Distribution of Subgingival Bacteria in Chronic Periodontitis Patients Correlated with IgA Nephropathy

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SUMMARY

Background: Several studies indicated that chronic periodontitis (CP) and its subgingival bacteria correlated with IgA nephropathy (IgAN). Previous research has shown that prevalence of IgAN in chronic periodontitis patients is significantly higher than that in non CP patients in Xinjiang especially in ethnic Uyghur. The aim of this study is to investigate the distribution of plaque bacterial microbes in CP and IgAN patients and to find correlation between CP and IgAN.

Methods: All of the subgingival plaque samples including 7 healthy controls (N group), 8 CP patients, 14 IgAN patients, and 14 CP with IgAN patients were obtained from ethnic Uyghur people. To investigate the distribution of plaque microbes in Uyghur CP and IgAN patients, the 16s rRNA sequencing and comparative analysis of subgingival bacteria were performed.

Results: There were no statistically differences in the community richness estimator (Chao) and the diversity estimator (Shannon index) among four groups. The abundance of Burkholderiales (order), Ottowia (genus) in the plaque microbes were significantly higher in CP with IgAN patients than CP patients. The abundance of Eubacterium (genus) was significantly higher in CP with IgAN patients than IgAN patients. The abundance of Veillonella (genus) was significantly higher while Streptococcus (genus), Tannerella (genus) were significantly lower in CP patients than healthy volunteers.

Conclusions: The composition and abundance of subgingival plaque microbes in Uyghur CP and IgAN patients were significantly different at several levels. Which suggested that abundance of subgingival bacteria is correlated to CP and IgAN.


KEYWORDS

chronic periodontitis, IgA nephropathy, subgingival bacteria, 16S rRNA gene

INTRODUCTION

Chronic periodontitis (CP) is a chronic infectious, inflammatory, and nonspecific disease caused by microorganisms in gingiva and periodontal tissue. Moderate or serious periodontitis especially untreated periodontitis, may correlated with several systematic diseases [1,2], such as cardiovascular diseases [3], diabetes [4], respiratory diseases [5], and chronic kidney disease.
IgA nephropathy (IgAN) is a common glomerular disease, usually presenting proteinuria with or without hematuria [6]. About 10% - 20% of patients will progressively develop to end-stage renal disease (ESRD) within 10 years. It is reported that pathogenesis of IgAN is referred to mucous membrane infection [7-9]. Recurrent or chronic bacterial infection, which is a potential stimulant of the mucosal IgA system, has been suggested as a risk factor for the development of IgAN. Tonsillectomy, for example, has been reported to have a therapeutic effect in animal models and patients with IgAN [10]. The prevalence of CP and aggressive periodontitis is higher in IgAN patients than non IgAN patients, which indicates that the occurrence and development of IgAN is correlated with CP [11,12]. Oral microbial infection and CP might be a risk factor for the occurrence and development of IgAN.

In this decade, our group focused on the study of CP and kidney diseases in Moyu. Moyu is located in south Xinjiang, 97.19% of the local resident is Uyghur. We observed that the prevalence of IgAN in ethnic Uyghur is higher than Han (20.06%, 27.27%, and 25.13% in Han, Uyghur, and other ethnic groups, respectively) and the prevalence of CP in rural Uyghur residents in Moyu increased from 66.6% in 2007 to 79.6% in 2013, in comparison with 37.5% in urban China and 49.5% in rural areas. In addition, the prevalence of IgAN in CP patients is significantly higher than non CP patients (53% vs. 29%) in Xinjiang province.

To investigate the correlation between oral bacteria distribution and IgAN in Uyghur CP patient, plaque samples from Uyghur IgAN patients, CP patients, and healthy Uyghur volunteers were collected. For all samples, high throughput 16S rRNA sequencing was used to analyze the plaque microbes. The subgingival micro-biome distribution of all subjects was observed to investigate the correlation of IgAN and CP.

MATERIALS AND METHODS

Ethics statement
Written informed consent was obtained from all patients in this study. The study design, protocol, and informed consent were approved by Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (20170605-16). The methods were carried out in accordance with relevant guidelines.

Collection of subgingival plaque samples
All of the samples were obtained from ethnic Uyghur people. A total of 43 samples including 7 healthy volunteers (N group), 8 CP patients (B group), 14 IgAN patients (C group), and 14 CP with IgAN patients (D group) were selected and examined at the First Affiliated Hospital of Xinjiang Medical University. All subjects were ≥ 20 years - < 60 years old, gender unlimited. The standard of healthy control group: oral examination except periodontitis, without albuminuria, without renal hematuria under the naked eye or microscope, serum creatinine within the normal range. Part of patients with CP were detected by Florida probe and software system while the rest were diagnosed by experienced stomatologists in accordance with the diagnostic criteria of periodontitis. All patients with IgAN were diagnosed after renal biopsy. Exclusion standard: patients with advanced tumor, dialysis, mobility disorder, chronic obstructive pulmonary disease, tuberculosis, AIDS. In each group, the plaque samples were scraped from the neck and root of the gingiva with aseptic excavated spoon handheld instruments, and then stored in 70% ethanol immediately.

Generation of amplicon libraries and Miseq sequencing
Metagenomic DNA was extracted from subgingival samples by using commercial kit. The concentration and purity was detected through the NanoDrop 2000 (Thermo Fisher Scientific, USA) and checked by regular 0.8% agarose gel electrophoresis. The bacteria genomic DNA was amplified with the forward primer: GACTA CHVGGGTATCCTAACCC and reverse primer: CCTAC GGGNGGCWGCAAG specific for the V3-V4 hypervariable regions of the 16SrDNA gene. Three PCR products per sample were pooled to mitigate reaction-level PCR biases. At the same time, the standard bacterial/fungal genomic DNA mix was used as a positive control. Amplification products were checked using gel electrophoresis and purified using the Agencourt AMPure XP kit. The products were quantified accurately by Invitrogen Qubit 3.0 Spectrophotometer (Thermo Fisher Scientific, USA), then mixed in proportion by mole ratio, and sequenced on Illumina MiSeq Benchtop Sequencer for generating 2 × 250 bp pair-end reads at Genesky Biotech, Shanghai. Each sample was at least 50,000 reads.

Data processing and statistical analyses
Raw sequencing reads were processed by Shanghai Genesky Biotech co., Ltd. (Shanghai, China) using Trim Galore and MOTHUR. The sequences were removed if they were shorter than 100 bp, had a low-quality score (≤ 20), using Trim Galore (v0.4.5). The overlapping paired-end reads were merged using FLASH 2 (v2.2.00). MOTHUR (v1.39.3) was used to identify and wipe off the index. The sequences were removed if they were shorter than 100 bp, had basic group error rate more than 2%, using Usearch (v10). Clean reads were sorted into different samples according to their barcodes and clustered into OTUs at 97% sequence similarity with UPARSE denova mode. These OTUs were used as a basis for calculating alpha-diversity and beta-diversity metrics using MOTHUR and R (v3.4.3). The sufficiency of the sampling effort was evaluated by drawing rarefaction curves, the bacterial community diversity within each individual sample was estimated using the Shannon-Wiener index, the species richness was estimated with the CHAO1 index. The R was used to classify all sequences into different taxonomic groups. The beta-diversity was performed with R (v3.4.3) to assess the differences of microbial
Subgingival Bacteria in CP and IgAN

communities among all groups based on their composition. A principal coordinate analysis (PCoA) of weighted UniFrac was performed to compare the overall structure of subgingival microbiome of all samples, based on the relative abundance of OTUs (at an 80% similarity level). The abundance of bacterial phyla for each group was expressed as the percentage of total sequences and the bacterial community structures of all groups were further compared at phylum, class, order, family, and genus level using Metastats. p-values were corrected using a false discovery rate (FDR) correction to account for correction of multiple testing.

RESULTS

Demographic characteristics of studied subjects
Subgingival plaque samples were collected from 7 healthy volunteers (N group), 8 CP patients (B group), 14 IgAN patients (C group) and 14 CP patients with IgAN (D group). The average age of B, C, D, and N groups were 48.63 ± 14.47, 36.71 ± 13.25, 40.57 ± 14.58, and 32.14 ± 9.0 years, respectively. No statistical difference was found (p > 0.05). The percentage of male and female were 58.1% and 41.9%, respectively.

Characteristics of MiSeq sequencing results
A total of 7,196,178 raw reads were obtained from all 43 subgingival plaque samples. After filtering, 6,123,256 clean reads and 776 OTUs were obtained. The Rarefaction Curve of all samples (Figure 1) indicated that our sequencing was deep enough.

Alpha diversity
There was no significant difference in the community richness estimator (Chao) and the diversity estimator (Shannon index) among all groups (Supplementary Figure 1).

Taxonomy at different levels in all groups
Overall bacterial compositions for all samples at phylum level were shown in Figure 2. A total of 25 phyla in all samples were obtained. The dominant phyla of all groups were Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria, representing 86.31% of the total sequences (Figure 2). The most dominant microbes were Firmicutes and Bacteroidetes in group B, D, and N, while in group C they were Firmicutes and Proteobacteria. Compared with the other three groups, Fusobacteria (18.7%) in B group, Proteobacteria (23.2%) in group C, Actinobacteria (8.1%), and Firmicutes (39.6%) in group N was higher, no significant difference was found (Figure 3).

There were 34 classes in all samples. Compared with other three groups, the abundance of Clostridia (6.9%), Negativicutes (10.0%), Bacteroidia (17.8%) in B group, Betaproteobacteria (15.8%) in group C, Actinobacteria (7.8%), Bacilli (28.6%) in group N was higher, while Betaproteobacteria (6.7%), Fusobacteria (11.6%) in group N were lower, no significant differences were found (Figure 3).

Comparative analysis of the microbe abundances at different levels between two groups
There was no significant difference between group D and group B at phylum and class levels. At order level, the abundance of Burkholderiales was significantly higher (D: 3.513%, B: 0.923%, p = 0.00999). At family level, the abundance of Comamonadaceae was significantly higher (D: 1.612%, B: 0.217%, p = 0.03796). At genus level, the abundance of Burkholderiales was significant higher (D: 1.388%, B: 0.187%, p = 0.02597) (Figure 4).

There was no significant difference between group D and group C, at phylum, class or order level. The abundance of Euabacteria (D: 0.807%, C: 0.413%, p = 0.02797) at family level and Eubacterium (D: 0.806%, C: 0.410%, p = 0.02897) at genus level was significantly higher (Figure 5).

There were no significant differences between Group B and group C, at class, order, family, and genus level. At phylum level, the abundance of Candidatus Saccharibacteria (C: 1.908 ± 0.531%, B: 4.384 ± 0.946%, p = 0.036963) was significantly higher.

There was no significant difference between group B and group C at phylum level. The abundance of Negativicutes at class level, Veillonellaceae, Atopobiales, Clostridiales Family XIII. Incertae_Sedis at family level and Veillonella at genus level were significantly higher, while Streptococcaceae, and Tannerellaceae at family level and Streptococcus, and Tannerella at genus level were significantly lower (Table 1).

There were no significant differences between Group C and group N, at order, family, and genus level. At phylum level, the abundance of Proteobacteria, and Synergistetes was significantly higher, while the abundance of Candidatus Saccharibacteria was significantly lower. At class level, the abundance of Proteobacteria, and Synergistetes was significantly higher (Table 2).

There were no significant differences between group D and group N, at phylum and class level. At order level, the abundance of Bacillales was significantly higher, while Burkholderiales was significantly lower. At family level, the abundance of Comamonadaceae was significantly higher. At genus level, the abundance of Ottowia, Eikenella and Corynebacterium was significantly higher, while the abundance of Gemella was significantly lower (Table 3).

Beta diversity
Weighted PCoA showed that the separation degree between group B and D, group C and D was slightly lower. Meanwhile, the separation degree between group B and C, group B and N, group C and N, and group D and N were obvious. Statistical analyses indicated that microbial composition of four groups were different.
Table 1. Relative abundance at class, family, genus level and statistical significance between group B (CP patients) and group N (normal volunteers).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>N group (%)</th>
<th>B group (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negativicutes (class)</td>
<td>6.078</td>
<td>10.003</td>
<td>0.044</td>
</tr>
<tr>
<td>Bacilli (class)/Streptococcaceae (family)</td>
<td>20.879</td>
<td>9.475</td>
<td>0.025423</td>
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<td>Negativicutes (class)/Veillonellaceae (family)</td>
<td>2.408</td>
<td>5.701</td>
<td>0.003225</td>
</tr>
<tr>
<td>Bacteroidia (class)/Tannerellaceae (family)</td>
<td>1.188</td>
<td>0.504</td>
<td>0.031746</td>
</tr>
<tr>
<td>Coriobacteriia (class)/Atopobiaceae (family)</td>
<td>0.112</td>
<td>0.459</td>
<td>0.035423</td>
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<tr>
<td>Clostridiales (class)/Clostridiales_Family_XIII_Incertae_Sedis (family)</td>
<td>0.125</td>
<td>0.436</td>
<td>0.022338</td>
</tr>
<tr>
<td>Streptococcaceae (family)/Streptococcus (genus)</td>
<td>20.540</td>
<td>9.061</td>
<td>0.022284</td>
</tr>
<tr>
<td>Veillonellaceae (family)/Veillonella (genus)</td>
<td>1.992</td>
<td>4.315</td>
<td>0.001863</td>
</tr>
<tr>
<td>Tannerellaceae (family)/Tannerella (genus)</td>
<td>1.187</td>
<td>0.504</td>
<td>0.026275</td>
</tr>
</tbody>
</table>

Table 2. Relative abundance at phylum, class level and statistical significance between group N (normal volunteers) and group C (IgAN patients).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>N group (%)</th>
<th>C group (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria (phylum)</td>
<td>13.789</td>
<td>23.199</td>
<td>0.04395</td>
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<tr>
<td>Candidatus_Saccharibacteria (phylum)</td>
<td>5.583</td>
<td>1.908</td>
<td>0.0444</td>
</tr>
<tr>
<td>Synergistetes (phylum)</td>
<td>0.118</td>
<td>0.494</td>
<td>0.0448</td>
</tr>
<tr>
<td>Proteobacteria (phylum)/Betaproteobacteria (class)</td>
<td>0.067362</td>
<td>0.157704</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Table 3. Relative abundance at order, family, genus level and statistical significance between group N (normal volunteers) and group D (CP with IgAN patients).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>N group (%)</th>
<th>D group (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacilli (class)/Bacillales (order)</td>
<td>4.196</td>
<td>1.945</td>
<td>0.044574</td>
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<tr>
<td>Betaproteobacteria (class)/Burkholderiales (order)</td>
<td>1.125</td>
<td>3.531</td>
<td>0.013979</td>
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<td>Burkholderiales (order)/Comamonadaceae (order)</td>
<td>0.286</td>
<td>1.612</td>
<td>0.014394</td>
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<td>Bacillales (order)/Gemella (genus)</td>
<td>4.193</td>
<td>1.942</td>
<td>0.04221</td>
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<tr>
<td>Corynebacteriales (order)/Corynebacterium (genus)</td>
<td>1.561</td>
<td>3.208</td>
<td>0.04979</td>
</tr>
<tr>
<td>Burkholderiales (order)/Ottowia (genus)</td>
<td>0.242</td>
<td>1.388</td>
<td>0.013495</td>
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<tr>
<td>Neisseriales (order)/Eikenella (genus)</td>
<td>0.254</td>
<td>0.560</td>
<td>0.042886</td>
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DISCUSSION

CP is a common oral chronic inflammatory disease, which is the main cause of tooth loss in adult patients. Almost all kinds of periodontal disease occur because of mixed microbial infections. Many bacterial species are recognized as putative periodontal pathogens, such as Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Fusobacterium nucleatum, and Synergistetes [11,13,14].

Previous study reported the simultaneous infection of P. gingivalis, T. forsythensis, P. intermedia, and T. denticola is found in the patients with chronic periodontitis, in which the levels of P. gingivalis and T. forsythensis are higher than the other two microorganisms in Chinese people [15]. While Porphyromonas gingivalis,
Figure 1. Rarefaction Curves of each subgingival sample from 43 patients.

B - B: CP patients; B - C: IgAN patients; B - D: CP with IgAN patients; B - N: normal control.

Tannerella forsythia, Epstein-Barr virus (EBV) type 1, cytomegalovirus (CMV), Aggregatibacter actinomycetemcomitans, and EBV type 2 were detected in European people [16]. In this study we found Burkholderiales, Ottowia Eubacterium Veillonell Streptococcus and Tannerella, which indicated that distribution of subgingival bacteria in chronic periodontitis is different depending on the region/country.

Recent studies indicated that periodontal disease is closely linked with systemic health. Treating periodontal disease can help with the prevention of several other chronic inflammatory diseases, including IgAN [11,17-21]. IgAN is the most common primary glomerulonephritis. Immune complexes consisting of IgA1 with galactose-deficient hinge region and anti-glycan antibodies deposit in glomeruli and induce renal injury [22]. Until now, knowledge about the influence of subgingival microbes in CP patients on IgAN incidence is still limited. In this study, in order to investigate the plaque bacterial microbe distribution characteristics and its correlation with CP and IgAN, we used high throughput 16S rRNA sequencing to identify and compare the composition and proportion differences of plaque microbes in Uyghur. The diversity of plaque microbes had no statistical difference, but the abundance of subgingival microbiome was significantly different among four
Figure 2. Relative abundance of plaque bacteria at phylum level in all samples.

Figure 3. Relative abundance of plaque bacteria at phylum, class level in B, C, D, N group.

B - B: CP patients; B - C: IgAN patients; B - D: CP with IgAN patients; B - N: normal control.
Figure 4. Relative abundance of plaque bacteria at order, family, genus level in B and D group.

B - B: CP patients; B - D: CP with IgAN patients.

Figure 5. Relative abundance of plaque bacteria at family, genus level in C and D group.

B - C: IgAN patients; B - D: CP with IgAN patients.
groups. At phylum level, the ratio between *Firmicutes*/*Proteobacteria* decreased in disease groups (N = 2.8725, B = 2.4744, C = 1.3595, D = 1.4620), the ratio of CP with IgAN patients (group D) was lower than CP patients (group B), which is consistent with previous study that has demonstrated a decreasing ratio of *Firmicutes*/*Proteobacteria* in IgAN patients’ saliva [23] and plaque microbiome [12], indicating CP patients with IgAN may decrease this ratio in plaque. Compared with healthy volunteers (group N), at genus level the abundance of *Veillonella* was significantly higher, while *Streptococcus* and *Tannerella* were significantly lower than CP patients (group B). *Streptococcus* belongs to *Firmicutes*, some studies found that *Streptococcus* was lower in gingivitis patients [24]. *Tannerella* belongs to *Bacteroidetes*. Karatas O et al. [25] found that the abundance of plaque *Tannerella* in CP patients was lower than that in normal people, which is consistent with our study. Our study also showed that the abundance of *Tannerella* in CP with IgAN patients (group D) was lower than that of normal weight with or without CP patients. Compared with healthy volunteers (group N), at family level the abundance of *Veillonellaceae* was significantly higher, while *Proteobacteria* was significantly lower than that of normal people, which need to be further studied. Compared with CP patients (group B), the abundance of *Burkholderiales* at order level, *Comamonadaceae* at family level, and *Ottowia* at genus level were significantly higher than that in CP with IgAN patients (group D). *Ottowia* belongs to *Proteobacteria*/*Betaproteobacteria*/*Burkholderiales*/*Comamonadaceae*. Cao Y et al. [12] found that the abundance of *Betaproteobacteria* in CP with IgAN patients was significantly lower than in CP patients, which is consistent with our results. Until now, the knowledge of correlation between *Ottowia* and CP or IgAN is still limited, it can be a new direction of relationship between subgingival microbes and CP or IgAN. Compared with IgAN patients (group C), the abundance of *Eubacteriaceae* at family level and *Eubacterium* at genus level were significantly higher than that in CP with IgAN patients (group D), *Eubacterium* belongs to *Firmicutes*/*Clostridia*/*Clostridiales*/*Eubacteriaceae*. A study showed that the abundance of *Eubacterium* in gingivitis, invasive periodontitis and CP was significantly higher than that in normal people [26]. Maciel SS et al. [27] found that the abundance of *Eubacterium* in obese with CP patients was significantly higher than that in normal weight with or without CP patients. Karatas O et al. [23] showed that the abundance of *Eubacterium* in CP patients was significantly lower than that in normal persons, which is the opposite of our study. This might be another characteristic of Uyghur patients, which needed further investigation.

In summary, we used high throughput 16S rRNA sequencing to analyze the composition and abundance of plaque microbes in CP and IgAN patients, and found that there were significantly difference at different level, such as *Streptococcus* decreased in CP patients (B group), *Veillonella* decreased in IgAN patients (C group), *Betaproteobacteria* and *Ottowia* increased in IgAN patients (C group), *Clostridia* increased in CP with IgAN patients (D group), and *Firmicutes*/*Proteobacteria* decreased in the disease groups, which suggested that changing abundance of plaque microbes were correlated with IgAN and CP. However, the correlation and mechanism of changing microbe structures in CP patients with occurrence and aggravation of IgAN is still unclear, and human subgingival microbiome is huge and complex, which is affected by many factors, thus the study should be continued.

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**Data Availability:**
Data is available from the corresponding author on request.

**Declaration of Interest:**
The authors declared no conflict of interest.

**References:**


Additional material can be found online at: http://supplementary.clin-lab-publications.com/230316/