

Letter to the editor

Changes in the salivary microbiota of oral leukoplakia and oral cancer



Oral cancer is one of the most prevalent cancers in the world, with oral squamous cell carcinoma (OSCC) accounting for 90% of all oral cancers [1]. The oral cavity is the second microbiome habitat in the human body and harbors numerous microbiota; an estimated 500–700 bacteria belonging to different species are present here [2]. To better understand the relationship between the salivary microbiota and oral leukoplakia (OLK) and OSCC, we evaluated changes in the salivary microbiota of patients with OSCC and OLK relative to the oral bacterial profiles in healthy controls (HCs) in present study. This study was approved by the Institutional Review Board of PKUSS (IRB No. PKUSSIRB-2012036).

Totally, 16 patients with OSCC, 10 with OLK, and 19 HCs were enrolled in this study from Peking University School and Hospital of Stomatology (PKUSS), Beijing, China. OSCC and OLK were diagnosed and confirmed by clinical practitioners and oral pathologists. Nonstimulated saliva was collected and DNA was extracted. The profiles of salivary bacteria in patients with OSCC and OLK were evaluated using the Illumina MiSeq prosequencing of 16S rRNA and compared with those for HCs. The R program and SPSS software (version 19.0) were used for all statistical analyses.

After excluding one OSCC patient during sequencing analysis due to a small number of reads, 44 samples were analyzed in present study. A total of 401,757 high-quality sequences were generated and 2861 operational taxonomical units (OTUs) were obtained. The number of OTUs ranged from 143 to 593 in the HC group (mean, 319 ± 135), 122–702 in the OSCC group (mean, 386 ± 158), and 275–705 (mean, 469 ± 150) in the OLK group. Three groups shared 920 common OTUs. Among the three groups, OTUs were the most abundant in the OLK group, with a significant difference from the number in the HC group ($p = 0.012$). The OLK samples exhibited higher richness values, while the HC group exhibited the lowest values. Significant differences were observed between the OLK and HC groups when the Chao index was used ($p = 0.023$), but no difference were found when the ACE index was used ($p = 0.081$) (Fig. 1a and b). With regard to diversities in bacterial communities, the Shannon and Simpson indices consistently revealed the highest diversity in the OSCC group, which was different from that in the HC group ($p_{sh} = 0.001$, $p_{si} = 0.002$). There was a significant difference between the OLK and OSCC groups only according to the Simpson index ($p = 0.046$), not the Shannon index ($p = 0.76$) (Fig. 1c and d).

The OTU analysis showed that the HC group samples tended to form close clusters, while the OSCC and OLK samples appeared separated from the HC samples in PCA (Fig. 2a). We then evaluated the microbiota profile in the 3 groups. Collectively, 14 phyla, 22 classes, 32 orders, 54 families, and 67 genera were detected. The phylum Firmicutes accounted for the majority in samples from all three groups, particularly the HC group (73%). Other dominant phyla included Proteobacteria, Bacteroidetes, Fusobacteria, and uncultured TM7; these occupied more than 90% of all phyla. The proportion of the phylum Firmicutes was lower in the OLK group (66.2%) than that in the HC group, while the relative abundance of the phylum Bacteroidetes (2.84%) and TM7 (3.42%) in OLK group was much higher in the HC group (2.25%, 0.37%). The phylum Firmicutes was the least abundant in the CA group (60.3%), while the phyla Bacteroidetes (3.88%) and TM7 (7.67%) were the most abundant. The genus *Streptococcus* was the most abundant in all three groups, particularly the HC group (60.5%). *Neisseria* was the second most abundant genus, with a proportion of approximately 20% in all three groups. *Granulicatella*, *Campylobacter*, *Capnocytophaga*, *Veillonella*, and *Fusobacterium* were also predominantly observed, each occupying more than 1% in all three groups. Four main genera were discovered with significant differences among the three groups (Fig. 2b). The genera *Streptococcus* and *Abiotrophia* were the most abundant in the HC group, with a significant difference between the HC (60.5%, 0.41%) and OSCC (42.8%, 0.15%) and OLK (49.4%, 0.06%) groups. *Haemophilus* was much more abundant in the OLK group (1.51%) than in the OSCC (0.54%) and HC (0.34%) groups, while *Bacillus* was the most abundant in the OSCC group (4.44%). Moreover, the proportion of *Bacillus* was higher in the OLK group (0.34%) than that in the HC group (0.002%).

Previous studies of the oral microbiota in patients with OSCC and precancerous lesions are limited and provide inconsistent results [3–5]. Saliva can be an optimal sample for the study of oral diseases because of convenient access and noninvasive collection. To the best of our knowledge, our study is the first to simultaneously analyze the salivary microbiota in OSCC and OLK patients using high-throughput sequencing. Changes in bacterial abundance and diversity in OSCC and OLK patients might be associated with bacterial metabolism. Microorganisms and their products are reportedly toxic to host cells and may induce mutations and signaling pathway alterations [6–9].

In conclusion, the results of our study primarily demonstrated OLK and OSCC might be associated with changes in the salivary microbiota. Evaluation of the oral microbiome can prove to be a promising diagnostic technique for OSCC and a novel monitoring technique for precancerous lesions.

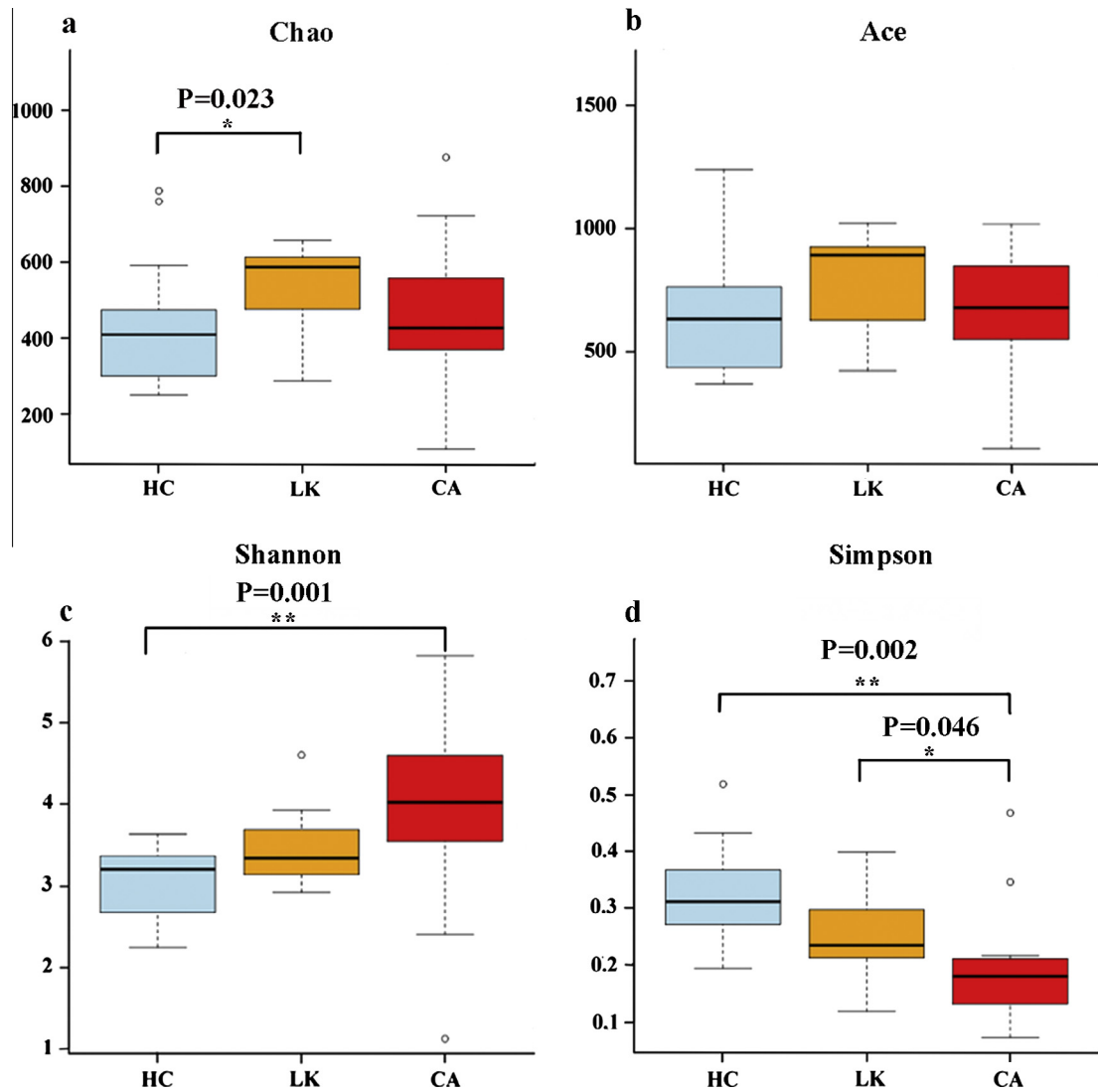


Fig. 1. Alpha diversities based on OTUs in the HC, OLK, and OSCC groups (a and b) represented the richness in each group by Chao and ACE indices, while (c and d) showed the diversity in each group by the Shannon and Simpson indices. The Kruskal–Wallis test was used to compare the significant difference within 3 groups. A p -value of <0.05 was considered statistically significant.

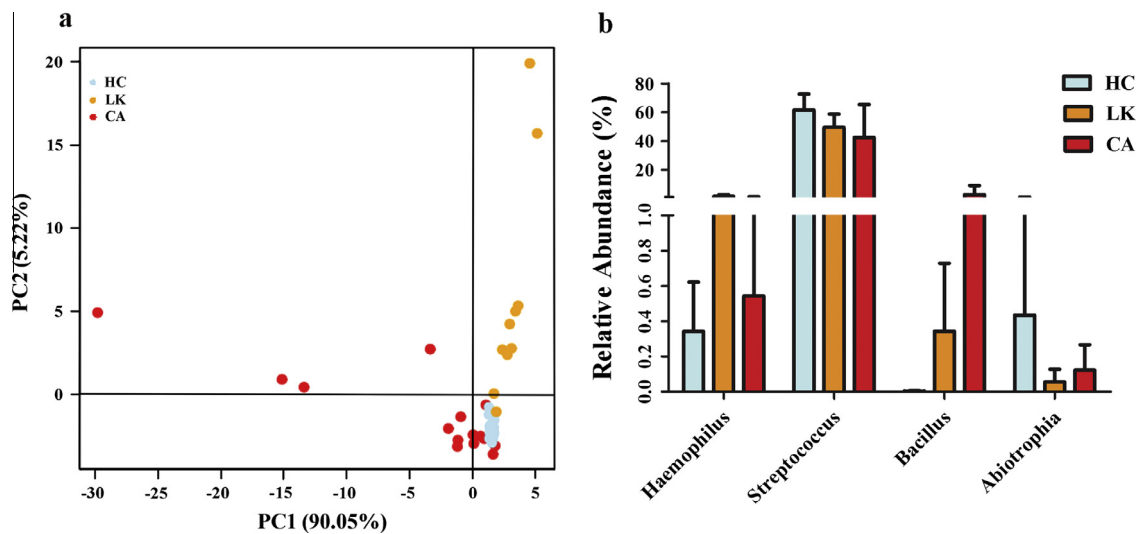


Fig. 2. (a) Principal component analysis (PCA) for the 44 samples based on the relative abundance of OTUs in each sample. PC1 and PC2 explain 95.27% of the total variability among the three groups. (b) Genera with significant differences among the three groups. The Kruskal–Wallis test was used to test the difference in the difference of structure in 3 groups. *Streptococcus* and *Abiotrophia* were the most abundant in the HC group, *Haemophilus* in the OLK group, and *Bacillus* in the OSCC group.

Conflict of interest statement

None declared.

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