Microbiological Evaluation of Dental Implants Using Quantification of *Porphyromonas gingivalis* in Dental Teaching Hospital Universitas Indonesia from 2009-2014

*Media Sukmalia Adibah[1], Yuniarti Soeroso[1], Hari Sunarto[1]**

1. Department of Periodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

Abstract

Dental implants provide excellent results in terms of survival and the success rates of oral rehabilitation. The Dental Teaching Hospital, Faculty of Dentistry, Universitas Indonesia (DTH UI) is among the leading dental hospitals that have offered dental implants since 2009, but dental implant treatments have not yet been fully evaluated. The aim of this study was to evaluate the implant success rate by quantification of levels of *Porphyromonas gingivalis* bacteria. Twenty-nine dental implant samples were taken from patients from the Periodontal Clinic of DTH UI from 2009–2014. Samples plaques were obtained from each dental implant using implant probes. The baseline group consisted of similar plaque samples taken from healthy teeth and periodontitis teeth. All samples were subjected to microbiological analysis by quantification of *P. gingivalis* using real time PCR. No significant differences were noted in numbers of *P. gingivalis* between the dental implant groups and the healthy tooth group (P value >0.05), but the numbers of bacteria were significantly lower in the dental implant group than in the periodontitis group (P value < 0.05). The success rate of dental implants was satisfactory, as determined by quantification of *P. gingivalis.*

Clinical article (J Int Dent Med Res 2018; 11(1): pp. 220-223)

Keywords: Dental implant, microbiological, evaluation, Porphyromonas gingivalis, real time PCR.

Received date: 20 September 2017 **Accept date:** 25 October 2017

Introduction

The fundamental goal in dental therapy is to achieve favorable esthetic, mastication, speech, and comfort functions. In the modern era, research and technological advancements in dental implants have revolutionized this therapy. Dental implants have good results in terms of the success and survival rate of oral rehabilitation and are currently the first line of treatment for the replacement of missing teeth.[1] The breakthrough for dental implants was the concept proposed by Branemark et al. in 1952 for osseointegration, which ultimately led to dental implants.[2] The success of a dental implant depends on successful osseointegration, which in turn can be adversely influenced by the presence of bacteria and inflammatory infections.[3] The survival of a dental implant can also be influenced by peri-implantitis due to the presence of plaque. Peri-implantitis is defined as an inflammatory lesion of bacterial etiology, characterized by the loss of supporting bone, as well as inflammation of the mucosa.[3,4,5] Published long-term evaluations of dental implants reveal 10 to 20-year survival rates ranging from 50 to 96%.[6,7,8] The success rate of dental implants can be evaluated in terms of clinical, radiograph, and microbiological aspects.[9,10] Microbiological analysis can be used to detect periodontal pathogens that could initiate peri-implantitis. Heuer and colleagues stated that *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were more abundant than other bacteria in patients with peri-implantitis.[11] Maruyama et al. also found *P. gingivalis* to be one of the most abundant bacteria associated with peri-implantitis.[12] These findings strongly implicate *P. gingivalis* in peri-implantitis; thus, the quantification of this bacterial species could be a useful periodontal pathogen marker for the evaluation of the condition of dental implants. The aim of this study was to evaluate the implant success rate by quantification of *P. gingivalis* in patients who received dental implants.

Corresponding author:
Hari Sunarto
Department of Periodontics
Faculty of Dentistry, Universitas Indonesia,
Jl. Salemba Raya No. 4 Jakarta Pusat
E-mail: harisunarto156@yahoo.co.id

Materials and methods

Twenty-nine plaque samples were obtained from dental implants that had been placed from 2009–2014 at DTH UI. All subjects were treated with implants from the same manufacturer (ITI Straumann- Switzerland). Seven samples with periodontally diseased teeth (pocket depth > 4mm) and five samples with healthy teeth were selected as baseline groups. All subjects were non-smokers and in good general health. This research was approved by the Ethics Committee of the Faculty of Dentistry, Universitas Indonesia, and written informed consent was obtained from all subjects. The sampling sites were isolated with sterile cotton rolls and then the microbial plaque around the implants was obtained for each dental implant using an implant probe (Colorvue Probe UNC 12, Hu-Friedy). The baseline groups of healthy teeth and periodontitis teeth had similar plaque samples collected with an excavator (Crown, Japan). The collected plaque samples were placed in a microtube containing 1000 µl phosphate buffered saline (PBS). All samples were transferred to a microbiological laboratory in the Oral Biology Laboratory, Faculty of Dentistry, Universitas Indonesia. Samples were stored in a -20 °C freezer until real time PCR (RT-PCR) was conducted.

DNA from the samples was extracted using a heat-shock technique and standardized by spectrophotometry (Metertech-Taiwan) to determine the DNA concentration and degree of purity. The *P. gingivalis* (PG) primers consisted of forward (TACCCATCGTCGCCTTGGT) and reverse (CGGACTAAAACCGCATACACTTG) primers. Real-time PCR amplification reactions were carried out in a microwell plate containing 5 µLSYBR Green; 0.5 µL forward PG primer (10 µM); 0.5 µL reverse PG primer (10 µM), and 1 µL H_2O in each well. The real time PCR amplification was conducted in a Step One Real Time PCR System (Applied Biosystems, USA) under the following conditions: initial denaturation at 95°C for 10 minutes, followed by 80 amplification cycles of denaturation at 95°C for 15 seconds, and annealing and elongation at 60°C for 1 minute. The data from the samples were gained at the end of RT-PCR process through cycle threshold (CT). The CT value was inserted into a standard curve equation, which was y= -0.25x+12.284 for *P. gingivalis.*

Results

Samples were obtained from 11 subjects (5 males and 6 females) with 29 implants, as well as from 5 healthy teeth and 7 periodontitis teeth. The mean subject age was 44.3 years (range 24–59 years). The dental implants had been functioning for a mean of 3 years (range 2–7 years). The Shapiro-Wilk normality test (Table 2) showed that the quantitative data for *P. gingivalis* in implant samples did not have a normal distribution.

Variable	Implant Sample		Healthy Teeth Sample		Periodontitis Sample	
	Mean (SD)	Min – Max	Mean (SD)	Min – Max	Mean (SD)	Min-Max
Quantitation of *Porphyromonas gingivalis* (log10 CFU/ml)	22.37 (3.52)	17.08–29.45	20.29 (3.80)	16.27–24.88	33.76 (2.37)	29.78–37.12

Table 1. Mean distribution, standard deviation, minimum and maximum value quantitative measurement of *Porphyromonas gingivalis.*

Variable	p value
P. gingivalis in implant sample	0.047
P. gingivalis in healthy teeth sample	0.342
P. gingivalis in periodontitis sample	0.816

Table 2. Results for normal distribution of the quantitative data for *Porphyromonas gingivalis.* Shapiro-Wilk Test; p> 0.05 = normal distribution

The Kruskal-Wallis test was conducted to compare the quantitative data for *P. gingivalis* in all three groups because of the lack of a normal distribution for these data. The Kruskal-Wallis test results showed a significant difference in the levels of *P. gingivalis* in the implant, healthy, and periodontitis samples (Table 3). A post-hoc test was needed to determine which group showed a significant difference in the comparative analysis between the three groups. The results of the Mann-Whitney post-hoc test are shown in Table 4.

P. gingivalis Level	N	Mean (SD)	p value
Implant sample	29	22.37(3.52)	0.000*
Healthy teeth sample	5	20.29 (3.80)	
Periodontitis sample	7	33.76 (2.37)	

Table 3. Comparative analysis of *Porphyromonas gingivalis* levels between implants, healthy teeth, and periodontitis samples. Kruskal-Wallis test;*p<0.05 = significant

	Implant	Healthy teeth	Periodontitis
Implant		0.158	0.000
Healthy teeth	0.158		0.004
Periodontitis	0.000	0.004	

Table 4. Post-hoc analysis of *Porphyromonas gingivalis* levels. Mann-Whitney test;*p<0.05 = significant.

Comparison of the results of the implant and healthy teeth samples showed no significant differences in terms of the levels of *P. gingivalis.* These levels were significantly lower in the implant group compared to the periodontitis samples, and in the healthy teeth compared to the periodontitis samples.

Discussion

Early transmission of periodontal pathogens from periodontal to implant sites is confirmed months after implant placement.[13] The microflora species *P. intermedia* and *P. gingivalis* begin to colonize the peri-implant sites three months after the exposure of the implants to the oral cavity.[14] Shibli et al. compared the microflora around peri-implantitis implants and healthy implants and found that the microflora, in terms of the types of bacteria, were the same in the both implant types, but the quantity of bacteria was increased in the peri-implantitis sites.[15] The microflora that colonize implants is generally similar to that of the teeth, which may reflect the transmission of periodontal pathogens from the residual dentition to the implant.[16,17] Similarly, studies have shown that the microflora in peri-implantitis sites resemble the microflora associated with periodontitis.[16]

The present research showed a significant difference between the quantitative levels of *P. gingivalis* in implants and in periodontitis samples. This means that the *P. gingivalis* levels in dental implant samples obtained from patients treated in the Periodontal Clinic of DTH UI did not exceed those found in periodontitis samples. This finding is consistent with previous observations that *P. gingivalis* was found more frequently and tended to be higher in peri-implantitis and periodontitis sites than in healthy peri-implant sites.[18] By contrast, our results indicated no significant differences in *P. gingivalis* levels between samples from dental implants and from healthy teeth.

This finding is similar to the research reported by Mombelli et al. and by Rismanchian et al., who indicated that the microflora in implant sulci was similar to that in the tooth sulci, when the depths of these sulci are normal (<4 mm).[16,19] However, Vered et al. reported significantly higher numbers of aerobic and anaerobic oral bacteria in samples taken from teeth than from implants within the same mouth.[20] Nowzari et al. also demonstrated a higher level and frequency of periodontal pathogens around clinically healthy teeth than around healthy peri-implant sites, but these differences were not statistically significant.[21]

Conclusion

In summary, dental implant samples have levels of *P. gingivalis* level that are significantly lower than periodontitis samples but not significantly different from the levels in healthy teeth samples. From this study, we can conclude that dental implants placed in the periodontal clinic of DTH UI show very well-established and satisfactory results. The implant evaluation is successful and the survival rate is excellent. Further clinical studies are needed to assess other periopathogens associated with dental implants.

Acknowledgement

This study was supported by a grant from PITTA Universitas Indonesia.

Declaration of Interest

The authors report no conflict of interest.

References

1. Misch, CE, Perel ML, Wang H-L, Sammartino G, Galindo-Moreno P, Trisi P, Steigmann M, et al. Implant Success, Survival, and Failure: The International Congress of Oral Implantologists (ICOI) Pisa Consensus Conference. Implant Dent. 2008; 17(1):5-15.
2. Parithimarkalaignan S, Padmanabhan TV. Osseointegration: An Update. *The Journal of the Indian Prosthodontic Society*. 2013;13(1):2-6.
3. Abullais SS, AlQahtani NA, Kudyar N, Priyanka N. Success of dental implants: Must-know prognostic factors. J Dent Implant. 2016;6:44-8.
4. Prathapachandran J, Suresh N. Management of peri-implantitis. *Dental Research Journal*. 2012;9(5):516-521.
5. Smeets R, Henningsen A, Jung O, Heiland M, Hammächer C, Stein JM. Definition, etiology, prevention and treatment of peri-implantitis – a review. *Head & Face Medicine*. 2014;10:34.

6. Setzer FC, Kim S. Comparison of Long-term Survival of Implants and Endodontically Treated Teeth. *Journal of Dental Research*. 2014;93(1):19-26.
7. Chappuis, V., Buser, R., Brägger, U., Bornstein, M. M., Salvi, G. E. and Buser, D. Long-Term Outcomes of Dental Implants with a Titanium Plasma-Sprayed Surface: A 20-Year Prospective Case Series Study in Partially Edentulous Patients. Clinical Implant Dentistry and Related Research. 2013,15: 780–790.
8. Rams TE, Balkin BE, Roberts TW, Molzan AK. Microbiological Aspects of Human Mandibular Subperiosteal Dental Implants. J Oral Implantology. 2013;39(6):714-22.
9. Karthik K, Sivakumar S, Thangaswamy V. Evaluation of Implant Success: A Review of Past and Present Concepts. J Pharm Bioallied Sci. 2013;5(Suppl 1):S117-9.
10. Simonis, P., Dufour, T. and Tenenbaum, H. Long-term implant survival and success: a 10–16-year follow-up of non-submerged dental implants. Clinical Oral Implants Research. 2010;21: 772–777.
11. Heuer W, Kettenring A, Stumpp SN, Eberhard J, Gellermann E, Winkel A, Stiesch M. Metagenomic Analysis of the Peri-Implant and Periodontal Microflora in Patients with Clinical Signs of Gingivitis or Mucositis. Clin Oral Investig. 2012;16(3):843-50.
12. Maruyama N, Maruyama F, Takeuchi Y, Aikawa C, Izumi Y, Nakagawa I. Intraindividual Variation in Core Microbiota in Peri-Implantitis and Periodontitis. Sci Rep. 2014;4:6602.
13. Kim K-K, Sung H-M. Outcomes of dental implant treatment in patients with generalized aggressive periodontitis: a systematic review. *The Journal of Advanced Prosthodontics*. 2012;4(4):210-217.
14. Dhir S. Biofilm and dental implant: The microbial link. *Journal of Indian Society of Periodontology*. 2013;17(1):5-11.
15. Shibli JA, Melo L, Ferrari DS, Figueiredo LC, Faveri M, Feres M. Composition of Supra- and Subgingival Biofilm of Subjects with Healthy and Diseased Implants. Clin Oral Implants Res. 2008;19(10):975-82.
16. Shahabouee M, Rismanchian M, Yaghini J, Babashahi A, Badrian H, Goroohi H. Microflora around teeth and dental implants. *Dental Research Journal*. 2012;9(2):215-220.
17. Stokman MA, van Winkelhoff AJ, Vissink A, Spijkervet FKL, Raghoebar GM. Bacterial colonization of the peri-implant sulcus in dentate patients: a prospective observational study. *Clinical Oral Investigations*. 2017;21(2):717-724.
18. Cortelli, SC, Cortelli JR, Romeiro RL, Costa FO, Aquino DR, Orzechowski PR, Araújo VC, Duarte PM. Frequency of Periodontal Pathogens in Equivalent Peri-Implant and Periodontal Clinical Statuses. Arch Oral Biol 2013;58(1):67-74.
19. Rismanchian M, Babashahi A, Goroohi H, Shahabouee M, Yaghini J, Badrian H. Microflora around Teeth and Dental Implants. Dent Res J. 2012;9(2):215.
20. Vered Y, Zini A, Mann J, Kolog H, Steinberg D, Zambon JJ, Haraszthy VI, DeVizio W, Sreenivasan P. Teeth and Implant Surroundings: Clinical Health Indices and Microbiologic Parameters. Quintessence International. 2011;42(4):339–44.
21. Nowzari H, Botero JE, DeGiacomo M, Villacres MC, Rich SK. Microbiology and Cytokine Levels around Healthy Dental Implants and Teeth. Clin Impl Dent Relat Res. 2008;10(3):166-73.