Original Article

Journal of Immunology (ISSN 2995-861X)

Homogeneity Gut microbiome Characteristics in Autoimmune Epithelitis: Primary Biliary Cholangitis and Primary Sjögren's Syndrome.

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Received Date : Dec 16, 2023
Accepted Date : Dec 18, 2023
Published Date : Jan 24, 2024

ABSTRACT

Background : Accumulating studies had revealed that dysbiosis of the gut microbiome was involved in the occurrence and development of Primary biliary cholangitis (PBC) and Primary Sjögren's syndrome (pSS).

Aims : To examine the similarities and differences in gut microbiome as well as the relationship with pathogenesis in PBC and pSS.

Methods : High throughput sequencing was conducted to analyze the 16S rDNA V3-V4 region of fecal microbial samples from 109 subjects including PBC (n=38), pSS (n=41) and healthy controls (HC, n=30) from the Affiliated Hospital of Qingdao University. The composition, diversity and markers of gut microbiome were compared between PBC, pSS and HC. Inter-group comparisons were conducted using analysis of variance and the Mann-Whitney U test. The similarity and differences in gut microbiome were compared by the heat-map and LEfSe analysis at the phylum and genus levels.

Results : The results showed that the microbiome of PBC and pSS patients were distinct from that of HC. Bifidobacteria in PBC and pSS patients were clearly reduced with significantly decreased alpha diversity ($P<0.05$). Strikingly, the majority of species that were differentially abundant in PBC and pSS were highly similar when compared to HC. However, there were specific pattern of micobiome imbalance between the two diseases in that Actinobacteria was higher in pSS patients whereas Proteobacteria was elevated in PBC patients ($P < 0.05$). Furthermore, Bifidobacteria was decreased in PBC patients with severe cholestasis while pathogenic bacteria (Shigella, Collinella, and Streptococcus) were increased. Among pSS patients, the proportion of Bifidobacteria was reduced in the active pSS group, whereas Rossella was increased in those with inactive pSS.

Conclusions : PBC and pSS shared similarities in gut microbiota composition and diversity but distinct from HC, suggesting common mechanistic microbial influences on their pathogenicity, but manifested differently under other confounding factors.

Keywords : Primary biliary cholangitis, Primary Sjögren's syndrome, Gastrointestinal microbiome, 16S rDNA sequencing, autoimmune epithelitis

Core tip : By comparing the composition of the gastrointestinal microbiome (GM) of patients with primary biliary cholangitis (PBC), primary Sjögren's syndrome (pSS) and healthy controls, we found that the GM of PBC and pSS patients was significantly different from that of HC. Moreover, it was firstly approved that PBC and pSS shared many similarities in their GM composition and diversity, suggesting common mechanistic microbial influences on their pathogenicity, but manifested differently under other confounding factors. This study lays a foundation for investigating how the GM may exert pathogenic effects on other organs through the gut-endocrine axis and signaling pathways.

Journal of Immunology (ISSN 2995-861X)

INTRODUCTION

Primary biliary cholangitis (PBC) and Primary Sjögren's syndrome (pSS) are both immunological disorders histopathologically depicted as autoimmune epithelitis [1]. PBC is a chronic organ-specific autoimmune diseases (AIDs) characterized by immune-mediated destruction of biliary epithelial cells (BEC) that causes non-suppurative damages in the intrahepatic duct, progressing to fibrosis, cirrhosis, and subsequent liver failure [2-4]. On the other hand, pSS primarily affects exocrine glands and other organs including the liver. Over 20% of pSS patients manifest liver function abnormalities and liver biopsy pathology similar to PBC [5, 6]. The prevalence of concomitant pSS with PBC is evident. Insert Reference (PMID: 35970319). Thus, PBC could be regarded as pSS of the liver, whereas pSS might be similarly considered as PBC of the salivary glands [7]. Genetics and environmental factors play key roles in the pathogenesis of PBC and pSS. The natural history of PBC and pSS involves the initiation of apoptosis of epithelial cells followed by presentation of autoantigens on biliary and exocrine gland epithelia that activate the cognitive autoreactive T cells. Subsequently, a multi-lineage autoreactive T and B cell response leads to massive infiltration of autoantigen specific CD4+ and CD8+ T to the target organs causing the immune-mediated injury of the salivary gland epithelial cells (SGEC) or BEC [8]. Increased levels of INF-γ, essential for Th1 differentiation, are observed in pSS and PBC patients [9]. Th17 cells are believed to involve in the pathogenesis of both diseases [10, 11]. Moreover, both pSS and PBC are related to the dysfunction of B cells as evident by the elevation of B cell activating factor (BAFF) levels, hyperIgM and presence of serum autoantibodies anti-mitochondrial autoantibodies (AMA) and Anti-SSA/SSB antibodies for PBC and pSS, respectively). In PBC, there is a significant relationship between BAFF and biochemical alteration levels, AMA titers, and disease stage. A recent study demonstrated that combination of anti-BAFF and anti-CD20 treatment was effective in reducing serum levels of AMA, total IgM and IgG as well as alleviating biliary damage in female ARE mice model of PBC. Although pSS is also associated with extra glandular presentations [12, 13], given the crucial role of the immune system in the pathogenesis of PBC and pSS, it is pivotal to comprehend the triggers of inflammatory responses and the possible contribution from the human gut microbiome. Dysbiosis can elicit the development of AIDs [14]. Accumulating studies had revealed that dysbiosis of the gut microbiome was involved in the occurrence and development of these two diseases [15-19]. Gut microbial diversity is an important indicator that reflects the stability of the gut microbiome. The species richness of the gut microbiome in patients with PBC or pSS is markedly different [20, 21]. This study is aimed to examine the composition and diversity of the intestinal microbiota of patients with PBC, pSS and HC by by 16S rRNA sequencing. Moreover, bioinformatic analysis was performed using Quantitative Insights Into Microbial Ecology (QIME) to address the relationship of the microbiome and their possible mechanistic impact on the pathogenesis of PBC and pSS.

MATERIALS & METHODS

Study group

Patients with PBC and pSS were recruited from the Department of Rheumatology and Immunology, an affiliated hospital of Qingdao University, China, from December 2019 to June 2020. Thirty-eight PBC patients were diagnosed according to the classification criteria of the 2000 American Association for the Study of Liver Diseases (AASLD) PBC guidelines[22]. PBC patients were further divided according to their serum alkaline phosphatase (ALP) levels into mild and severe groups with ALP level ≤ 3 times the upper limit of normal value and ALP > 3 times the upper limit of normal value respectively. Forty-one patients with pSS were enrolled based on the 2012 American Rheumatism Association Classification Standard for Sjogren's Syndrome[23]. pSS patients with the EULAR Sjögren's syndrome disease activity index (ESSDAI) scores >3 were assigned to the active group, and those having scores ≤ 3 were in the inactive group. 30 healthy subjects with routine health examinations and medical records in the health management centre of the hospital were recruited as healthy controls (HC). All HC subjects met the following in inclusion criteria: (1) normal blood pressure; (2) normal range of urine, stool, blood glucose, lipid, and kidney function tests; (3) free of hepatitis B/C virus antigen; (4) and not taking antibiotics and other gut microbiome regulators (prebiotics and/or probiotics) within 3 months. This research adhere to the principles of the Helsinki Declaration and was approved by the ethics committee of the affiliated hospital of Qingdao University (approval number: QYFY WZLL 26798). All study participants provided written informed consent for sample collection and participation.

DNA extraction, 16S rDNA gene amplicon sequencing and data processing

Fresh stool samples from all participants were collected and frozen at −80^{O}C within 2 hours after collection. Microbial genomic DNA was extracted from fecal samples by a QIA amp DNA Stool Mini Kit following the manufacturer's protocol .16S rDNA gene sequencing analysis was performed using QIIME (Quantitative Insights into Microbial Ecology, v1.8.0, http://qiime.org/) and the R package 3.5.1 (https://www.r-project.org/). Forward primer (338F 5'-ACTCCTACGGGAGGCAGCA-3') and reverse primer (806R 5'-GGACTACHVGGGTWTCTAAT -3') were used to amplify the V3-V4 regions of the 16S rRNA gene.

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Bioinformatic analysis

The quality filtering of the readers, as well as their taxonomic classification, was performed via QIIME software, version 1.9.1(http://qiime.org/1.9.1). The quality-filtered readers were clustered into the operational taxonomic unit (OTU) at 97% similarity threshold and the taxonomic assignment was performed with the genes database (Release 13.8, http://greengenes.secondgenome.com). Four α diversity metrices including Chao1, observed species, Shannon, and Simpson were assessed to estimate fecal microbial community richness, information content, and evenness via Mothur software (version: v.1.30.1). Based on the biological evolution information of sequences from each sample, the weighted Unifrac metric principal coordinates analysis (PCoA) was performed to estimate the Beta diversity of the gut microbiota, which reflected differences of the intestinal microflora between groups. Linear discriminant analysis effect size "LEfSe" was used to determine the genera that best characterize each study group. LEfSe was performed to detect differentially abundant taxa at the phylum, family, and genus levels across groups using the default parameters. Heatmap was plotted using R package 3.5.1.

Statistical analyses

Continuous variables were represented as mean ± standard deviation (SD) or interquartile range and analyzed using ANOVA and Mann-Whitney U test for comparisons among multiple groups. Categorical variables were represented as percentages (%) and analysed via the chi-square test to compare differences among multiple groups. The significant difference in Beta diversity was tested with Adonis non-parametric test. ANOVA and the Mann-Whitney U tests were performed by SPSS software, version 25.0 (IBM Corp, New York, NY, USA). The Adonis non-parametric test and Student's t-test were performed through R software, version 3.4.1 (https://www.r-project.org). The similarity and differences in gut microbiome were compared by the heat-map and LEfSe analysis at the phylum and genus levels. P-values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of the study population

The demographics and clinical characteristics of participants are summarized in **Table 1.** After quality control, a total of 109 fecal samples were obtained from PBC, pSS patients, and HC subjects. There was no significant difference in age, gender, height, weight, and BMI index among all participants.

Table 1. Characteristics of PBC, pSS patients, and HC

		PBC n=38	**pSS n=41**	**HC n=30**	**F/χ^2**	**P**
Age in years, mean ± SD		47.66±13.69	45.46±11.02	45.97±8.16	0.39	0.68
sex, n	Male	2	3	2		0.93
					0.14	
	Female	36	38	28		
Height (cm)		161.08±3.92	162.22±3.90	159.73±5.78	2.65	0.08
Weight (kg)		56.61±4.81	58.37±4.90	56.80±6.48	1.26	0.29
BMI(kg/m^2)		21.80±1.32	22.16±1.44	22.24±1.98	0.82	0.44

P-values for PBC, pSS, and HC statistical comparisons were calculated with the t-test or Fisher's exact test. P-values for the overall study population were calculated with the one-way ANOVA or Chi-squared test.

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Diversity of gut microbiota in PBC, pSS patients and HC

64,141 sequenced operational taxonomic units (OTU) were processed from all the fecal samples. The sequenced OTU obtained from PBC, pSS and HC were 18,243, 19,913, and 25,985 respectively.

The pSS and PBC cohort had reduced gut microbiome α diversity as measured by abundance when compared with the HC (Chao1, P= 0.045, P=0.02; Shannon, P=0.00034, P=0.0067; Simpson, P=0.0014, P=0.0036; and Observed Species, P=0.0044, P=0.0025 respectively) (Fig 1A). On the other hand, there were no differences in the evenness and richness in the gut microbiota between the PBC and pSS patients (Chao1, P= 0.66; Shannon, P= 0.59; Simpson, P=0.66; and Observed Species, P=0.77. Fig 1A).

Intestinal microflora in PBC, pSS patients and HC

A weighted UniFrac Principle Coordinate Amalysis (PCoA) was performed to identify the difference in fecal intestinal microflora composition between patient with PBC, pSS and HC. As shown in Fig 1B there were significant differences in clustering from PBC and pSS patients compared with HC (P= 0.001, P = 0.027and P=0.027) and the total intestinal microflora were significantly different between these groups.

Composition of relative abundance of gut microbiota at phyla level in PBC, pSS patients and HC

According to the sequencing analysis, the gut microbiota of the PBC, pSS, and HC consisted of four bacterial phyla mainly Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. Firmicutes (58.45%, 62.42% vs. 64.21%; P=0.145,0.639) and Bacteroides (14.25%, 16.17% vs. 26.77%; p=0.378,0.062) in PBC and pSS were lower than HC. The Proteobacteria in PBC were higher than pSS and HC (16.36% vs. 8.15%, 5.51%; P=0.025,0.001). The Actinobacteria in PBC and pSS were higher compared with HC (8.45%, 14.31% vs. 3.16%; P=0.039,0.000) (Table 2, Fig 2A). These results indicate that the composition of the gut microbiota was different in the two groups for Actinomycota and Proteobacteria when compared with HC.

Table 2. Analysis of gut microbiota at phyla in patients and HC

Group	n	Actinobacteria (%)	Proteobacteria (%)	Bacteroidetes (%)	Firmicutes (%)
PBC	38	8.45±14.51	16.36±24.22	16.17±20.11	58.45±25.55
pSS	41	14.31±19.94	8.15±16.86	14.25±16.83	62.42±23.08
HC	30	3.16±3.98	5.51±8.30	26.77±22.71	64.21±21.05
P value	PBC vs. HC	0.039*	0.001*	0.378	0.145
	pSS vs. HC	0.000*	0.101	0.062	0.639
	pSS vs. PBC	0.015*	0.025	0.288	0.250

Abbreviations: PBC, primary biliary cholangitis; pSS, primary sjögren's syndrome; HC, healthy controls.

Composition of relative abundance of gut microbiota at genus level in PBC, pSS patients and HC

Analysis of the top 10 of gut microbiota in relative abundance at the genus level showed that the Bifidobacterium in PBC and pSS were significantly reduced compared with HC. The reduction in PBC group was more obvious (P<0.05) compared with other groups. The proportion of Faecalibacterium in pSS was significantly higher than PBC and HC groups (Table 3, Fig 2B).

To determine the differences in gut microbiota among the three groups, gut microbiota abundances at the genus level were compared by LEfSe (Fig 2C). 22 biomarkers were found in PBC patients where the top 5 were: p-Proteobacteria, c-Gamaproteobacteria, c-Bacilli, f-Enterobacteriaceae, and o-Enterobacterales. p-Proteobacteria was the most significant among all. They were f-Ruminococcaceae, g-Faecalibacterium, p-Gemmatimonadetes, f-ACK-M1 and Xanthomonadales in pSS, and Lachnospiraceae, g-Clostridium, Acorus, g-Mitsuokella and g-Hyphomicrobium in HC.

The similarity and differences in community composition in pSS, PBC and HC were further investigated by heat map (Fig 2D, 2E) and noted there were key changes in the abundance of the gut microbiota between them. The relative abundance of Saccharibacteria (TM7), Proteobacteria, and Fusobacteria was higher in PBC. In pSS, Synergistes, Actinobacteria, Verrucomicrobia, tenericutes, and gemmatimonadetes were higher. In HC the relative abundance of Firmicutes, Bacteroidetes, Chloroflexi, and caldatribacterium (OP9) was higher (Fig 2D). The relative abundance of the top 20 gut microbiota at the genus level (Fig 2E) revealed that Streptococcus, Collinsella, Psychrobacter, and Shigella were dominant in PBC whereas Prevotella, Gemmiger,

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and Faecalibacterium were dominant in pSS. In HC, Bifidobacterium, Roseburia, Alistipes, Coprocccus, and Dorea were most dominant. Based on this data, the abundance of pathogenic bacteria (Shigella, Collins) in the gut flora in patients with PBC were than in pSS and HC.

Table 3. Analysis of gut microbiota at genus in PBC, pSS patients and HC

genus	PBC	pSS	HC	P	Pa	Pb	pc
Bacteroides	13.04%	15.77%	14 .84%	0.837	0.848	0.718	0.498
Bifidobacterium	5.08%	6.69%	16.07%	0.176	0.019*	0.007	0.621
Faecalibacterium	5.01%	13.17%	7.34%	0.005*	0.041	0.418	0.001*
Blautia	8.76%	6.95%	8.88%	0.396	0.442	0.964	0.380
Shigella	9.01%	4.17%	3.93%	0.083	0.950	0.198	0.131
Roseburia	4.05%	3.98%	6.04%	0.435	0.248	0.271	0.966
Coprococcus	2.04%	3.12%	3.43%	0.119	0.767	0.185	0.201
Gemmiger	1.93%	3.24%	1.56%	0.119	0.112	0.725	0.135
Streptococcus	3.25%	1.04%	1.29%	0.051	0.883	0.241	0.106
[Ruminococcus]	2.13%	2.33%	1.89%	0.211	0.207	0.579	0.396

P^a = relative abundance difference between pSS group and HC group, P^b = relative abundance difference between PBC group and HC group, and P^c = comparison of relative abundance of top 10 gut microbiota between pSS group and PBC group. *= P<0.05, **= P<0.01.

Serlogical chemistry and gut microbiota between mild and severe cholestasis group in PBC patients

As expected, the levels of bile acid, aspartate aminotransferase (AST), alanine transaminase (ALT), ALP, and gamma-glutamyl transferase (γGGT) of PBC patients group were significantly higher in PBC patients with severe cholestasis than those with mild cholestasis. The levels of platelets (PLT) in the severe group were significantly lower than that in the mild one (Table 4, Fig 3A). The relative abundance of Bifidobacteria, in severe group was significantly lower in those with mild cholestasis ($p<0.05$). Pathogenic bacteria (such as Shigella, Streptococcus, and Collinsella) were more abundant whereas Facealibacterium and Corprococcus were less in PBC patietns with severe cholestasis , but the difference was not statistically significant (Table 5, Fig 3B).

Table 4. Comparison of serological indexes between mild and severe cholestasis PBC patients

	Mild(n=26)	Severe(n=12)	F/χ^2	P
WBC	6.29±2.39	5.95±1.60	1.169	0.661
Hb	117.73±27.10	124.42±10.91	1.023	0.076
PLT	183.38±81.25	131.92±36.15	16.08	0.010
ALT	35.15±18.11	52.83±22.13	0.989	0.013
AST	32.33±13.24	52.83±22.13	0.989	0.013
GGT	65.40±37.18	172.17±45.26	0.803	0.000
ALP	123.59±74.28	361.17±132.74	2.272	0.000
TBA	9.92±5.33	19.92±8.32	4.637	0.002

WBC: white blood cells; Hb: hemoglobin; PLT: platelet; ALT: alanine aminotransferase; AST: Aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase; TBA: Total bile acid.

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Table 5. Comparison of gut microbiota at genus level between mild and severe cholestasis PBC patients

Genus	Mild	Severe	P
Bacteroides	16.73%	19.91%	0.744
Bifidobacterium	20.50%	4.82%	0.033
Blautia	5.64%	10.61%	0.077
Faecalibacterium	8.97%	4.78%	0.179
Shigella	2.30%	9.40%	0.124
Roseburia	2.75%	3.30%	0.717
Coprococcus	3.97%	2.14%	0.245
Streptococcus	1.25%	3.95%	0.382
Gemmiger	2.21%	1.96%	0.787
Collinsella	0.36%	2.92%	0.468

The influence of pSS disease activity on the gut microbiota

At the phylum level, the relative composition of the gut microbiota of pSS patients in the active and inactive groups were similar (Fig 3C). Among the top ten abundant bacteria, their relative quantity were highly comparable with the exception that Bifidobacteria was less the proportion of Roseburia was significantly higher in patients with active pSS (P= 0.002) (Table 6, Fig 3D).

Table 6. Comparison of gut microbiota at genus level between active and inactive pSS patients

Genus	SS—S	SS—M	P
Faecalibacterium	12.02%	11.92%	0.982
Bifidobacterium	9.34%	15.13%	0.401
Bacteroides	11.63%	11.96%	0.954
Blautia	6.94%	7.59%	0.839
Roseburia	7.25%	2.24%	0.002*
Shigella	3.72%	4.87%	0.753
Gemmiger	4.61%	2.68%	0.273
Coprococcus	2.50%	3.53%	0.504
Ruminococcaceae_Ruminococcus	3.01%	1.87%	0.332
[Ruminococcus]	1.28%	1.17%	0.872

SS-S: pSS disease activity group; SS-M: pSS disease inactivity group. *=P<0.05

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Figure

Figure 1

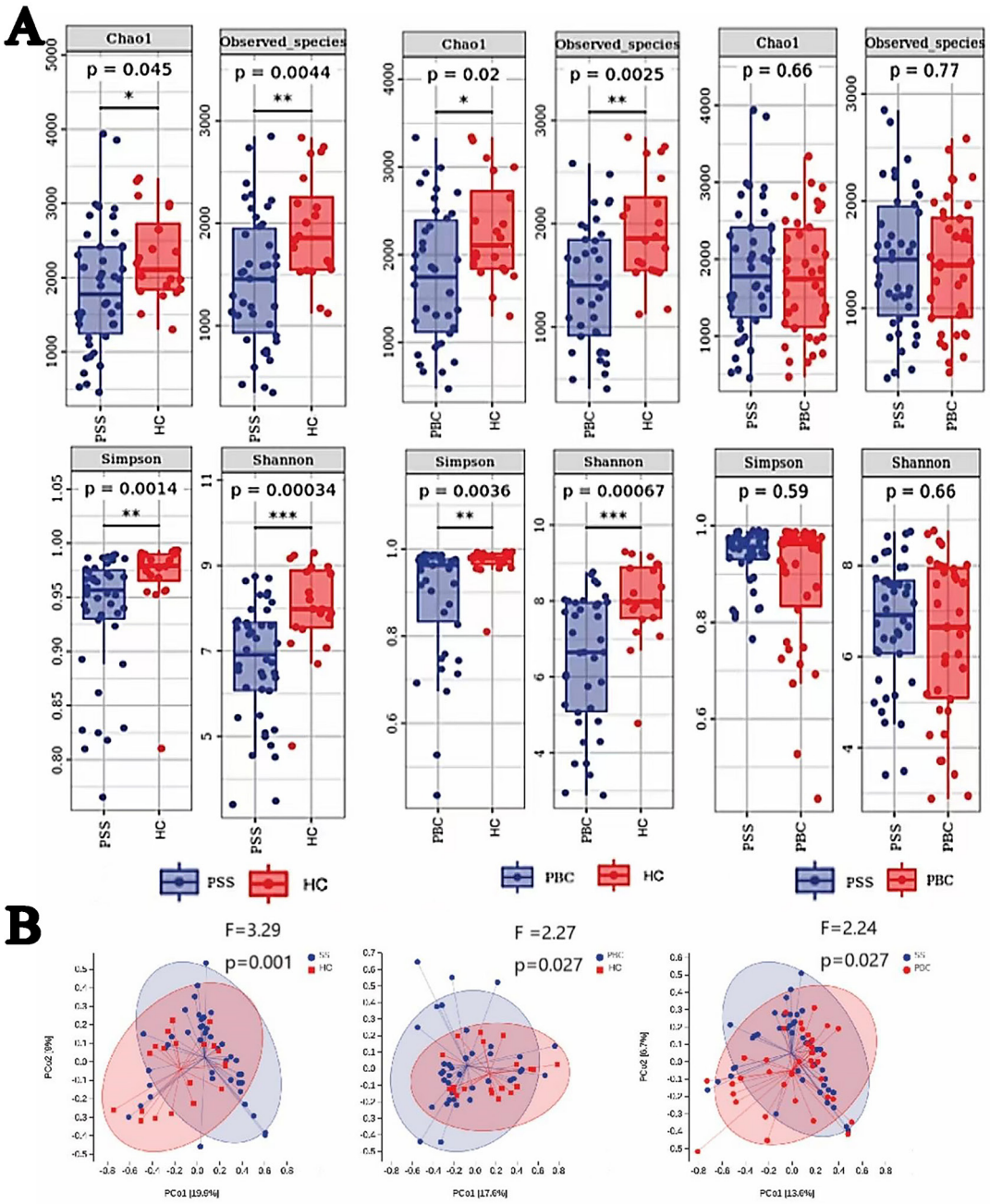

Figure 1. Diversity of gut microbiota in PBC, pSS patients and HC. A: Comparison of Alpha diversity index between pSS, PBC and HC groups. *Significantly higher than the HC, $p < 0.05$ (* $p < 0.05$; ** $p < 0.01$; ***$p<0.001$). Box plot representation of gut microbiota richness and diversity distribution across the histogram of alpha diversity indexes. The Chao1 estimator and Observed species estimator were used to identify community richness. Shannon and Simpson indexes were used to identify community diversity for both diversity and evenness between subgroups of patients and HC. B: Principal coordinates analysis plots of PBC, pSS patients and HC. The plots were based on weighted UniFrac distances.

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Figure 2.

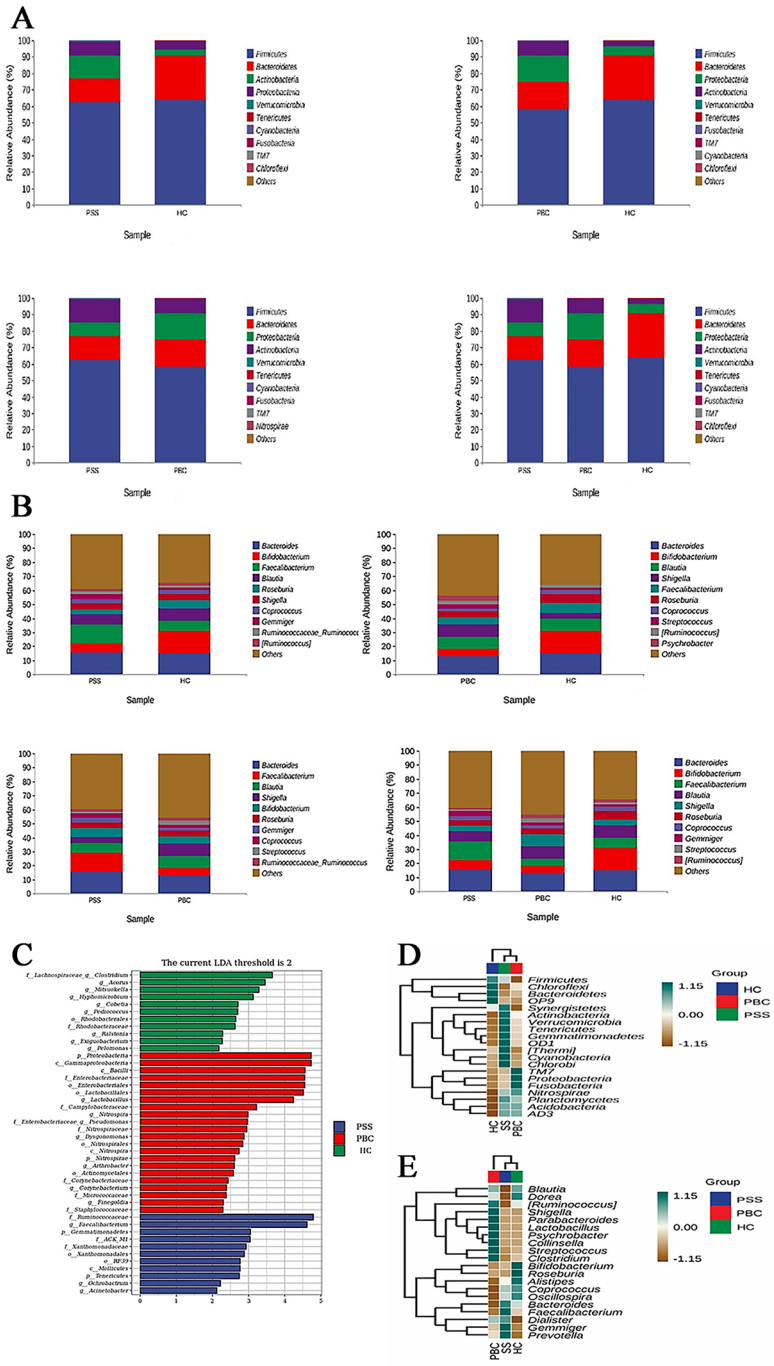

Figure 2. Composition of relative abundance of gut microbiota at phyla/genus level in PBC, pSS patients and HC. A: Comparison of relative abundance of gut microbiota in top 10 at phyla level in pSS, PBC and HC patients. B: Comparison of relative abundance of gut microbiota in top 10 at genus level in pSS, PBC and HC patients. C: Linear discriminant analysis (LDA) demonstrated distinct bacterial genera enriched in PBC, pSS and HC. Genera with P<0.05 and LDA score >2 were considered significant and were shown here with notation for their corresponding phylum(p), family(f), order(o), class(c), and genus(g) level. D: variations in the heatmap of the top 20 abundances of fecal bacterial taxa at the phyla level. E: variations in the heatmap of the top 20 abundances of fecal bacterial taxa at the genus level.

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Figure 3.

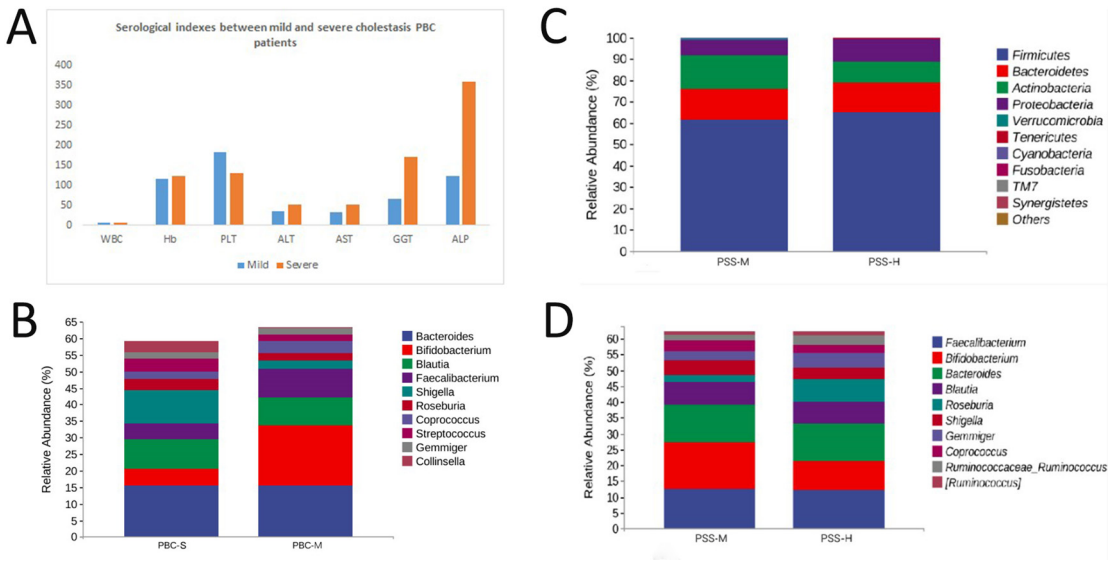

Figure 3. A-B: Serological indexes and Relative abundance composition of the fecal microbiome between mild and severe cholestasis group in PBC patients. A: Serological indexes between mild and severe cholestasis PBC patients. B: Relative abundance composition of the fecal microbiome at genus level in patients with mild and severe cholestasis. C-D: Relative abundance composition of the fecal microbiome in patients with pSS from the active and inactive group. C: The composition of the top 10 abundances of fecal bacterial taxa in the pSS active and inactive group at the phylum level. D: The composition of the top 10 abundances of fecal bacterial taxa in the pSS active and inactive group at the genus level.

DISCUSSION

PBC and pSS are both typical AIDs orchestrated by immune-mediated destruction of epithelial cells [13]. For a long time, it has been much debated whether PBC and pSS were the same, closely associated, or different AIDs. Like other AIDs, genetic, epigenetic, environmental, and infectious factors have been demonstrated critical for the development PBC and pSS. Hirschfield et al. reported an association between PBC and the IRF5-TNPO3 locus, at chromosome 17q12-21, and at loci MMEL1 in a cohort of PBC patients of European descent. This finding was intriguing because IRF5-TNPO3 has been linked to various other AIDs-related conditions, namely pSS lupus, and systemic sclerosis (SSc) [24]. Various studies suggested that changes in the structure or composition of gut microbiota might be one of the mechanisms that induced these AIDs [17, 21, 25]. Although the species richness of the gut microbiome in patients with PBC or pSS is markedly different, the similarities and differences in the dysbiosis between them require further investigation [20, 21].

Our study found that both PBC and pSS patients had lower alpha diversity in their gut microbiota compared to the HC, but there was no significant difference between the two diseases in our research. Reduced gut bacterial diversity may be favourable to the expansion of pathogenic bacteria niches, which could disrupt the mucosal barrier and activate mucosal cells in intestinal lamina propria and mesenteric lymph nodes to release inflammatory mediators, aggravate inflammatory effects, and lead to AIDs [25, 26]. Mice with pSS living in a sterile environment were found to have more severe damage to the mucosal barrier of the cornea than that those in a normal environment, and can develop clinical symptoms of pSS earlier in life [27]. The dry eye phenotype of pSS mice was significantly worse than the normal one, and interestingly its incidence was inversely proportional to the diversity of the fecal flora [28]. The species diversity and abundance of gut microbiota in pSS patients were significantly lower than those in HC [29]. Our study results were in agreement with the results of these previous studies in that pSS and PBC patients had a lower proportion of beneficial bacteria and a higher proportion of opportunistic pathogens [17]. Similarly, the diversity of gut microbiota was found to be lower in a constructed PBC mouse model than that in HC. Moreover, T cell-mediated inflammatory infiltration and bile duct damage were significantly reduced after drug intervention [30]. These findings suggest that the decrease in gut microbiota diversity may be related to the pathogenesis of PBC and pSS.

Data on species annotation analysis on the OTU showed that the relative abundance of Actinomycota in PBC and pSS patients was more than that in HC [31]. In our study, PBC patients were enriched in Proteobacteria, which contains numerous AIDs-causing pathogens. Animal studies also

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confirmed that an increased in Proteobacteria disrupted the stability of the gut microbiota community, and was could be used as a biomarker of flora imbalance and potential diseases [32, 33]. Pathogenic Proteobacteria could produce endotoxins, cause antigen exposure that leads to activation of dendritic cells, promote the synthesis of pro-inflammatory mediators in the intestinal lamina propria, mediate the hyperactivation of lymphoid cells and subsequently induce AIDs. Therefore, it is likely that Proteobacteria are involved in the onset of PBC.

Our data suggests that dysbiosis of gut microbiome is an initiating factor in the pathogenies of autoimmune epithelitis in PBC and pSS. The amount of Bifidobacterium, which regulates intestinal immunity and maintains mucosal barrier function, were decreased in PBC and pSS patients when compared with HC. Secondary metabolites of Bifidobacteria in the intestine could regulate the adaptive immune response mediated by B cells, reduce the production of pro-inflammatory factors, and inhibit inflammatory response via the nuclear factor kappa-B (NF-κB) pathway. Some strains could inhibit the binding of lipopolysaccharide (LPS) and CD14 receptors, reduce the activation of NF-κβ, and exert certain anti-inflammatory effects [34, 35]. Bifidobacterium could also inhibit the oxidation reaction of free radicals, reduce cell damage and enhance the body's immune regulation ability [36]. Thus, the decrease in the abundance of this type of Bifidobacterium, as noted at the genus level in our cohort, strongly indicate the impact of microbial composition on the etiology and pathogenesis of AIDs [37]. Several studies have confirmed that some strains of this genus play an effective role in the treatment of diarrhoea, irritable bowel syndrome, and Crohn's disease [38-40]. Therefore, the decrease in intestinal Bifidobacterium population might be an adverse factor affecting the development of PBC and pSS. Supplementing Bifidobacterium to regulate the gut microbiome might be a new strategy for the treatment of these two diseases.

Gut microbiome can generate short-chain fatty acids (SCFA) and have significant immunological implications. Lachnospirillaceae, Faecococcus, and Clostridium, were significantly reduced in the PBC and pSS patients. Such bacterial types could be beneficial as they can produce SCFA to participate in the differentiation and regulation of a variety of immune cells [41, 42]. SCFA and their metabolites also regulate the balance of Th17/Treg cells and the production of IL-10, thereby enhancing the immune barrier function of the intestinal mucosa and inhibiting the release of pro-inflammatory factors IL-2, IL-8, and TNF-α to exert anti-inflammatory effects [43, 44]. In B cells, SCFA accelerates cellular metabolism, producing energy and building blocks to support antibody production [45]. Reduced flora in PBC and pSS may lower SCFA levels and trigger immunological dysfunction. The reduction in this type of flora promotes the enrichment of potential opportunistic pathogens (such as Enterococcus), which can evade the immune system surveillance, adhere to host cells to form biofilms, and stimulate the intestinal mucosal epithelium or inflammatory cells to produce inflammatory mediators and exacerbate the inflammatory response [46, 47]. Supplementing mice with SCFA can not only reduce the local intestinal inflammatory response in intestines but also improve the condition of allergic respiratory diseases [48]. In addition, symptoms of SSc patients could be improved after oral administration of probiotics [49]. Therefore, it is suspected that the use of intestinal microecological preparations might improve the gut microbiota disorder and have a potential in the prevention and treatment of PBC and pSS .

Bacterial infection might be a potential mechanism in the development of PBC. Several large-scale, case-control studies have found that urinary tract infections caused by Escherichia coli are associated with the onset of PBC [50, 51]. Animal studies and molecular mimicry analysis of the PBC mitochondrial autoantigen and E. coli proteins showed that E. coli was a key factor in breaking immunological tolerance against the mitochondria, resulting in the production of AMAs . Novosphingobium aromaticivorans, an ubiquitous xenobiotic-metabolizing bacterium, is another candidate that might be involved in the etiology of PBC. Moreover, a variety of pathogenic bacteria could induce PBC bile duct damage through molecular simulation mechanisms. Similarly, certain antibodies in the serum of PBC patients also have cross-immune reactions with peptides produced by intestinal bacteria [52]. Our study also found that the relative abundance of pathogenic bacteria (such as Collinsella and Shigella) in the dominant flora in PBC patients was increased, and these bacteria were significantly enriched in PBC patients with severe cholestasis which was similar to that observed in rheumatoid arthritis (RA) and Graves' disease patients [53-55]. Metabolites of these genus could reduce the expression of tight junction protein ZO-1 in epithelial cells and up-regulate the expression of RORα and chemokines as well as cause changes in the permeability of the intestinal mucosa, allowing pathogenic bacteria to opportunistically invade and trigger the body's immune response [56]. Shigella was also a dominant bacterium in PBC patients; it could inhibit the secretion, recovery, and endocytosis of host cells by secreting specific virulence factors IpaJ and VirA to promote its invasion of the colonic epithelium [57]. Bacteria and their metabolites penetrate the portal venous system and enter liver circulation, producing inflammation and exacerbating the liver disease. Hence, we speculate that strains of Collinsella and Shigella may be involved in the development of PBC.

The more severe the dysbiosis of intestinal microflora, the more severe the disease. The proportion of beneficial bacteria

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such as Bifidobacteria was significantly reduced in severe cholestasis PBC group than that the mild PBC group. The reason for cholestasis in PBC might be the damaged small bile ducts, and the accumulation of bile acids in turn aggravates the damage to the bile duct and intestinal mucosa. Bile duct cells could also experience innate immune reactions with gut microbiota in the bile excretion and enterohepatic circulation, causing or promoting inflammatory reactions and aggravating organ damage [58]. The accumulation of bile acid itself could alter the bile duct and intestinal microenvironment, leading to the growth of certain pathogenic bacteria, which is in agreement with our research findings. In contrast, the changes in bile acids of PBC patients might be related to the abnormal expression of bile acid transporters and nuclear receptors, which are related to the biological enzymes produced by some gut microbiota [59]. Gut microbiota enzymes (bile acid hydrolases and induced enzymes) play an important role in maintaining bile acid homeostasis. In addition, actinomycetes and Bacteroides (such as Lactobacillus, Bifidobacteria, and Clostridia) strains have special bacterial enzymes that can act on the 3, 7, and 12 hydroxyl groups of bile acids and change their structure and reduce hydrophobicity or toxicity, thereby protecting the liver from invasion [60]. The reduced proportion of Bifidobacteria in severe cholestasis PBC patients might cause decreased enzyme levels, weaken the detoxification of bile acids, and aggravate organ damage. Therefore, the interaction between bile acids and some strains of the gut microbiome might play a crucial role in the process of PBC [61].

Among patients pSS with active disease, Bifidobacterium was reduced while Roseburia was increased compared with the inactive group. A recent meta-analysis showed that probiotic supplements could reduce the disease activity of ulcerative colitis [62]. Similarly, Bifidobacteria supplementation decreased the inflammatory cell count in multiple sclerosis patients [63]. Roseburia is a potentially beneficial bacteria that can produce butyric acid and improve the levels of atherosclerosis and alcoholic fatty liver in mice [64, 65]. We reason that the “good” and “bad” intestinal bacteria are not in static state but in fact in dynamic state. In this study, our data first revealed that the proportion of Roseburia bacteria in the active pSS group was significantly increased. However, the specific mechanism remains unclear and needs to be further explored.

This study also has various limitations such as the immunologic and lymphocyte subset analysis or cytokines levels were not assessed between the three groups. in addition, the association of inflammation and microbiome alterations was not studied in detail. In addition, the type of diet followed by patients and HC was not fully assessed and compared.

CONCLUSION

In conclusion, the gut microbiota in PBC and pSS patients showed a significant increase in pro-inflammatory bacteria and opportunistic pathogens and a reduction in beneficial bacteria. However, PBC and pSS patients exhibited a number of similar changes in gut microbiome. Based upon our data, we hypothesise that intestinal dysbiosis can be one of the pathogenic factors in the development of autoimmune epithelitis in PBC and pSS. Our research provides data supporting the need for further detailed studies on PBC and pSS gut microbiome to examine the alteration in gut microbiome and precisely identified the relevant bacterial strains underlying the etiology of their mechanistic role in the disease development. Modulation of the gut microbiome can become a therapeutic strategy in PBC and pSS.

Acknowledgments

We would like to thank these patients and their family for providing their consent to publish this report.

Funding

This work was supported by National Natural Science Foundation of China [grant number 8167060249, 81241094]; the Natural Science Foundation of Shandong Province, China [grant number ZR2016HM13] and the Science and Technology Project of Qingdao West Coast, China [grant number 2020-50, 2022-49].

Footnotes

Institutional review board statement : This research adhere to the principles of the Helsinki Declaration and was approved by the ethics committee of the affiliated hospital of Qingdao University (approval number: QYFY WZLL 26798).

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

Data Availability Statement : All data generated in the study are presented within the manuscript. Additional de-identified data can be obtained from the corresponding author upon reasonable request.

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