—52.
2. World Health Organization. *Global hepatitis report 2024: action for access in low- and middle-income countries*. Geneva, Switzerland: World Health Organization, 2024.
3. Fanning GC, Zoulim F, Hou J, Bertoletti A. Therapeutic strategies for hepatitis B virus infection: towards a cure. *Nat Rev Drug Discov* 2019;18:827—44. Erratum in: Nat Rev Drug Discov 2020;19:291.
4. European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017;67:370—98.
5. Pabst O, Hornef MW, Schaap FG, Cerovic V, Clavel T, Bruns T. Gut-liver axis: barriers and functional circuits. *Nat Rev Gastroenterol Hepatol* 2023;20:447—61.
6. Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: pathophysiological basis for therapy. *J Hepatol* 2020;72:558—77.
7. Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014;513:59—64.
8. Ling Z, Liu X, Cheng Y, et al. Decreased diversity of the oral microbiota of patients with Hepatitis B virus-induced chronic liver disease: a pilot project. *Sci Rep* 2015;5:17098.
9. Martin P, DiMartini A, Feng S, Brown Jr R, Fallon M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* 2014;59:1144—65.
10. Grønkjær LL, Holmstrup P, Schou S, et al. Presence and consequence of tooth periapical radiolucency in patients with cirrhosis. *Hepat Med* 2016;8:97—103.
11. World Health Organization. *Oral health surveys: basic methods*, 5th ed. Geneva, Switzerland: World Health Organization, 2013.
12. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019;37:852—7.
13. Plessis C, Jeanne T, Dionne A, Vivancos J, Droit A, Hogue R. ASVmaker: a new tool to improve taxonomic identifications for amplicon sequencing data. *Plants* 2023;12:3678.
14. Oeda S, Tanaka K, Oshima A, Matsumoto Y, Sueoka E, Takahashi H. Diagnostic accuracy of FibroScan and factors affecting measurements. *Diagnostics* 2020;10:940.
15. Xiao H, Shi M, Xie Y, Chi X. Comparison of diagnostic accuracy of magnetic resonance elastography and Fibroscan for detecting liver fibrosis in chronic hepatitis B patients: a systematic review and meta-analysis. *PLoS One* 2017;12:e0186660.
16. Hou K, Wu ZX, Chen XY, et al. Microbiota in health and diseases. *Signal Transduct Targeted Ther* 2022;7:135.
17. Kilian M. The oral microbiome - friend or foe? *Eur J Oral Sci* 2018;126(Suppl 1):S5—12.
18. Krishnan K, Chen T, Paster BJ. A practical guide to the oral microbiome and its relation to health and disease. *Oral Dis* 2017;23:276—86.
19. Acharya C, Bajaj JS. Altered microbiome in patients with cirrhosis and complications. *Clin Gastroenterol Hepatol* 2019;17:307—21.
20. Acharya C, Sahingur SE, Bajaj JS. Microbiota, cirrhosis, and the emerging oral-gut-liver axis. *JCI Insight* 2017;2:e94416.
21. 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.
22. Albuquerque-Souza E, Sahingur SE. Periodontitis, chronic liver diseases, and the emerging oral-gut-liver axis. *Periodontol 2000* 2022;89:125—41.
23. Alexopoulou A, Karayiannis P. HBeAg negative variants and their role in the natural history of chronic hepatitis B virus infection. *World J Gastroenterol* 2014;20:7644—52.
24. Deo PN, Deshmukh R. Oral microbiome: unveiling the fundamentals. *J Oral Maxillofac Pathol* 2019;23:122—8.
25. Kato I, Vasquez A, Moyerbrailean G, et al. Nutritional correlates of human oral microbiome. *J Am Coll Nutr* 2017;36:88—98.

26. Shi Y, Zheng M. Hepatitis B virus persistence and reactivation. *BMJ* 2020;370:m2200.
27. Yuen MF, Chen DS, Dusheiko GM, et al. Hepatitis B virus infection. *Nat Rev Dis Prim* 2018;4:18035.
28. Verma D, Garg PK, Dubey AK. Insights into the human oral microbiome. *Arch Microbiol* 2018;200:525—40.
29. Sedghi L, DiMassa V, Harrington A, Lynch SV, Kapila YL. The oral microbiome: role of key organisms and complex networks in oral health and disease. *Periodontol* 2000 2021;87:107—31.
30. Huang C, Shi G. Smoking and microbiome in oral, airway, gut and some systemic diseases. *J Transl Med* 2019;17:225.
31. Yamashita Y, Takeshita T. The oral microbiome and human health. *J Oral Sci* 2017;59:201—6.
32. Baker JL, Mark Welch JL, Kauffman KM, McLean JS, He X. The oral microbiome: diversity, biogeography and human health. *Nat Rev Microbiol* 2024;22:89—104.