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Microbiological and aMMP-8 findings depending on peri-implant disease in patients undergoing supportive implant therapy

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ABSTRACT

The aim of this study was to evaluate microbiological findings and aMMP-8 level of peri-implant mucositis (M) and peri-implantitis (P) in patients undergoing supportive implant therapy (SIT). Eighty-nine patients with 171 implants were included. The case definitions were as follows: M: PPD ≥4 mm, BOP; P: PPD ≥ 4 mm, BOP, radiographic bone loss ≥3.5 mm. Samples of peri-implant sulcular fluid (PISF) were taken from all peri-implant pockets at each implant to detect periodontal pathogens using PCR and aMMP-8 level with ELISA. Only *Treponema denticola* (Td) and *Prevotella intermedia* (Pi) showed significantly higher prevalence in P (healthy implants [HI]: Td = 27%, Pi = 17%; M: Td = 26%, Pi = 15%; P: Td and Pi = 50%; $P < 0.05$). The mean aMMP-8 level at implant sites did not show any significant difference ($P = 0.05$) among HI (5.2 ± 8.1), M (9.9 ± 19.0), and P (4.9 ± 7.7). Microbiological findings and aMMP-8 levels are not reliable criteria to distinguish between HI, M, and P in patients undergoing SIT.

1. Introduction

Peri-implant diseases, including peri-implant mucositis (M) and peri-implantitis (P), are inflammatory infectious diseases primarily caused by bacteria (Mombelli et al., 2012). Moreover, peri-implant diseases are also multifactorial and have different risk factors (Tomasi and Derks, 2012). M is an inflammatory lesion of the mucosa, while P also affects the supporting bone (Lang and Berglundh, 2011; Sanz and Chapple, 2012). Similar to gingivitis, M is a reversible condition (Lang and Berglundh, 2011; Salvi et al., 2012). Furthermore, there are several similarities between peri-implant and periodontal diseases, especially regarding clinical features and etiology (Berglundh et al., 2011). However, critical differences are noticeable in histopathological factors, the structure of bone loss, and a potentially higher aggressiveness of P lesions (Berglundh et al., 2011; Mombelli et al., 2012).

Moreover, the diagnostics of peri-implant diseases match those of periodontal disease. In a review by Heitz-Mayfield (2008), probing depth (light force 0.25 N), presence of bleeding on probing (BOP positive), and suppuration are described as the main clinical diagnostic criteria of peri-implant diseases. Additionally, radiographic examinations are necessary to evaluate bone loss (Heitz-Mayfield, 2008). In recent years, the range of clinical diagnostics for periodontal diseases was extended to include microbiological analysis of subgingival biofilm (plaque), assessment of matrix-metalloproteinase 8 (aMMP-8) levels, and assessment of genetic risk factors such as interleukin-1 (IL1) polymorphism (Loos et al., 2005; Sorsa et al., 2004; van Winkelhoff, 2003). The microbiology of P has also been investigated repeatedly (Dabdoub et al., 2013; Mombelli and Décaillet, 2011; Zhuang et al., 2016). In some studies the main finding was that the bacterial composition seems to be more diverse than it is for periodontitis (Charalampakis and Belibasakis, 2015; Mombelli and Décaillet, 2011). A recent review by Faveri et al. (2015) showed significant differences between periodontal and peri-implant microbiota and also concluded that further investigations about the diversity of peri-implant bacteria were necessary. Moreover, a further study of this working group showed no correlation between potentially periodontal bacteria findings and peri-implant diseases, especially M and P (Schmalz et al., 2016).

Apart from the microbiological flora, aMMP-8 may serve as a potential marker for inflammation or progressive bone loss in P. Available results suggest higher concentrations of aMMP-8 in P (Arakawa et al., 2012; Ramseier et al., 2016). Furthermore, genetic risk factors that potentially correlate to peri-implant inflammation have been discussed (Cosgarea et al., 2012). However, conflicting results do not allow a clear determination of the relevance of genetic risk factors (IL-1) for the development and progression of P (Dereka et al., 2012; Huynh-Ba et al., 2008).

Based on the heterogeneity of the available results, the clinical relevance of these supplemental diagnostic tools remains unclear and

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requires further evaluation. However, these options might bring great opportunities to replace conventional diagnostics such as x-rays, which are currently indispensable.

Therefore this practice-based cross-sectional study aims to evaluate the microbiological findings and aMMP-8 of M and P in partially edentulous patients undergoing SIT/SPT (supportive implant/periodontal therapy). The hypothesis was formed that aMMP-8 but not microbiological findings might be correlated to peri-implant diseases. In the present study, patients from an earlier evaluation by Rinke et al. (2011) were investigated to detect periodontal pathogens and aMMP-8 levels. Additionally, potential factors influencing P and IL1 polymorphism were evaluated as potential risk factors.

2. Materials and methods

2.1. Study design

This clinical study was performed as a practice-based cross-sectional observational study. It is based on the patients and clinical data of a previous retrospective study (Rinke et al., 2011). The detection of microbiological and aMMP-8 findings as well as IL1 polymorphism was performed when the clinical data were assessed. However, statistical analysis and interpretation of the data were executed in 2015. The study was reviewed and approved by the ethics committee of the University Medical Center Goettingen, Germany (No. 3/2/10). All patients received informative communication (verbally and in writing) and provided written informed consent.

2.2. Patients

Between January 1, 1999 and June 30, 2006, 134 partially edentulous patients were treated with the same type of implant by a dentist in a private dental office (*SR*). Patients who met the following criteria were included in the study:

- implant system: Ankylos, Dentsply Friadent, Mannheim, Germany;
- fixed superstructures (single crowns);
- functional period of restoration >2 years;
- panoramic radiograph (PT) immediately after surgery;
- participation in SPT: regular or irregular prophylaxis (SIT/SPT); and
- complete medical history, including smoking/non-smoking.

Furthermore, the following exclusion criteria were defined:

- aggressive periodontitis;
- no systematic post-therapeutic therapy at all;
- inadequate radiograph;
- no osseointegration of implant;
- function time documented <2 years;
- other missing data.

According to the previous study by Rinke et al. (2011), patients were defined as smokers if they smoked at the time of the follow-up examination or had quit smoking less than 5 years prior (Lang et al., 2003), and were classified as having a "periodontal history" if they had pre-existing periodontal therapy (scaling and root planning or surgical therapy) within 5 years of implant placement. SPT/SIT included peri-implant and periodontal diagnostic, oral hygiene assessment and instructions as well as professional tooth cleaning in a risk adapted, patient specific interval. Patients who did not exceed the recommended interval for SPT by more than 100% after implant placement were classified as "regular SPT". If they exceeded the recommended interval at least once by more than 100%, they were classified as "irregular SPT" (Eickholz et al., 2008; Rinke et al., 2011).

2.3. Dental examination

All patients meeting the inclusion criteria attended a final dental examination carried out by an experienced practitioner (*SR*). The examination covered PPD and bleeding on probing (BOP). The PPD measurement was performed with a millimeter-scaled periodontal probe (PCP 15, Hu-Friedy, Chicago, IL, USA) at four sites per implant (mesio-buccal, disto-buccal, mesio-oral, and disto-oral), and BOP was documented 30 seconds after probing.

Radiographs (PT: panoramic radiographs) were taken from patients with implants showing positive BOP and a PPD ≥ 5 mm, and radiographic bone loss was determined (Frisch et al., 2013; Rinke et al. 2011). All PTs were obtained using the same digital x-ray device (Orthophos, Sirona Dental Systems, Bensheim, Germany). The first x-ray was taken immediately after surgery and served as the baseline-reference for analysis. Data were analyzed with the corresponding PC program (Sidexis XG, Sirona Dental Systems, Bensheim) and a calibrated monitor (SyncMaster 2443SW, Samsung, Schwalbach, Germany) as described by Rinke et al. (2011).

After dental examination, samples of gingival crevicular fluid (GCF) were taken from all peri-implant pockets to detect periodontal pathogens (PCR) and aMMP-8 levels (ELISA). In addition, oral mucosal membrane cells were taken by a cheek smear to detect available interleukin-1 polymorphism.

2.4. Case definition for peri-implant diseases

M and P were the dependent variables in the study and were recorded at each implant site. M was diagnosed at sites with a PPD ≥ 4 mm and positive BOP (Roos-Jansaker et al., 2006a). P was diagnosed if progressive bone loss was determined in addition to the symptoms of M: PPD ≥ 4 mm, positive BOP, and radiographic bone loss ≥3.5 mm (Karoussis et al. 2004; Roos-Jansaker et al., 2006b). If all criteria for P were found in at least one implant site, the diagnosis P was assigned to the whole implant. If all criteria for M were found in least at one implant site but the criteria for P were not found at any site, the diagnosis M was assigned to the whole implant. Implants exhibiting criteria for neither M nor P at any site were defined as healthy implants (HI).

2.5. Molecular biological analysis of aMMP-8

Samples for aMMP-8 and microbiological analyses were collected on the same day. Sampling for aMMP-8 was performed first as follows: Samples of the gingival crevicular fluid/peri-implant sulcular fluid (GCF/PISF) were taken from the deepest peri-implant pockets at each implant using sterile GCF/PISF strips (30 seconds). Prior to GCF sampling, supragingival plaque was carefully removed using a hand scaler. The GCF samples were taken after a little waiting time to ensure that there was no bleeding. The aMMP-8 level of each implant was determined by enzyme-linked immunosorbent assay (ELISA; quantitative aMMP-8 laboratory test, Dentognostics, Jena, Germany). The aMMP-8 analysis (GCF) was performed in a professional laboratory (Dentognostics Jena, Germany).

2.6. Microbiological analysis of periodontal pathogenic bacteria

Following the collection for aMMP-8 analysis, samples for microbiological diagnostic were collected and analyzed. After the removal of supragingival plaque from the implant restorations, three sterile paper tips were used to obtain subgingival biofilm samples (20 seconds) from all peri-implant pockets at each implant and were pooled for each implant. The microbiological analysis of the periodontal pathogens was carried out using polymerase chain reaction analysis (PCR) in the clinical laboratory of the Department of Preventive Dentistry, Periodontology and Cariology, University Medical Center, Goettingen (*DG*). For the semi-quantitative detection of the bacterial colonization of the