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Detection of *Actinobacillus actinomycetemcomitans* DNA in Patients with Partial and Complete Dentures by Real-Time PCR

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Abstract

Background: The purpose of the present study was to detect *Actinobacillus actinomycetemcomitans* (Aa) using RT-PCR in patients with complete and partial edentulism before (T0) and three months after (T3) treatment with removable partial dentures (RPD) and complete dentures (CD), respectively, to compare the data between these two research groups.

***Methods and Results*:** The sample comprised 60 patients: 33 men and 27 women, aged 48 to 76 years. The patients were divided into two groups. Group 1 included 30 patients with partial edentulism who were treated with RPD. Group 2 included 30 patients with complete edentulism who were treated with CD. The samples from Group 1 were taken from the gingival sulcus of the abutment teeth by means of sterile paper points. For Group 2, the samples were taken with a sterile swab from the dorsum of the tongue. The samples were taken in T0 and T3 intervals. To detect Aa DNA, we used RT-PCR and ParodontoScreen REAL-TIME PCR Detection Kit (DNA-TECHNOLOGY). Bacterial load levels of species were conventionally represented in logarithm (Lg) of genome equivalents per sample. The results were also presented in three ranges depending on the level of bacterial load: normal (<4.0 Lg), mild/moderate (≥4.0 Lg), and severe (>5.0 Lg).

The study found a significant difference in the amount of Aa between the T0 and T3 intervals only in patients treated with RPD (0.87±1.58 Lg vs. 1.28 ±1.96 Lg, *P*=0.004). Patients treated with CD, however, did not differ significantly in the amount of Aa between the T0 and T3 intervals (0.03±0.16 Lg vs. 0). The average bacterial load in patients with RPD was significantly higher than in those with CD three months after treatment (*P*=0.02). Of the 30 patients with RPD, 2(6.7%) had a severe range, 2(6.7%) had a mild/moderate range, and 26(86.7%) had a normal range. The 30 CD patients all had a normal range. There was no significant difference in the prevalence range of bacterial load level with Aa between groups (Fisher's Exact Test = 3.537, *P*=0.113) / Monte Carlo Sig. (2-sided) / 0.105–0.121). However, in general, RPD causes a significant increase in Aa, so the level of periodontal pathogens may be higher in RPD patients than in CD patients.(**International Journal of Biomedicine. 2023;13(1):141-145.**)

Keywords: *Actinobacillus actinomycetemcomitans* • real-time PCR • dentures

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Abbreviations

CD, complete dentures; **RPD**, removable partial dentures; **RT-PCR**, real-time polymerase chain reaction.

Introduction

The oral cavity is a suitable environment that provides multiple habitats for colonizing microorganisms, including viruses, bacteria, and fungi. The role of some of them is already known in the development of various oral diseases, including caries and periodontitis. Still, recent research has shown that these microorganisms are also implicated in heart disease, gastrointestinal infections, and malignant diseases.[1-3]

Researchers have observed that certain microorganisms colonize specific areas, including removable partial dentures (RPD) and complete dentures (CD). Due to the properties of the dentures' acrylic surface and the acidic action that saliva has on the prosthesis, affecting the creation of pores, the dentures

become a suitable area for the adhesion of microorganisms.[4] These microorganisms have the potential to develop biofilm, thus influencing the appearance of local changes in the oral cavity.

The clinical condition that most often appears in users of removable dentures is prosthetic stomatitis, which is characterized by inflammatory changes in the oral mucosa. Different factors affect the development of prosthetic stomatitis, including a poor adaptation of prostheses, long-term treatment with antibiotics, and fungal infection resulting from *Candida albicans*.[5]

Although most of the literature in this area has focused on *Candida albicans*, there is growing evidence to suggest that the use of CD and RPD also causes microbial changes, including changes in the level of periodontal pathogens.[6-9]

Some of these studies have reported changes occurring in the level of red-complex bacteria. Still, the literature is deficient in reporting the level of *Actinobacillus actinomycetemcomitans* (Aa), considering the risk factors associated with it, which are not limited to the area of the mouth but also extend to the general condition of a person›s organism.[10,11]

Accordingly, the purpose of the present study was to detect Aa using RT-PCR in patients with complete and partial edentulism before (T0) and three months after (T3) treatment with RPD and CD, respectively, to compare the data between these two research groups.

Materials and Methods

The sample comprised 60 patients: 33 men and 27 women, aged 48 to 76 years. The patients were divided into two groups. Group 1 included 30 patients with partial edentulism who were treated with RPD. Group 2 included 30 patients with complete edentulism who were treated with CD.

Inclusion criteria: patients' ability and willingness to cooperate, an indication for treatment with RPD and CD. Exclusion criteria: antimicrobial therapy and using immunosuppressants in the previous 90 days, temporomandibular joint problems, severe periodontal conditions.

CD was made of acrylic resin, and RPD was made of acrylic resin with a metal frame.

Sample collection

The samples from Group 1 were taken from the gingival sulcus of the abutment teeth by means of sterile paper points. For Group 2, the samples were taken with a sterile swab from the dorsum of the tongue. The samples were taken in T0 and T3 intervals. We placed the samples in sterile test tubes containing the physiological solution and sent them to the appropriate microbiological laboratory.

RT-PCR

To detect Aa, we used RT-PCR and ParodontoScreen REAL-TIME PCR Detection Kit (DNA-TECHNOLOGY). The laboratory stages are described in detail on the company's website.[12] Bacterial load levels of species were conventionally represented in logarithm (Lg) of genome equivalents per sample. The results were also presented in three ranges depending on the level of bacterial load: normal (<4.0 Lg), mild/moderate (≥4.0 Lg), and severe (>5.0 Lg).

Statistical analysis was performed using statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). For descriptive analysis, results are presented as mean (M) ± standard deviation (SD), 95% Confidence Interval (95% CI), Minimum, and Maximum. Differences in attributive series between the patient groups were tested using Pearson Chi-square / Monte Carlo Sig. (2-sided), Fisher's Exact Test / Monte Carlo Sig (2-sided). The Kruskal-Wallis H test/one way ANOVA was used to compare groups. In all cases, a probability value of $P<0.05$ was considered statistically significant.

Ethical approval for this study was obtained from the Ethical Committee of the University Clinical Center of Pristina, Pristina, Kosovo (protocol number 378/19). All participants provided written informed consent.

Results

Table 1 provides descriptive statistics on the value of Aa bacterial load in patients with RPD and CD. The study found a significant difference in the amount of Aa between the T0 and T3 intervals only in patients treated with RPD (0.87±1.58 Lg vs. 1.28±1.96 Lg, $P=0.004$). Patients treated with CD, however, did not differ significantly in the amount of Aa between the T0 and T3 intervals (0.03±0.16 Lg vs. 0).

Table 1.

The value of the Aa bacterial load before (T0) and 3 months after (T3) of treatment with RPD and CD.

Dentures	T	n	Mean	-95% CI	+95% CI	Min	Max	SD	Z	P
	T0	30	0.87	0.28	1.46	0	5.2	1.58		
RPD	T3	30	1.28	0.55	2.01	0	6.1	1.96	2.1	0.04
	T0	30	0.03	-0.03	0.09	0	0.9	0.16		
CD	T3	30	0			0	0	0	/	/

The average bacterial load in patients with RPD was significantly higher than in those with CD three months after treatment ($P=0.02$) (Table 2).

Table 2.

The difference in the Aa bacterial load 3 months after prosthetic treatment.

Actinobacillus actinomycetemcomitans (3 months after treatment)	n	A	RPD	CD
			R:53.23	35
RPD	30	1597		0.02
CD	30	1050	0.02	

R: statistical variables; A: amounts

The prevalence range of bacterial load levels in the groups are presented in Table 3. Of the 30 patients with RPD, 2(6.7%) had a severe range, 2(6.7%) had a mild/moderate range, and 26(86.7%) had a normal range. The 30 CD patients all had a normal range. There was no significant difference in the prevalence range of bacterial load level with Aa between groups (Fisher's Exact Test = 3.537, *P*=0.113) / Monte Carlo Sig. (2-sided) / 0.105–0.121). However, in general, RPD causes a significant increase in Aa, so the level of periodontal pathogens may be higher in RPD patients than in CD patients.

Table 3.

The prevalence range of the Aa bacterial load levels in the study groups.

		Actinobacillus actinomycetemcomitans (3 months after)			Total
		Severe	Mild/ moderate	Normal	
RPD	Count	2	2	26	30
	%	6.67%	6.67%	86.67%	100.0%
CD	Count	0	0	30	30
	%	0%	0%	100%	100.0%
Total	Count	2	2	56	60
	%	3.33%	3.33%	93.33%	100.0%

Discussion

The main characteristic of the RT-PCR method used in our research is that it monitors the amplification of the DNA molecule in real time, not at the end, as in the conventional polymerase chain reaction. Using the molecular genetic method to detect Aa by isolating bacterial DNA is significantly more suitable than the culture method used in previous studies, due to the molecular method's sensitivity, speed, and reliability.[13] Therefore, the use of RT-PCR was suitable for this study.

In this study, we initially detected Aa in both groups of patients, namely 1(3.3%) of the edentulous patients and 8(26%) of the partially edentulous patients. The higher number of patients with Aa in the latter group may be attributed to the periodontal space in patients with teeth, providing a favorable environment in the oral cavity for anaerobic species. This theory is consistent with previous research: O'Donnell et al.[14] found higher levels of certain periodontal pathogens on the tooth surface than on the oral mucosa, and Gazdek et al.(15) observed lower levels of Aa in edentulous patients than in partially edentulous ones. Moreover, our results are in agreement with a series of studies,[6,16,17] which found that Aa colonizes even in edentulous conditions; however, our results are in contrast to the previous study by Danser et al.,[18] which did not identify the presence of Aa in bacterial samples collected from edentulous patients. These data provide significant information that the presence of Aa in the oral cavity of edentulous patients may be a major source of future bacterial colonization of dental implants.[19] This information may influence how peri-implants are developed. At any rate, Aa is an anaerobic bacterium that plays a role in the destruction of the periodontium and has a significant impact on the development of systemic diseases such as heart disease, diabetes mellitus, and dementia;[10,11] accordingly, we strongly encourage patients to maintain proper oral hygiene and denture care.

Another important objective of this study was the detection of Aa after treatment with removable prostheses. Based on our microbiological analyses, Aa was absent in edentulous patients three months after CD insertion. In contrast to our study, Andjekovic et al. observed an increase in Aa six months after treatment with CD. This difference may be attributed to the shorter follow-up time in our study than in the previous study.[20]

On the other hand, in our study the level of Aa increased significantly in patients treated with RPD. Our results are consistent with the findings of Costa et al., who observed a general increase of microorganisms, including Aa, six months after treatment with RPD.[21] Thus, based on the study's results three months after beginning therapy with RPD, the future risk of developing periodontal diseases in the supporting teeth is high when using RPD. These findings suggest more excellent oral hygiene and denture care over time. Moreover, we found that the bacterial load of Aa in patients after treatment with RPD is significantly higher than after treatment with CD. This may be attributed to the greater microbial diversity of dental plaque compared to the oral mucosa, since dental plaque may be a more hospitable environment for the growth of microorganisms.[22]

However, dietary changes that occur due to the placement of RPD are also important factors that could influence the level of bacterial load. Al-Hamd et al. found that dietary changes and poor oral hygiene significantly impact the composition of oral microflora.[23] In addition, removable prostheses can be a source of infection in cases where, after laboratory procedures, the prosthetic appliance has not been adequately disinfected, resulting in the transfer of bacteria from the dental laboratory to the new prosthesis.[24] Other factors that could influence the level of Aa and were not considered in the study are the degree of porosity of the prosthesis and the design of the framework of RPD. Both factors have been observed to play an important role in microbial diversity over time.[25,26] Thus, the proper management of removable prostheses to reduce bacterial colonization should be a major focus of future research.

Conclusion

Within the limits of this study, we can state: (1) RT-PCR analysis detected Aa in both study groups at the T0 time interval, but three months (T3) after the initial sample collection, Aa was detected only in patients treated with RPD; and (2) at T3, a significant difference emerged between the two groups, with RPD wearers having a higher level of Aa than CD wearers.

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Competing Interests

The authors declare that they have no competing interests.

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