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TONGUE MICROFLORA AND PERIODONTAL DISEASE

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Abstract

Aim of the study. To determine the role of bacteria on the dorsal surface of the tongue in periodontal disease. **Material and methods.** Fifthy-two patients aged 18 to 25 were enrolled in the study; the subjects had to complete a questionnaire on general health, diet and dental hygiene habits. Decay-missing- filled teeth index (DMFT), the periodontal screening index (PSI), bleeding on probing (BOP) and plaque index were recorded for all subjects. After clinical examination, samples from dental plaque and tongue microflora were harvested by swabbing with sterile cotton sticks. **Results.** Clinical data obtained correlate with tongue microflora. The microflora does not depend on age and sex of the pacient. The counts of *S.mutans* was found to be corelated with DMFT index and also, the BOP with *P.gingivalis* (p=0.0006). We found the presence of *P.gingivalis* in approximately 12% of the subjects. **Conclusions.** The tongue surface can be an important reservoir for pathogens bacteria which can have a direct influence in the development of dental caries, oral halitosis or periodontitis.

Keywords: Streptococcus mutans, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, tongue, periodontitis

Introduction

Periodontitis is an infectious disease which, if left untreated, results in progressive attachment and bone loss, ultimately leading to tooth loss (Berezow & Darveau, 2011). This disease appears to have multiple etiologies, such as microbial and immunological ones. Microbial species

inhabiting human mouth are associated with oral health, dental caries, periodontitis and gingivitis (Socransky & Haffajee, 2005). Within the oral cavity, the tongue, soft and hard palates, buccal mucosa, supra-gingival and sub-gingival surfaces and saliva may present a wide diversity of microorganisms. The oral microbiota can shift from one consisting of gram-positive *Streptococci* to one consisting of gram-negative anaerobes such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*. The tongue is a complex organ and from a microbiological point of view it is an important bacterial reservoir. So, it represents a key factor affecting bacterial colonization level on the tooth surfaces (Marsh, 1994).

In this study we focused on the role of the bacteria that are resident on the dorsal surface of the tongue in periodontal disease.

Material and methods

Subjects. Fifty-two patients aged 18 to 25 were enrolled in this study. Informed consent was obtained from each enrolled subject.

Subjects were interviewed concerning their diet, oral hygiene and smoking habits and examined by the same calibrated dental examiner. The inclusion criteria were as follows: no antibiotic therapy for the last 6 months, no systemic diseases or orthodontic treatment.

The decay-missing-filled teeth index (DMFT) and the periodontal screening index (PSI) was assessed for all selected subjects. Periodontal status was characterized by plaque index (PI), probing depths (measured with a periodontal probe UNC 15, Hu-Friedy) and clinical periodontal attachment level. Bleeding on probing (BOP) was recorded as the percentage of positive sites per subject based on measurements in four sites per tooth.

Microbiological sampling

Plaque samples from the first and second molar in each quadrant were collected. A paper point (ISO 50) was inserted into the selected gingival sulcus for 20s to obtain plaque. Microbiological samples from the dorsum of the tongue were taken by swabbing with sterile cotton sticks and stored at -20^0C util analyzed. Two bacterial species associated with periodontitis, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, were assessed by means of real-time PCR, and a cariogenic species (*Streptococcus mutans*) was assessed with GoTaq PCR Master Mix (Promega Corporation, USA).

Data analysis

All collected data were statistic analyzed using SPSS program, version 17. For statistic analysis we used Chi-square test and the level of

significance was set to $p<0.05$.

Results

Clinical finding. Clinical parameters of study population are presented in Table 1. The study population consisted of young people, with an average age of 20,3 ± 1,9SD, from which 71.2% were non-smokers. All subjects were diagnosed with gingivitis; none was diagnosed with periodontitis. BOP received a value = 1 in 48.5% cases, and in 36.0% of cases a value = 2.

Tabel 1. Clinical parameters of the study population

Variable		**Percent %**
Gender	Female	46.2%
	Male	53.8%
Smoking habits	Non-Smoker	71.2%
	Smoker	28.8%
Plaque index	0	3.8%
	1	59.6%
	2	30.8%
	3	5.8%
BOP	0	1.2 %
	1	48.5%
	2	36.0%
	3	13.5%
PSI	0	96.2%
	1	3.8%

43.3% of the subjects had no clinical evidence of carious lesions, missing teeth or fillings (DMFT = 0); in 4% of subjects a DMFT > 10 was found.

Microbiological finding

Detection of different species by real-time PCR is presented in Table 2 and Figure 1 and 2.

Tabel 2. Detection of different bacteria

Species	Plaque positive ($\geq 10^2$) (%)	Tongue positive ($\geq 10^2$) (%)	*p*
S.mutans	43%	29%	0.001
A.actinomycetemconitans	11%	10%	0.422
P.gingivalis	5%	7%	0.615

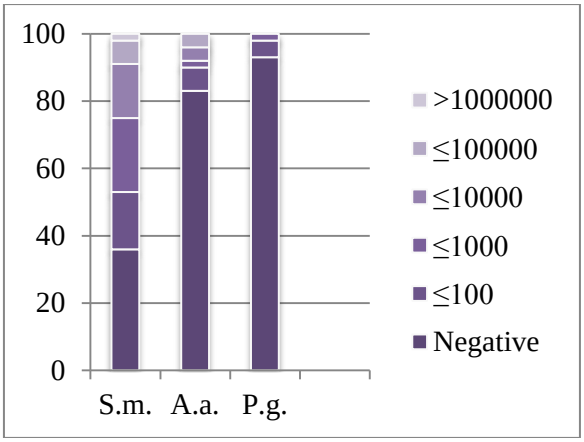

Figure 1. Detection of bacteria in plaque samples

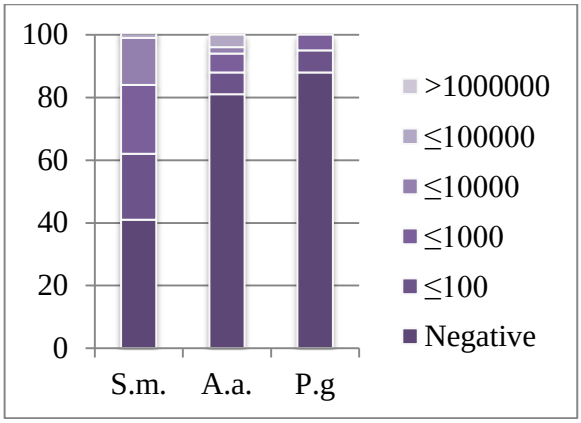

Figure 2. Detection of bacteria in samples from the dorsum of the tongue

The microbiological results showed that the detection frequencies of periodontal pathogensin tongue samples were: *S.mutans* 29%, *A.actinomycetemcomitans* 10%, *P.gingivalis* 7%. In 32 of all subjects, *S.mutans* was detected in plaque and on the dorsum of the tongue. *A. actinomycetemcomitans* was found in 8 tongue samples and *P.gingivalis* in 6 tongue samples. Clinical data associated with microbiological results showed no gender differences as regards microbiological parameters and smoking habits (p=0.04). The counts of *S.mutans* was found to be corelated with DMFT index and also, the BOP with *P.gingivalis* (p=0.0006). We found the presence of *P.gingivalis* in approximately 12% of the subjects.

Discussions

The present study investigated the presence of periodontal pathogens on the dorsum of the tongue. The counts of two periodontal bacteria (*A.actimycetemcomitans, P.gingivalis*) and of a cariogenic bacteria (*S.mutans*) suggested that either can cause periodontitis directly. *A.*

actonomycetemcomitans was found in approximately 25% of the subjects.

Various species of bacteria detected on the tongue of young adults have been previously reported. Higher species-detection frequencies from tongue, compared with tooth associated samples have been reported for *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia*, by means of immunofluorescence (Timmerman et al., 1998). This suggest that the dorsum of the tongue houses organized biofilm to which anaerobic bacteria may locate and thrive to seed anaerobic locations around teeth.

In a study carried out on adolescents, Cortelii and al. (2008), analize *T. fortsythia*, *P. gingivalis*, *A. actinomycetemcomitans* from the tongue and gingival sulcus. The results in our study were consistent with those in Cortelli's concerning the prevalence of the three species. However, Cortelli also observs a certain affinity of these species for the gingival areas which our study could not confirm. Van Assche (2009) was able to detect pathogen bacteria on the tongue surface a month after tooth extraction, thus confirming the favorable conditions for anaerobic growth on this area. In his study, Eick et colab (2013) found no difference in periodontal pathogens between smokers and non-smokers, which also confirms the results in our study. The observation could be explained by the young age of the subjects. On the other hand, Van de Velden (2003), found clear differences between smokers and non-smokers' subgingival microflora.

Our study detected simptoms of gingivitis in each subject. Huang et al. (2011) in their study on microbiota of Chinese adults, compare the diversity and population structure of microbiota associated with gingivitis. They suggest that oral samples from patient population of gingivitis can be characterized via plaque microbiota.

On the other hand, in this study we did not determine the serotypes of *A.actinomycetemcomitans*. Socransky and al. (1991) and Jentsch and al. (2012) show that serotype b is associated with periodontal disease, especially with aggressive periodontitis, and the serotype distribution depends on geographical regions.

Further studies are needed in order to understand the changes in oral microflora that foretell the early stages of periodontitis and dental caries, the most prevalent chronic oral diseases, which may allow better diagnosis and treatment planning.

Conclusion

The present study shows no gender related differences in terms of microbiological parameters.The clinical and microbiological results relate the presence of gingivitis, but no periodontitis in young people included in our study. The dorsum of the tongue houses organized biofilms and represents an important reservoir for pathogen bacteria directly influencing

the development of dental caries, periodontitis or halitosis.

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