MICROBIAL ASSESSMENT IN SALIVA VERSUS SUBGINGIVAL PLAQUE SAMPLES IN PERIODONTITIS

Hady Haririan[1], Kristina Bertl[1], Markus Laky[2], Michael Matejka[1], Oleh Andrukhov[1] and Xiaohui Rausch-Fan[1]

[1] Department of Periodontology, Bernhard Gottlieb School of Dentistry, Medical University of Vienna, Austria
[2] Department of Dental Education, Bernhard Gottlieb School of Dentistry, Medical University of Vienna, Austria

BACKGROUND AND OBJECTIVES

Periodontitis is an inflammatory disease caused by periodontopathic bacteria in the dental biofilm leading to destruction of the tooth surrounding tissues.

Subgingival plaque sampling is a common way for the determination of periodontopathic bacteria in patients suffering from periodontal disease, but the diagnostic potential of microbial assessment for periodontitis has been under discussion. Subgingival plaque is often collected by paper point sampling and assessed by polymerase chain reaction (PCR), Real-Time PCR or checkerboard DNA-DNA hybridization. Semi-quantitative determination by PCR was shown to be suitable to distinguish between healthy and periodontitis subjects (Haffajee et al., 2009).

Detecting periodontopathic bacteria in saliva would be a more comfortable way to identify individuals susceptible to periodontal disease than the time-consuming and delicate paper point sampling (Saygun et al., 2011).

The aim of the present study was to investigate if the results of microbial assessment in stimulated whole saliva is comparable to the determination of bacteria collected in the periodontal pocket.

MATERIALS AND METHODS

23 periodontitis patients (8 females, 15 males; mean age ± SD: 37.78 ± 7.79 years) participated in saliva and subgingival plaque sampling before conservative periodontal therapy. Saliva was collected using the Saliva Collection System® (SCS®, Greiner Bio-One) after an overnight fast between 8.00 and 10.00 am. Patients were not allowed to brush their teeth or smoke on the day of saliva sampling. Subgingival plaque samples were collected with sterile paper points from the 4 deepest pockets, inserted for 15 seconds. 79 bacterial species from the periodontal pocket were analyzed by checkerboard DNA-DNA hybridization (University of Bern). 20 bacterial species in the periodontal pocket sample and in whole saliva were determined by PCR (ParoCheck®, Lambda) using GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma Aldrich®) for DNA extraction.

SALIVA vs. PERIODONTAL POCKET

bacterial species	R	p
A.a.	0.637	0.001
A. viscosus	0.253	0.245
T. forsythia	0.680	0.000
C. rectus/showae	0.385	0.069
T. denticola	0.591	0.003
E. corrodens	0.291	0.178
P. intermedia	0.390	0.066
P. micros	0.467	0.025
P. gingivalis	0.350	0.102
F. nucleatum	0.696	0.000
A. odontolyticus	0.249	0.253
Capnocytophaga sp.	0.069	0.753
E. nodatum	0.165	0.450
S. constellatus group	0.098	0.657
C. gracilis	0.691	0.000
S. mitis group	0.097	0.659
P. nigrescens	0.340	0.112
S. gordonii group	0.027	0.904
V. parvula	0.286	0.187

Table 1. Regression analysis of 20 bacterial species between whole saliva and subgingival plaque samples.

CONVENTIONAL MICROBIAL ASSESSMENT

A

Bacterium	SNR	Result	Level
A. actinomycetemcomitans	0	negative	-
A. viscosus	0	negative	-
T. forsythia	182.7	positive	++
C. rectus/showae	0	negative	-
T. denticola	74.4	positive	++
E. corrodens	14.5	positive	(+)
P. intermedia	56.1	positive	++
P. micros	48.2	positive	++
P. gingivalis	0	negative	-
F. nucleatum	0	negative	-
A. odontolyticus	22.6	positive	+
Capnocytophaga sp.	23.6	positive	+
C. concisus	0	negative	-
E. nodatum	0	negative	-
S. constellatus group	0	negative	-
C. gracilis	0	negative	-
S. mitis group	0	negative	-
P. nigrescens	0	negative	-
S. gordonii group	0	negative	-
V. parvula	36.6	positive	+

B

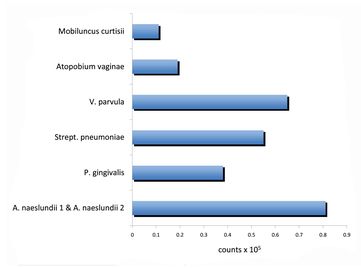

Table 2. PCR analysis of periodontal pocket sampling (A) and checkerboard DNA-DNA hybridization results (B) for a patient with generalized aggressive periodontitis.

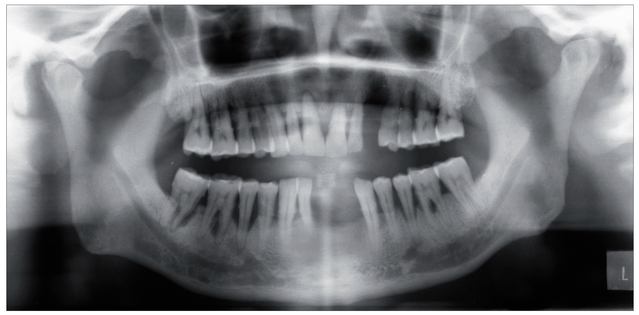

Figure 1. Panoramic radiograph of a patient suffering from generalized aggressive periodontitis (male, 38 years old).

MICROBIAL ASSESSMENT IN SALIVA

Bacterium	SNR	Result	Level
A. actinomycetemcomitans	69.9	positive	++
A. viscosus	48.5	positive	++
T. forsythia	0	negative	-
C. rectus/showae	0	negative	-
T. denticola	0	negative	-
E. corrodens	0	negative	-
P. intermedia	176.2	positive	++
P. micros	27.3	positive	+
P. gingivalis	0	negative	-
F. nucleatum	29.6	positive	+
A. odontolyticus	747.9	positive	+++
Capnocytophaga sp.	91.1	positive	++
C. concisus	0	negative	-
E. nodatum	0	negative	-
S. constellatus group	164	positive	++
C. gracilis	0	negative	-
S. mitis group	229.2	positive	+++
P. nigrescens	0	negative	-
S. gordonii group	199.1	positive	++
V. parvula	1212.3	positive	+++

Table 3. PCR analysis of whole saliva samples.

Picture 1. SCS®

Clinical Implication

Adjunctive antibiotic treatment - if yes, which one?

Antibiotic	Limitation
Metronidazole	not effective against A.a.
Amoxicillin Azithromycin	not effective against Fusobacterium spec.

Table 4. Limitations of different antibiotics (Shaddox and Walker, 2009).

BACTERIA - DISTRIBUTION

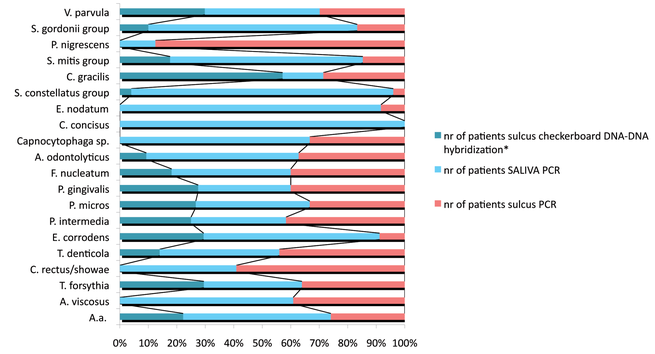

Figure 2. Percentage of bacterial species detected with different methods.
* raw data of checkerboard DNA-DNA hybridization

RESULTS

Regression analysis of the results of PCR showed that 6 bacterial species had significant correlations between saliva and subgingival plaque samples (Table 1). Namely, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Peptostreptococcus micros* and *Campylobacter gracilis* had the highest rank correlations (0.467-0.696). The presence of periodontal pathogens was detected to a higher amount in saliva compared to subgingival plaque samples (Figure 2). *F. nucleatum*, *A. odontolyticus*, *S. constellatus*, *S.mitis* and *V. parvula* were detected in 23/23 patients in whole saliva.

CONCLUSION

Detecting some species of periodontopathic bacteria by collecting saliva from periodontitis patients is identical or even more sensitive than by sampling in the periodontal pocket (Umeda et al., 1998). This may simplify microbial assessment in periodontitis patients, however, for certain microorganisms, the combination of different sampling methods should be preferred.

Salivary diagnostic may be a potential way for the early screening of periodontitis patients by combining the detection of bacteria and specific periodontal disease markers. It may also have an implication for evaluating the transmission of periodontopathic bacteria through saliva (Slots et al., 2011).

ACKNOWLEDGMENTS AND FINANCIAL SUPPORT

The authors thank Mrs. Phuong Quynh Nguyen and Mrs. Hedwig Rutschek (Department of Periodontology, Bernhard Gottlieb School of Dentistry, Medical University of Vienna, Austria) for their help in preparing and performing the experiments. Greiner Bio-One and Lambda supported this study by financing materials and sample analysis.

REFERENCES

Haffajee AD, Yaskell T, Torresyap G, Teles R, Socransky SS. Comparison between polymerase chain reaction-based and checkerboard DNA hybridization techniques for microbial assessment of subgingival plaque samples. J Clin Periodontol 2009;36:642–649.

Saygun I, Nizam N, Keskiner I, Bal V, Kubar A, Açikel C, Serdar M, Slots J. Salivary infectious agents and periodontal disease status. J Periodont Res 2011;46:235–239.

Shaddox L, Walker C. Microbial testing in periodontics: value, limitations and future directions. Periodontology 2000 2009;50:25–38.

Slots J, Slots H. Bacterial and viral pathogens in saliva: disease relationship and infectious risk. Periodontology 2000 2011;55:48–69.

Umeda M, Contreras A, Chen C, Bakker I, Slots J. The utility of whole saliva to detect the oral presence of periodontopathic bacteria. J Periodontol 1998;69(7):828–833.