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

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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.

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

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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.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.⁶,¹⁰ 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.
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 (CGGACTAAACCGCATACACTTG) 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 H2O 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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Implant Sample</th>
<th>Healthy Teeth Sample</th>
<th>Periodontitis Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Min – Max</td>
<td>Mean (SD)</td>
<td>Min – Max</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>22.37 (3.52)</td>
<td>20.29 (3.80)</td>
<td>24.88 (3.27)</td>
</tr>
</tbody>
</table>

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

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.

<table>
<thead>
<tr>
<th>P. gingivalis Level</th>
<th>N</th>
<th>Mean (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implant sample</td>
<td>29</td>
<td>22.37 (3.52)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Healthy teeth sample</td>
<td>5</td>
<td>20.29 (3.80)</td>
<td></td>
</tr>
<tr>
<td>Periodontitis sample</td>
<td>7</td>
<td>33.76 (2.37)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Comparative analysis of Porphyromonas gingivalis levels between implants, healthy teeth, and periodontitis samples.

Kruskal-Wallis test; *p<0.05 = significant
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). 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. 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.

**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.

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**Declaration of Interest**

The authors report no conflict of interest.

**References**


