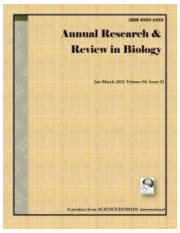

Annual Research & Review in Biology
4(15): 2450-2461, 2014

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Periodontal Pathogens in Screw and Cement Retained Prosthetic Appliances

B. Recani[1], N. Dulcic[2], S. Cimic[2] and A. Catic[2*]

[1]Private Practice, Albert-Rosshauoter Strasse 110, München, Germany.
[2]Department of Prosthodontics, School of Dental medicine, University of Zagreb, Croatia.

Authors' contributions

This work was carried out in collaboration between all authors. Author AC designed the study. Author ND performed the statistical analysis. Author AC wrote the protocol and author SC wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 12th January 2014
Accepted 7th April 2014
Published 16th April 2014

ABSTRACT

Background: Although implants are widely used in everyday practice there are insufficient data on the type of suprastructure fixation (screw versus cement) of the prosthetic appliance and presence of microbial species.
Aim: The aim of the study was to analyse the relationship between the type of prosthetic suprastructure fixation (screwed or cemented) and the presence of *A. actinomycetemcommitans, P. gingivalis, T. forsythia, P. intermedia, T. denticola* and *F. nucleatum*. Furthermore, the aim of this study was to evaluate possible correlation between the presence of systemic diseases and the type of the investigated bacteria.
Materials and Methods: In fifty one dentate patient, 136 implants were inserted either in the upper or lower jaw in the place where molars were missing. Cemented suprastructure was put in 32 patients and screw retained suprastructure in 19 patients. Samples were taken with sterile paper points before abutment fixation and six months later (three times during ten seconds from the gingival sulcus) and analyzed with real-time polymerase chain reaction. Plaque index, Papillla bleeding index and Community Periodontal Treatment Need Index as well as the presence of systemic diseases was recorded.
Shapiro – Wilks test was used when samples were smaller than 30 and Kolmogorov – Smirnov test when they were more than 30. P value under 0.05 was significant.
No differences in the presence of *A. actinomycetemcommitans, P. gingivalis, T. forsythia,*

**Corresponding author: Email: catic@sfzg.hr;*

P. intermedia, T. denticola and *F. nucleatum* with regard to the type of fixation (screwed versus cemented) were seen. There was a significant increase in bacterial count in persons with cardiovascular, rheumatic diseases and in those who took medications and were older. Preoperatively, there was no correlation between periodontal pocket depth with the number of the bacteria. Postoperatively, patients with more periodontal pockets of ≥4 mm harvested higher number of investigated bacteria.

Keywords: Dental implants; screwed or cemented suprastructure; bacteria; systemic diseases.

1. INTRODUCTION

The principles of abutment fixation and fixed prosthetic implant borne replacements have significantly changed with the development of implant prosthetics dentistry. Some ten years ago or even fifteen years ago, the screw was the main and the most common principle of fixation. Although the paradigm has changed in favour of the cement fixation, adherents of the old paradigm are still numerous, their arguments being a better control of soft tissues, easier removal of the replacement and less complicated and a higher quality repair outside the oral cavity. However, in the anterior segments of dental arches, due to position of the fixation screw with regard to the longitudinal axis of the implant and the crown, a significant advantage has been given to cement fixation. An additional argument in favour of the cement fixation is the development of high quality adhesive cements which, apart from excellent retention, offer a possibility to use individual zirconium-oxide ceramic abutments as well as highly aesthetic all-ceramic replacements. Therefore, some clinicians use cement fixation of replacements in the anterior segment of the dental arch and they also use screw fixation in the lateral segments. Another argument of those who prefer cement fixation is that in screw fixation a gap remains between the abutment and the replacement which can be colonised by bacteria thus becoming the source of bacterial infection and a sort of reservoir of infection [1].

The role of microorganism leakage through microgaps has been described in previous studies [2,3,4] which leads to the development of periimplantitis. O'Mahony et al. [5] also consider microgap to be responsible for periimplantitis and implant loss. Orsini et al. [6] presented a case of bacterial colonisation and dental plaque in a region with microgaps which were the cause of periimplant tissue inflammation in the microgaps region. Ericsson et al. [7] showed that clinically non-infected two-part implant system almost always has a moderate inflammatory reaction in the microgap region. All authors agree on the fact that microgaps and the microorganisms related to them are responsible for periimplantitis and have a certain importance in its development and maintenance.

European Academy for Periodontology [8] concluded that infections around implants are more frequently seen in patients with poor oral hygiene, in persons who suffered from periodontal disease and in smoking patients. The same group [8] concluded that there are insufficient data on genetic inheritance, diabetes mellitus, alcohol consumption and surface of the implants to predict the implant failure. Koyanagi et al. [9] reported that *Fusobacterium spp.* and *Streptococcus spp*. were dominant pathogens in both periimplantitis and periodontal disease, however, *Parvimonas micra* was found only in patients with periimplantitis. The same authors [9] concluded that biofilm in the periimplantitis is more complex with regard to the biofilm seen in periodontal disease. Cortelli et al. [10] found that

frequency of P. *gingivalis* and red complex bacteria was more pronounced in periimplantitis with regard to perimucositis sites. Furthermore, *T. forythia* and *T. denticola* were most frequently seen in periodontal diseases, less frequently in gingivitis and even less in healthy tissues. The same authors [10] reported that *P. gingivalis* and *A. actinomycetemcommitans* were seen equally in the periodontal disease and periimplantitis. Finally, they [3] concluded that these bacteria were seen more often on healthy teeth in comparison to the implant tissues. Casado et al. [11] found that periodontal disease was connected with periimplantitis and that *A. actinomycetemcommitans, P. gingivalis, P. intermedia, T. denticola* and *T. forsythia* were present both in healthy tissues as well as in the periimplanitis and periimplant mucositis tissues.Sumida et al. [12] reported that there is a transmission of *P. gingivalis* and *P. intermedia* from the periodontal pockets of the remaining natural teeth to the areas around implants.The same authors [12] concluded that colonisation by *P. gingivalis* and *A. actinomycetemcommitans* significantly correlated with periodontal pockets and areas around implants. The study of Botero et al. [13] showed that there were significant differences in subgingival pathogens between lesions around implants and stabile implants, i.e. *P. gingivalis* was detected in the lesions around implants but not within stable implants. Furthermore, more Gram negative rods were seen around implants as well as *P. gingivalis*. More Gram negative rods (75%) and *P. intermedia/nigrescens* (25%) were found in lesions around implants.

There are only few studies with regard to the type of fixation regarding microbial load [14,15,16,17]. Assenza et al. [14] reported that two-piece implants have gaps and cavities between the implant and the abutment, and these hollow spaces can act as a trap for bacteria. The same authors [14] concluded that hermeticity of the cement-retained implant-abutment assembly and the high prevalence of bacterial penetration of screw-retained implant-abutment assemblies. As there are insufficient data on the type of fixation and presence of certain microbial pathogens, the aim of this study was to determine the correlation between the type of prosthetic appliance fixation together with detailed medical history, smoking, alcohol consumption, and medication intake with regard to the microbial pathogens, specifically presence of *A. actinomycetemcommitans, P. gingivalis, T. forsythia, P. intermedia, T. denticola and F. nucleatum*.

2. MATERIALS AND METHODS

This study was approved by Ethics Committee of the School of Dental Medicine, University of Zagreb, Croatia and every participant signed an informed consent according to the Helsinki II. Exclusion criteria were missing teeth in any other part of the mouth except molar area, patients with periodontal diseases. A questionnaire was made for the purpose of this study where data regarding age, gender, systemic diseases, medication intake, smoking and alcohol consumption as well as periodontal status and number of aforementioned bacteria were recorded. In 51 patients, age range 22-86 years, median 56.5 years, 136 implants were inserted. In 32 patients, prosthetic appliance was cemented and in 19 patients they were screw retained (one person had one or more implants inserted). All the implants were Nobel Biocare-Nobel Replace and Bränemark UNIT III. Flat to flat connection between the abutments and implants was used.

Plaque index was determined according to O´Leary et al. [18] and papilla bleeding index according to Saxer and Mühlemann, [19] whereas Community Periodontal Index of Treatment Need (CPITN) was measured according to the World Health Organisation (WHO) guidelines [20]. Microbial samples were taken before abutment fixation and six months after, three times during 10 seconds with sterile paper points in gingival sulcus, periimplant tissue

and abutment seating. Samples were taken with paper points and analysed with real-time PCR (Carpegen® GmbH, Münster, Germany).

The frozen culture (1.5 ml) was sent to Carpegen GmbH, and 0.5 ml of the defrosted dilution was used for real-time PCR analysis. The cells were harvested by centrifugation (15,000 g at 41C) for 10 min and immediately subjected to the automated process of the Meridol Perio Diagnostics (GABA International, Munchenstein, Switzerland) analysis. This real-time PCR based analysis was developed and validated by Carpegen GmbH. Specificity of Meridol, Perio Diagnostics was verified with purified genomic DNA from several bacterial and fungal species as well as with human DNA. Even closely related species, such as P. intermedia and P. nigrescens, did not show any cross reactivity. The main validated test parameters of Meridol Perio Diagnostics are the detection limit for each of the five pathogens is 100 bacteria within a patient's sample; the linear range for quantification includes seven orders of magnitude for each pathogen; the coefficient of variation is 15%.

The test method detects and quantifies six periodontal pathogens (*A. actinomycetemcomitans*, *F. nucleatum ssp., P. gingivalis, P. intermedia, T. Forsythensis* and *T. denticola*) and the total bacterial load. Results for T. Denticola and total bacterial load were not used for the comparative study because the corresponding data from cultivation were not available.

The bacterial genomic DNA was isolated and purified with the AGOWA mag DNA Isolation Kit Sputum (AGOWAGmbH, Berlin, Germany). The protocol followed the manufacturer's instructions with minor changes to adjust the procedure to the automated isolation with a pipetting robot (Tecan, Genesis Workstation; Tecan Schweiz AG, Switzerland). Primers and probes for Meridol, Perio Diagnostics were designed to match highly specifically to ribosomal DNA (rDNA) of the five bacterial pathogens. The exact primer and probe were selected with the Primer Express software (Applied Biosystems, Foster City, CA, USA), which checks the primer and probe sets for matching the guidelines that are recommended for real-time PCR with TaqMan probes. The primers and probes were purchased from Applied Biosystems. Real-time PCR was carried out with 2 ml of the isolated DNA as template in a reaction mixture containing the appropriate primer probe sets and the TaqMan, Universal PCR Mastermix. The PCR was carried out in a ABI 7900 HT (Applied Biosystems) real-time PCR cycler in 384 well plates [21].

Three months after implant placement, crowns were installed.

2.1 Statistical Analysis

Shapiro – Wilks test was used when samples were smaller than 30 and Kolmogorov – Smirnov test when they were more than 30. Statistical significance between two binary variables was done by use of Fisher's test while the ones between two variables with more than two categories were performed by χ^2 test. The appearance of bacteria was tested by logistic regression analysis and odds ratio with 95% confidence interval for every variable. Correlation between binary dependant variables with more predictors, independent variables measured with various scales, was determined with multivariate (adjusted) binary logistic regression. All statistical data were analysed by SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

Table 1 shows that there were no significant differences in the bacterial count relative to gender, smoking and alcohol consumption.

Table 1. Difference in the bacterial count relative to gender, smoking and alcohol consumption

Gender	n	K-S/ S -W P*	Median (IQR)	p
Males	28	0.036	2 (1.25-4)	0.055
Females	23	0.090	4 (2 -5)	
Smoking				
No	42	0.027	3 (2-4.3)	0.305
Yes	9	0.187	4 (1.5-5.5)	
Alcohol consumption				
No	32	0.002	2 (1.3-4)	0.117
Yes	19	0.480	4 (2-5)	

Abbreviations: K-S/S-W P = Kolmogorov-Smirnov test or Shapiro - Wilk; IQR = interquartile median; P = Mann - Whitney U test

In the Table 2 data regarding participants and according to the presence of investigated bacteria are shown.

Table 2. Participants according to the presence of investigated bacteria

Presence of bacteria	N (%)
No	3 (5.9)
Yes	48 (94.1)
Aggregatibacter actinomycetemcomitans	
No	33 (64.7)
Yes	18 (35.3)
Porphyromonas gingivalis	
No	25 (49)
Yes	26 (51)
Tanerella forsythia	
No	17 (33.3)
Yes	34 (66.7)
Prevotella intermedia	
No	32 (62.7)
Yes	19 (37.3)
Treponema denticola	
No	24 (47.1)
Yes	27 (52.9)
Fusobacterium nucleatum	
No	19 (37.3)
Yes	32 (62.7)

Table 3. Participants according to the number of investigated bacteria.

Aggregatibacter actinomycetemcomitans	**N (%)**
-	33 (64.7)
+	8 (15.7)
++	2 (3.9)
+++	4 (7.8)
++++	4 (7.8)
Porphyromonas gingivalis	
-	25 (49)
+	12 (23.5)
++	6 (11.8)
+++	5 (9.8)
++++	3 (5.9)
Tanerella forsythia	
-	17 (33.3)
+	16 (31.4)
++	11 (21.6)
+++	7 (13.7)
++++	0
Prevotella intermedia	
-	32 (62.7)
+	11 (21.6)
++	3 (5.9)
+++	3 (5.9)
++++	2 (3.9)
Treponema denticola	
-	24 (47.1)
+	12 (23.5)
++	11 (21.6)
+++	3 (5.9)
++++	1 (2)
Fusobacterium nucleatum	
-	19 (37.3)
+	12 (23.5)
++	6 (11.8)
+++	11 (21.6)
++++	3 (5.9)

- Number of detected bacteria was not recorded
- + Number of detected bacteria was ≤10^3
- ++ Number of detected bacteria was 10^4
- +++ Number of detected bacteria was ≥10^5
- ++ ++ Number of detected bacteria was more than 10^6

Table 3 shows presence of the investigated bacteria in the studied participants.

Significant difference was determined between the number of detected bacteria and cardiovascular diseases (p=0.007) and rheumatic diseases (p=0.022). Patients with cardiovascular and rheumatic diseases had significantly higher number of bacteria in comparison to the patients not suffering from the above mentioned diseases. Also, patients

who used medication had significantly more bacteria in comparison to the ones who did not take any medication (p = 0.024) (Table 4).

Table 4. Difference in the investigated bacterial count regarding medical history

Cardiovascular diseases	n	K-S/ S -W P	Median (IQR)	p
No	28	0.028	2 (1.3-3)	0.007; 0.28
Yes	23	0.068	4 (3 -5)	
Respiratory diseases				
No	43	0.003	3 (2-5)	0.406
Yes	8	0.273	4 (2.3-4.8)	
Metabolic disturbances				
No	36	0.158	3 (1-4)	0.088
Yes	15	0.004	2 (2-3)	
Allergies				
No	40	0.037	3 (1-5)	0.476
Yes	11	0.029	2 (2 -3)	
Rheumatic diseases				
No	41	<0.001	2(1.5-4)	0.022; 0.27
Yes	10	0.067	4 (3-5)	
Mental disturbances				
No	48	0.003	3 (2-4.8)	0.834
Yes	3	0.463	4 (2.5-4.5)	
Medication intake				
No	25	0.129	2 (1.5-4)	0.024; 0.32
Yes	26	0.028	3.5 (2-5)	

Abbreviations: K-S/S-W P = Kolmogorov-Smirnov test or Shapiro - Wilk; IQR = interquartile median; P = Mann - Whitney U test

There were no significant differences in bacterial count between the number of teeth with probing depth of more than 4 mm, prior to the implant placement (p =0.016). There were no significant correlations between the plaque index (p = 0.380), and bleeding on probing with the number of investigated bacteria (p = 0.268) (Table 5).

Table 5. Difference in bacterial count regarding one diseased tooth preoperatively

Periodontal pockets ≥ 4 mm	n	K-S/ S -W P	Median (IQR)	p
No	26	0.107	2 (2-4)	0.162
Yes	25	0.084	3 (2 -5)	
Plaque				
No	5	0.254	4 (1.5-5)	0.686
Yes	46	0.007	3 (2-4.3)	
Bleeding				
No	7	0.037	4 (1-5)	0.765
Yes	44	0.010	3 (2-4)	

Abbreviations: K-S/S-W P = Kolmogorov-Smirnov test or Shapiro - Wilk; IQR = interquartile median; P = Mann - Whitney U test

Six months after implant placement there was a significant difference in bacterial count between the number of teeth with probing depth of more than 4 mm (p = 0.043). There were

no significant correlations between the plaque index (p = 0.579) and bleeding on probing with the number of investigated bacteria (p =0.336) (Table 6).

Table 6. Difference in bacterial count regarding one diseased tooth postoperatively

Periodontal pockets ≥ 4 mm	n	K-S/ S -W P	Median (IQR)	p
No	33	0.001	2 (1.5-4)	0.059
Yes	18	0.297	4 (2.8-5)	
Plaque				
No	2	x	x	x
Yes	49	x	xx	
Bleeding				
No	4	0.683	2 (1.3-2.8)	0.210
Yes	47	0.013	3 (2-5)	

Abbreviations: K-S/S-W P = Kolmogorov-Smirnov test or Shapiro - Wilk; IQR = interquartile median; P = Mann - Whitney U test

There was no significant difference in the number of bacterial species with regard to the replacement type (Table 7). Demographic characteristics, medical and dental history along with indicators of the periodontal status, were included in the analysis as covariates. The variables which were used as covariates were as follows: gender, age, smoking, alcohol consumption, drugs, positive medical history, periodontal pockets (pre and post surgery status), plaque (pre and post surgery status) and bleeding (pre and post surgery status). After the covariates had been included in the model, the difference in the number of different bacterial species residing in two types of fixations was not found to be statistically significant.

Table 7. Difference in bacterial species number regarding the typeand number of fixations

Fixation type	n	K-S/ S -W P	Median (IQR)	p
Cemented	32	0.004	2.5 (1.3-4.8)	0.367
Screwed	19	0.532	3 (2-5)	

Abbreviations: K-S/S-W P = Kolmogorov-Smirnov test or Shapiro - Wilk; IQR = interquartile median; P = Mann - Whitney U test

4. DISCUSSION

The results of Heitz-Mayfield [22] showed that there was a significant correlation between poor oral hygiene, previous data on periodontal disease and smoking and risk factors for periimplantitis on the basis of the retrospective study regarding published articles on the available literature. Contrary to our results, Alissa and Oliver [23] reported significant correlation between implant failure and smoking as well as alcohol consumption. However, the number of smokers within this study was relatively small (9 smokers when compared to 42 non-smokers) therefore the significant differences were not obtained. On the other hand, most of the non-smokers were smokers previously. Renvert et al. [24] reported that there were no significant differences between smoking and gender regarding the development of periimplantitis, however there was a significant correlation between cardiovascular diseases and anamnestic data on periodontal disease together with periimplantitis, which was consistent with our results. In another study, Renvert et al. [25] found significant correlation between patients who developed periimplantitis and who had previously developed periodontal disease and suffered from systemic diseases. The same authors [25] concluded

that there were no significant differences regarding the implant surface (TioBlast AstraTech™ and machine etched Branemark Nobel Biocare®) and frequency of periodontal disease.

De Boever and De Boever [26] reported that there were no differences between colonization of *A. actinomycetemcommitans, P. gingivalis, P. intermedia, T. forsythia* and *T. denticola* before implant placement and six months after and that investigated bacteria did not cause periimplantitis, mucositis and bone loss, which was supported by clinical findings by use of DNA-probes (micro-IDent) and radiological findings. De Souza et al. [27] found no significant correlation between periimplantitis and systemic diseases.

We might only speculate that due to a lack of saliva (its composition and quantity) in elderly patients and patients who took medication there was a greater number of bacteria present, however, salivary measurements were not performed. The reason why cardiovascular and rheumatic diseases positively correlated with greater number of bacteria but not metabolic diseases might be that diabetes was well controlled, unlike cardiovascular and rheumatic diseases. Cardiovascular and rheumatic diseases might influence vascular structures within tissues resulting in insufficient immune mechanisms in cardiovascular and rheumatic diseases might explain higher number of bacteria found in these patients. Furthermore, medications for treatment of rheumatic diseases can, due to their immunosuppressive effects, also negatively affect tissue healing. Besides, drugs used by these patients can have an effect on decreased salivation and the increase in pathogenic bacterial count since the mechanism of rinsing by saliva does not function and the enzymes lose their antibacterial effects due to the decrease in saliva.

Regarding the microflora in the microgap between abutment and screw retained suprastructures there are few published studies. Keller et al. [28] showed that the International Team for Implantology (ITI) implant mode of fixation (screw versus cemented) had little influence on the microbial and clinical parameters by use of continuous anaerobic techniques. Quiryanen and van Steenberghe [29] also had the same conclusions regarding Bränemark implants by means of differential phase-contrast microscopy. However, they pointed out that implant gaps might serve as a reservoir for the microorganisms which can leak into a pocket and interfere with the implant prognosis. On the contrary, in a recent study, Assenza et al. [7] reported that there was very low permeability to *P. aeruginosa* and *A. actinomycetemcomitans*of the conical implant-abutment connection, but found high prevalence of these bacteria in screw-retained implant-borne prosthodontic appliances. Piatelli et al. [8] also concluded that cement retained crowns offered better results relating to fluid and bacterial permeability compared to screw-retained crowns by means of fluid and bacterial penetration.

The results of the Assenza et al. [14] and Piatelli et al. [15] are not consistent with our results since we found no differences in the number of periodontal pathogens regarding cement or screw retained appliances. King et al. [23] reported that the size of the microgap does not have influence on the significant bone loss around implants. Contrary to these results, Mombelli et al. [24] reported that despite periimplantitis treatment, after a short period of time, microorganisms identical to those prior to the treatment were present. This finding suggests that microgaps serve as reservoirs of microorganisms.

The results of this study show that pathological pocket depth increased after the implant placement and an increased quantity of bacteria was found in them. There is a possibility that the individuals who underwent implant placement had poor oral hygiene.

5. CONCLUSION

There were no significant differences in bacterial count regarding the type of fixation (cement vs. screw) as well as regarding gender, smoking and alcohol consumption. However, older participants had more bacteria as did persons suffering from cardiovascular and rheumatic diseases and those who were older and who took medications. Preoperatively, there was no correlation with the number of teeth affected with plaque and bleeding on probing as well as periodontal pocket depth with the number of various bacteria. Postoperatively, patients with more periodontal pockets of ≥4 mm harvested higher number of investigated bacteria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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