Myeloperoxidase level around dental implants as an indicator of an inflammatory process

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Abstract

Aim: Myeloperoxidase (MPO) has been widely used as an inflammatory marker of both acute and chronic conditions. The aim of the present study was to analyze MPO found in the peri-implant sulcus fluid of implants (PISF) and gingival cervicular fluid (GCF) of natural teeth in healthy or diseased states. **Material and Methods:** A total of 107 dental implant sites, either healthy/noninflamed, inflamed or affected by periodontitis, were classified and GCF/PISF samples were obtained. GCF/PISF MPO was spectrophotometrically determined. **Results:** Both the GCF and the PISF volumes exhibited a gradual increase with gingival inflammation ($P < 0.05$). PISF from inflamed sites ($P = 0.0001$) and GCF from the gingivitis and periodontitis sites showed significantly higher total MPO levels ($P < 0.05$) in comparison with the noninflamed sites. The volumetric similarities of PISF and GCF in terms of response to inflammation were seen in the present study. However, some differences in PISF and GCF were observed. **Conclusion:** PISF may be suggested to have a considerable diagnostic potential as it exhibits the biologic changes around load-bearing endosseous dental implants.

Key words: Gingival cervicular fluid, implants, myeloperoxidase, peri-implant sulcus fluid

INTRODUCTION

Implant placement in the edentulous (either partial or full prosthesis) is the new trend in dentistry. It has a high success rate but, with increasing cases of implant placement, failure reports are also increasing. The success of dental implants is decided by the operator skill, quality and quantity of the bone available at the site and the patient›s oral hygiene. Failure of a dental implant means failure of the implant to osseointegrate; it may be lost or mobile or may show bone loss of >1.0 mm in the first year and >0.2 mm 1 year later. Implant success criteria given by The American Academy of Periodontology in 2000 include: "(1) absence of persistent signs/symptoms such as pain, infection, neuropathies, paresthesias, and violation of vital structures; (2) implant immobility; (3) no continuous peri-implant radiolucency; (4) negligible progressive bone loss (<0.2 mm annually) after physiologic remodeling during the first year of function; (5) patient/dentist satisfaction with the implant-supported restoration".[1] Thus, the early recognition of any peri-implant pathology including peri-implant soft tissue inflammation is vital for proper functioning of dental implants in the long term. The development of simple and reliable diagnostic tools for the early detection of the initial peri-implant inflammatory process and prevention of any irreversible host reactions, such as destructive peri-implant disease, may be of particular importance.

Atici suggested that the gingival crevicular fluid (GCF) intracytoplasmic enzyme profile is related to periodontal status and successful periodontal treatment, in addition to clinical improvement, and has a significant effect on this profile.[2] Analysis of biochemical events, more specifically intracytoplasmic enzyme levels in the GCF, are likely to offer a sensitive measure of periodontal pathology that may help in overcoming the existing limitations of clinical parameters. An enzyme, myeloperoxidase (MPO), in the GCF is reported to increase in infectious periodontal diseases,[3-5] initiated by bacteria that colonize the supra- and subgingival

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environments.[6] But, the present status of the role of MPO in peri-implantitis is not clearly defined, and this study was therefore undertaken to reveal the involvement of MPO in periodontal disease and to determine the probability of use of MPO estimation as a reliable diagnostic tool for the early detection of the initial peri-implant inflammatory process and, henceforth, chances of implant failure.

MATERIALS AND METHODS

A total of 107 sites in 21 subjects were included in the present cross-sectional study. Of these, 21 patients (mean age 44 years), 10 men and 11 women, were treated with screw-type endosseous dental implants. Ten of 21 patients had both dental implants and natural teeth and 51 peri-implant and periodontal sites in these patients were measured. Eleven subjects had only dental implants or natural teeth, and 58 sites in these patients were measured. Of the sites, 42 were dental implant sites, while 67 were natural tooth sites with clinical health or some state of inflammation. Patient participation in this study did not endanger their health. They underwent procedures deemed necessary for their oral health. Participation did not involve them in any extradental procedures. Participation was voluntary. All patients provided informed consent. The project was approved by the local ethics committee of the participating institutes.

GCF samples are an accepted method for the evaluation of clinical periodontal status of the natural dentition and for better understanding of the pathogenesis of periodontal disease. Thus, studies on potential similarities or discrepancies between peri-implant circular fluid from dental implants and GCF may contribute to our understanding of the peri-implant inflammatory process and thus to the success or failure of dental implants.

INCLUSION CRITERIA AND SELECTION OF PARTICIPANTS

- The patients were required to be healthy with no allergies or metabolic bone diseases
- No history of antibiotic use in the prior 3 months
- Patients with dental implants and natural teeth were preferred in order to minimize the interpatient variation. However, patients with only implants or teeth were not excluded
- All the patients who were included in the study in clinical periodontal health should have one site of each of the following:
  - Healthy site

- Gingivitis
- Periodontitis
- Dental implant restoration needed to be in function for at least 6 months, and
- Patients attending faculty for periodontal care and implant maintenance.

Determination of the clinical status of the soft tissue

The clinical status of peri-implant soft tissues and clinical periodontal status of natural tooth sites were evaluated by assessing the probing depth (PD), Plaque Index (PI) score, Gingival Index (GI) score and Gingival Bleeding Time Index (GBTI). These measurements were used to assess the presence/extent of both periodontal and peri-implant inflammatory destruction. All measurements were performed at four sites around each implant and natural tooth (mesial, distal, buccal and lingual) and were carried out to the nearest millimeter using a Michigan "O" probe.

To avoid any volumetric disturbance, all of the clinical measurements were recorded after peri-implant sulcus fluid of implants (PISF) and GCF sampling.

Determination of experimental groups

A GI score of 0 was considered to represent the state of clinical health (noninflamed), while a GI score >0 represented inflammation. Radiographic analysis of all tooth and dental implant sites did not demonstrate any alveolar bone loss. Dental implants and natural teeth were further divided into three subgroups according to the severity of the clinical inflammation: (1) Clinically healthy (noninflamed; GI = 0), (2) slightly Inflamed (GI ≤ 1) and (3) moderate/severely inflamed (GI > 1). Patients diagnosed as having chronic periodontitis were also examined as a group. While all other groups reflected the state of clinical inflammation, this particular group represented natural teeth with periodontal destruction and allowed analysis of the potential changes in biochemical parameters at sites with destructive periodontitis.

PISF/GCF sampling

PISF/GCF sampling was performed at the dental implant and natural tooth sites as guided by Rüdin et al., Chapple, Atici et al. and Tözüm et al. PISF/GCF samples were obtained according to the method described by Rüdin et al. using standardized paper strips (Periopaper, no. 593525; Ora Flow, Amityville, NY, USA). Briefly, following the isolation of the sampling area with sterile cotton rolls, supragingival plaque was removed and the site was gently air-dried to reduce any contamination with plaque and saliva. Care was taken to minimize the level of mechanical irritation during PISF/GCF sampling, as this is known to affect the actual fluid volume in a given site. Therefore, paper

strips were placed at the entrance of the peri-implant sulcus or natural tooth crevice and were inserted to a standardized depth of 1 mm at each site regardless of the PD. In order not to affect the actual fluid volume, sampling time was also standardized as 30 s. Samples with evidence of gingival bleeding were not included. To eliminate the risk of evaporation, paper strips were immediately transported to a previously calibrated Periotron 8000 (Ora Flow, USA) located chairside for electronic volume determination. Before sampling, the Periotron 8000 was switched on and allowed to warm up. A blank paper strip was placed in the device and the reading dial was set to 0.33. To increase reliability, the calibration of the device was checked periodically by triplicate readings, as described previously. The PISF/GCF was measured electronically in Periotron units, which were converted to microliters (μL) by MLCONVRT.EXE software (Ora Flow, USA). The PISF/GCF samples were then placed in sterile, wrapped Eppendorf tubes and stored at −20°C until the day of laboratory analysis.

Determination of MPO level of PISF/GCF

The MPO level of the PISF/GCF was measured using a spectrophotometric MPO assay, a modification of the method reported by Suzuki et al.[7] Briefly, the assay mixture consisted of 50 mmol/L phosphate buffer (ph 5.4), 1.6 mmol/L synthetic substrate tetramethyl benzidine (TMB), 0.5% hexadecyltrimethyl ammonium bromide, 1 mmol/L H_2O_2 and 50 μL GCF extract. The reaction was initiated by the addition of H_2O_2 and the rate of TMB oxidation was followed at 655 nm using a recording spectrophotometer. The initial linear phase of the reaction was used to determine the change in absorbance per minute. One unit of MPO activity was expressed as the amount of enzyme producing 1 absorbance change under assay conditions. MPO activity in the PISF/GCF samples was calculated and expressed as both enzyme concentration and the total enzyme activity.

RESULTS

Analysis of MPO level of natural teeth and dental implants grouped by state of inflammation

Despite the higher GCF total MPO levels at the periodontitis sites compared with healthy sites ($P = 0.0001$), such a difference was not observed for GCF MPO concentration. Where dental implant sites were concerned, PISF total MPO level presented a trend of increase at inflamed sites. Concentration mode of data presentation for MPO was nonsignificant. While no difference was observed in any of the laboratory parameters between the inflamed GCF and PISF samples, a

pattern of increase was evaluated in GCF total MPO levels [Table 1]; sites with moderate/severe inflammation provided more GCF MPO than slightly inflamed sites. Differences in MPO concentration were not significant ($P > 0.05$, Table 2), with the increased severity of inflammation compared with healthy sites and presence of periodontal breakdown. At dental implant sites, higher PI and GBTI scores were observed at moderately/severely inflamed sites than both the clinically noninflamed sites ($P = 0.0001$). Moreover, a significant correlation ($P < 0.05$) was found between total MPO level and MPO concentration at natural tooth and dental implant sites (healthy, inflamed and periodontitis, Table 3).

MPO is demonstrated to be a significant ingredient of GCF and to be involved in the pathogenesis of inflammatory periodontal diseases.[8-10] The vast majority of previous studies demonstrate the close association of MPO activity with the clinical and microbial signs of periodontal disease.[6-11]

Increased GCF MPO levels have been shown at sites with gingivitis and chronic and aggressive periodontitis.[9-14] A significant decrease in GCF MPO activity following successful periodontal treatment has also been observed.[13-14] Polymorphonuclear leukocytes that accumulate at sites of gingival

Table 1: Analysis of myeloperoxidase level of natural teeth and dental implants grouped by state of inflammation

	Myloperoxidase		
	Mean±SD	Median	Min-max
Healthy, *n*=14	0.239±0.229	0.114	0.028-0.662
Gingivitis, *n*=27	0.751±0.723	0.345	0.044-0.338
Periodontitis, *n*=24	0.752±0.512	0.628	0.168-0.712
Noninflamed, *n*=20	0.184±0.184	0.066	0.020-0.575
Inflamed, *n*=22	0.432±0.270	0.307	0.054-0.977
	χ^2=29.114, d.f.=4, P=0.00001		

***Healthy, gingivitis and periodontitis groups belong to natural teeth group, while the noninflamed and the inflamed belong to the implanted teeth group, SD: Standard deviation

Table 2: Analysis of myeloperoxidase concentration of natural teeth and dental implants grouped by state of inflammation

	MPO concentration		
	Mean±SD	Median	Min-max
Healthy, *n*=14	1.431±1.188	0.916	0.195-4.681
Gingivitis, *n*=27	1.841±1.850	0.578	0.035-5.408
Periodontitis, *n*=24	0.596±0.312	0.544	0.190-1.340
Noninflamed, *n*=20	0.312±0.257	0.205	0.033-0.883
Inflamed, *n*=22	0.708±0.519	0.582	0.107-1.733
	χ^2=22.525, d.f.= 4, P=0.00016		

***Healthy, gingivitis and periodontitis groups belong to the natural teeth group, while the noninflamed and the inflamed belong to the implanted teeth group, SD: Standard deviation, MPO: Myeloperoxidase

Table 3: Analysis of myloperoxidase level and total myloperoxidase concentration of natural teeth and dental implants grouped by state of inflammation

Groups	Total MPO level (U)		MPO concentration	
	Z	P value	Z	P value
Healthy vs. gingivitis	−2.764	0.006*	−0.327	0.744
Healthy vs. periodontal	−3.727	0.001*	−2.678	0.007*
Gingivitis vs. periodontal	−0.793	0.428	−1.34	0.18
Noninflamed vs. inflamed	−2.997	0.003*	−2.77	0.006*
Healthy vs. noninflamed	−1.337	0.181	−4.075	0.001
Healthy vs. inflamed	−2.602	0.009*	−2.158	0.031
Gingivitis vs. noninflamed	−3.4	0.001*	−3.077	0.002*
Gingivitis vs. inflamed	−0.844	0.399	−1.568	0.117
Periodontal vs. noninflamed	−4.196	0.001	−3.253	0.001*
Periodontal vs. inflamed	−2.177	0.029*	−0.22	0.826

***Healthy, gingivitis and periodontitis groups belong to the natural teeth group, while the noninflamed and the inflamed belong to the implanted teeth group), MPO: Myeloperoxidase, *$P<0.05$

inflammation release various products, including MPO, as a result of the bacteria–host interaction. Thus, increased GCF MPO at periodontally diseased sites is attributed to the increase in gingival inflammation as a result of leukocytes entering the gingival sulcular area. The higher GCF MPO production at both the inflamed and the periodontitis sites observed in the present study is generally in line with these previous studies, which underline MPO as an ingredient of GCF and as a specific enzyme related to the pathogenesis of periodontal diseases.[9-14] Although the MPO content of PISF has not been analyzed to the same extent as the MPO content of GCF, studies have also indicated the presence of MPO in PISF and demonstrated higher PISF MPO levels at inflamed peri-implant sites and peri-implantitis sites.[15,16] Briefly, Boutros *et al*., reported that the MPO level was lower at successful dental implant sites than at failing implant PISF sites.[1] Further, there were no statistically significant differences between healthy natural tooth sites and successful dental implant sites, and the authors concluded that MPO may be a good candidate as a risk marker of implant failure.[15] Liskmann *et al*. demonstrated that total MPO level was significantly higher in PISF of inflamed dental implant sites than in that of healthy sites and that MPO could be a promising marker of inflammation around endosseous implants.[11,16] The present study also revealed that inflamed peri-implant sites demonstrated a pattern of increased total MPO level compared with the noninflamed implant sites. Further, a gradual increase was also noticed with the severity of clinical inflammation. Therefore, the present study supports an association between MPO and the peri-implant inflammatory process at dental implant sites. Based on the findings of the present study, which analyzed both PISF and GCF MPO levels

and demonstrated a similarity of PISF and GCF MPO activity in response to inflammation, a similar role for MPO in the pathogenesis of both periodontal diseases and peri-implant disorders is likely.

Comparing the present study with our previous similar study taking nitrite level and nitrite concentration in account,[17] we found that with respect to total activity level, nitrite and MPO concentrations in inflamed peri-implant and natural teeth with gingivitis or periodontitis were lower than healthy sites. This contrast between two modes of data presentation suggests the volume-dependent nature of the concentration expression.[9,18,19] As concentration expression is affected by the available PISF or GCF volume in a given site, it may be suggested that GCF and PISF share similar volumetric features with respect to the appropriate mode of data presentation.[9,18,19] Although a detectable amount of nitrite was available at all GCF and PISF samples, MPO was not detectable at 13% of the sites. All these MPO-lacking sites were healthy/noninflamed natural tooth or dental implant sites. Based on these findings, MPO and nitrite do not appear to be equal measures of the inflammatory process. As an indicator of leukocyte migration,[20] presence/absence of MPO in either GCF or PISF samples seems to be a better marker of clinical periodontal or peri-implant health and inflammatory status when compared with the nitrite level. Further, NO metabolism may be affected by force and loading.[9,21,22] Thus, besides the inflammatory process, PISF nitrite levels may also be affected by the loading of dental implants. It is possible that the design of the implant-supported prosthesis (e.g. a complete mandibular prosthesis supported by a ball attachment) may affect NO production at dental implant sites and the subsequent PISF nitrite levels.

The results should be interpreted with caution due to the limited number of samples analyzed. Further studies on that to evaluate and compare the components of PISF and GCF, especially with respect to the inflammatory process and bone metabolism, are needed to increase our understanding of the role of each component and the diagnostic potential of PISF for peri-implant pathologies as a biological fluid.

CONCLUSIONS

The findings of the present study support the contribution of both MPO and NO,[17] metabolism to the inflammatory process around both natural teeth and dental implants. Despite their similar volumetric increase with inflammation, the inflammatory

response of PISF and GCF at the molecular level does not seem to be identical in terms of their nitrite and MPO content, probably because of the variety of factors that regulate these two molecular measures. PISF appears to have diagnostic potential for the discrimination between peri-implant health/disease and for a better understanding of the peri-implant biological mechanisms on a molecular level.

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