Saliva Versus Peri-implant Inflammation: Quantification of IL-1\(\beta\) in Partially and Totally Edentulous Patients

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The aim of this study was to investigate the potential use of salivary IL-1\(\beta\) in early-stage diagnostics of peri-implant inflammation in partially and totally edentulous patients rehabilitated with dental implants. Patients were classified according to peri-implant probing depth and bleeding upon probing in groups of healthy individuals or in groups of individuals with peri-implant inflammation. Data on plaque index, clinical attachment loss, suppuration, and mobility were also assessed. Saliva was collected without stimulation, and the levels of IL-1\(\beta\) were determined by ELISA. Healthy groups demonstrated significantly lower levels of IL-1\(\beta\) compared with the inflammation groups. No difference in IL-1\(\beta\) levels was observed between partially edentulous or totally edentulous patients. Salivary IL-1\(\beta\) may be useful for the diagnosis and monitoring of early peri-implant inflammation, particularly in edentulous patients.

Key Words: peri-implant sulcular fluid, cytokines, dental implant, inflammation

INTRODUCTION

Oral rehabilitation with implant-supported prostheses in total and partial edentulous patients is a well-documented procedure.\(^1\),\(^2\) However, implant failures do occasionally occur. In general, implant loss following successful osseointegration (late failure) is most likely a result of peri-implant disease.\(^3\)

The development of simple and reliable diagnostic tools for the early detection of peri-implant inflammation and the prevention of irreversible problems is of particular importance.\(^4\) Various clinical and radiographic parameters, such as probing depth, gingival index, plaque accumulation, assessment of bleeding on probing, suppuration, and mobility, are used for this purpose\(^4,5\). However, no measure is currently suggested to be sensitive enough to monitor peri-implant conditions at the desired level.

Recent research has focused on certain complementary diagnostic tools that are based on saliva compounds.\(^6\)–\(^8\) The analysis of saliva offers an interesting model to evaluate peri-implant status due to its noninvasive nature and the simple method of collection. In addition, saliva is generally readily abundant, compared to sulcular fluid.\(^4\)

The salivary pro-inflammatory cytokine interleukin-1\(\beta\) (IL-1\(\beta\)) has already been associated with periodontal tissue destruction;\(^9,10\) however, no previous study suggests its use as a marker of peri-implant disease. This cytokine is secreted in order to up-regulate inflammatory reactions in concert with other components of the local immune response.\(^11\) In this context, the aim of this study was to investigate the potential use of salivary...
IL-1β in early-stage diagnostics of peri-implant inflammation in partial and total edentulous patients rehabilitated with dental implants.

Materials and Methods

Patient population

Fifteen partially edentulous patients (6 men and 9 women) and 15 totally edentulous patients (10 men and 5 women) who had been treated with osseointegrated dental implants at the Department of Oral and Maxillofacial Surgery and Implantology of the Dentistry School of Federal University of Uberlandia participated in this study. Five dentate patients (1 man and 4 women) from the same school made up the control group. Inclusion criteria were as follows: (1) the presence of all teeth and a decayed, missing, and filled teeth score of 0 for Group I, the control group; (2) the presence of at least 1 dental implant restored with an appropriate prosthesis in function for at least 6 months for Group II, the partially edentulous patients; (3) the presence of a full denture in the upper jaw and an implant-retained overdenture with a barr attachment system in the lower jaw that has been in function for at least 6 months for Group III, the totally edentulous patients; (4) no history of antibiotic or anti-inflammatory treatment for 3 months prior to the study; (5) no history of systemic disease or of the use of medications that might influence periodontal or peri-implant status; (6) the absence of smoking habits; and (7) the absence of any periodontal or peri-implant therapy within the last 6 months. The present study was approved by the Ethical Committee of Federal University of Uberlandia, Brazil (Protocol n°217/08). All participating individuals signed an informed consent form prior to the onset of the study.

Clinical examination

The clinical status of the natural teeth and implants was evaluated by a single calibrated examiner at four selected sites (mesial, distal, lingual, and buccal). Periodontal probing pocket depth and peri-implant probing pocket depth (IPPD) were measured from the free mucosal margin to the bottom of the pocket using a conventional periodontal probe (Golgran/Brasil, São Paulo, SP, Brazil), positioned parallel to the long axis of the teeth/implant. Bleeding on probing around teeth and implants was also evaluated (with a score of 0 indicating the absence of bleeding and a score of 1 indicating the presence of bleeding). A modified plaque index score (mPI) was used to record plaque accumulation (with a score of 0 indicating the absence of plaque; a score of 1 indicating that plaque was recognized only by running a probe across the marginal teeth/implant surface; a score of 2 indicating visible plaque; and a score of 3 indicating abundant plaque). Periapical X rays were analyzed and revealed no bone loss in any patient. Data on the presence of clinical attachment loss, suppuration, and mobility were also assessed but were not used as criteria for the healthy and inflammation subgroups. According to these parameters, clinical diagnosis was applied as shown in Table 1.

Saliva samples

Whole, unstimulated saliva (3 mL) was collected prior to clinical evaluation. Patients were instructed to expectorate whole saliva into sterile collector tubes (Salivette, Sarstedt, Newton, NC). No antiseptic mouth rinse was used prior to collection. The samples were immediately placed on ice and then centrifuged (FANEM – Mod. 243, São Paulo, SP, Brazil) at 3000g for 5 minutes. The supernatant was aliquoted and stored at −20°C until laboratory analysis.

| Table 1
<table>
<thead>
<tr>
<th>Established criteria for group distribution</th>
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<tr>
<td>Group I – Control</td>
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<tr>
<td>Healthy</td>
</tr>
<tr>
<td>DMFT = 0, PPPD ≤ 3 mm and BOP = 0</td>
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<td></td>
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<tr>
<td>Group II – Partially Edentulous Patients</td>
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<tr>
<td>Healthy</td>
</tr>
<tr>
<td>All peri-implant sites with BOP = 0 and</td>
</tr>
<tr>
<td>IPPD ≤ 3 mm</td>
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<tr>
<td>Group III – Totally Edentulous Patients</td>
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<tr>
<td>Healthy</td>
</tr>
<tr>
<td>All peri-implant sites with BOP = 0 and</td>
</tr>
<tr>
<td>IPPD ≤ 3 mm</td>
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<tr>
<td>mPI ≥ 1</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Inflammation</td>
</tr>
<tr>
<td>More than 2 peri-implant sites with</td>
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<tr>
<td>BOP &gt; 0 and mPI ≥ 1</td>
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Cytokine assay
The cytokine concentration was determined in duplicate by means of appropriate commercially available ELISA-based capture assay kits for IL-1β (eBioscience, San Diego, CA) in accordance with the manufacturer’s instructions.

Statistical analysis
Statistical analysis was performed with statistical software (GraphPad Prism version 5.0 for Windows, San Diego, CA). Mann-Whitney and Kruskal-Wallis tests (post-test Dunn’s) were used to evaluate the differences between the groups. Correlations between clinical parameters and cytokine levels were evaluated by means of the Spearman correlation coefficient test. Differences were considered to be statistically significant if \( P < .05 \).

RESULTS
No significant differences were found among IPPD with respect to the presence of inflammation (Group II – Healthy: 1.59 ± 0.04 mm; Group II – Inflammation: 1.73 ± 0.05 mm; Group III – Healthy: 2.05 ± 0.08 mm; Group III – Inflammation: 2.15 ± 0.09 mm). However, a significant difference in IPPD was determined for the clinical condition (partial edentulous or total edentulous patients; \( P < .01 \); Figure 1). Moreover, a significant difference was found between the evaluated groups in the mPI score (\( P < .01 \); Figure 2).

Salivary IL-1β was detected in all of the samples. Patients from Group II – Healthy (328.7 ± 32.35) and Group III – Healthy (284.4 ± 37.97) had significantly lower levels of IL-1β than those in Group II – Inflammation (663.1 ± 124.1; \( P < .05 \)) and Group III – Inflammation (1043.0 ± 264.2; \( P < .01 \)) (Figures 3 and 4). No difference was observed in IL-1β levels between partial edentulous and total edentulous patients (Figure 5).

A significant positive correlation between salivary IL-1β levels and changes in the clinical parameter IPPD was exclusively observed in Group II – Inflammation (\( r = 0.5602 \); \( P = .02 \); Table 2).

DISCUSSION
In the present study, it was interesting to note that in the same group of patients, implants with deep pockets did not demonstrate a greater extent of inflammation than implants with shallow pockets. However, a deeper IPPD was observed in total edentulous patients than in partial edentulous patients. A possible explanation for these results is the slight degree of inflammation observed in inflammation subgroups, in which none of the patients displayed actual signs of peri-implantitis. In failing implants, a progressive deepening of IPPD is always found, but pocket depth itself is not always indicative of implant failure, particularly in total edentulous patients, as hyperplasia of the peri-implant mucosa is frequently observed in these patients. In this study, the discovery of a significant positive correlation between salivary levels of IL-1β and the clinical parameter IPPD in only Group II – Inflammation led us to consider the potential influence of mucosa morphology on these results. Although no studies have evaluated the degree of relief on hearing of the capture clip (clip-bar attachment systems) and its relationship with an increase in the mucosa volume and, consequently, in IPPD, this clinical data is relevant to analysis in cases of overdentures. According to Bonachela and Rossetti,13 the load that is transmitted to the bar next to the mucosa during the insertion of the prosthesis could generate a vacuum situation. This would result in tissue hyperplasia under the bar, although this circumstance may not always be clinically evident.

The significant differences in mPI scores between the evaluated groups are in agreement with other studies, which determines a positive correlation between the plaque index and inflammation.14 Although only early stages of peri-implant disease were evaluated, poor oral hygiene remains an important factor in the development and progression of inflammation.14 Good plaque control may be associated with stable peri-implant conditions despite the subjective nature of measuring the plaque index.

The balance between inflammatory mediators and their counter-regulatory molecules may be crucial in determining the outcome of immune pathology of peri-implant disease. In peri-implant sulcus fluid, higher levels of IL-1β have already been associated with peri-implantitis.11,15,16 In concordance with the former, in this study, although the samples analyzed differed, the levels of IL-1β in saliva from subjects with inflammation were signif-
significantly greater than those observed in healthy or control groups. This finding suggests that the overproduction of IL-1β may be implicated as an important factor in the clinical manifestation of peri-implant disease. Nevertheless, this implication should be approached with caution, as no cytokine level threshold is currently known that could differentiate a stable site from one in which a pathologic process has initiated in periodontal and peri-implant tissues.\textsuperscript{17}

In the present study, the production of IL-1β was observed in control and healthy patients. Schultze-Mosgau et al.\textsuperscript{18} previously reported a build-up in cytokine expression due to the implant insertion in a clinical setting, but a specific explanation for this finding is not available. According to Nowzari et al.,\textsuperscript{17} this build-up in cytokine expression may be representative of an inevitable and continuous microbial challenge around teeth and implants,\textsuperscript{17} even in the clinical absence of inflammation. The same authors also demonstrated that the presence

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|}
\hline
 & Group II – Partially Edentulous Patients & Group III – Totally Edentulous Patients \\
\hline Healthy & Inflammation & Healthy & Inflammation \\
\hline
\end{tabular}
\end{table}

$ r = -0.0829 \quad r = +0.5602^* \quad r = +0.8000 \quad r = +0.1445$

$^*P < .05$
of periodontopathogenic bacteria is accompanied by increased cytokine levels, which could help explain the higher levels of IL-1β in the saliva of subjects with inflammation.

It is important to emphasize the deviation in IL-1β levels between the two inflammation groups and the absence of differences in IPPD with respect to the presence of inflammation. The extent and severity of peri-implant inflammation is known to be the result of multiple factors, and high cytokine expression may be a host trait and not exclusively a function of clinical parameters. In light of these factors, the pathogenesis and development of peri-implant disease may differ among individuals. Ideally, diagnostic tests should demonstrate high specificity and sensitivity. Given the complex nature of peri-implant disease, it is unlikely that a single marker will prove to be both. Indeed, a combination may provide a more accurate assessment of peri-implant status. In this study, we were unable to produce conclusive evidence that IL-1β can serve as a predictable marker of peri-implant inflammation. However, the findings suggest the promising potential of utilizing this cytokine to evaluate peri-implant status.

**CONCLUSION**

Salivary IL-1β evaluation may be useful for diagnosing and monitoring early peri-implant inflammation, particularly in edentulous patients. However, future studies conducted with large and diverse patient populations are required in order to validate these findings for clinical application.

**ABBREVIATIONS**

IL-1β: interleukin-1β  
IPPD: peri-implant probing pocket depth  
mPl: modified plaque index

**REFERENCES**