ORIGINAL RESEARCH

Assessment of Myeloperoxidase and Nitric Levels around Dental Implants and Natural Teeth as a Marker of Inflammation: A Comparative Study

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ABSTRACT

Introduction: Dental implants form the mainstay of dental treatment involving rehabilitation of missing teeth. One of the major concerns for the clinicians doing dental implants is the postsurgical failure of dental implants. Success of dental implants is dependent upon the skills of the surgeon and the amount and quality of the bone remaining at the edentulous area where dental implant has to be placed. Myeloperoxidase (MPO) and nitrites are few of the enzymes and molecules which are said to be altered in inflammation. However, their exact role in the inflammatory processes around natural tooth and dental implant is still unclear. Hence we comparatively evaluated the levels of MPO and nitrites in the areas around the dental implants and natural teeth.

Materials and methods: The present study comprises 42 patients who underwent prosthetic rehabilitation by dental implants from 2011 to 2014. Depth of probing value (DP), score of plaque index (SPI), gingival index (GI), and index of gingival bleeding time (GBT) were evaluated for the assessment of the periimplant soft tissue changes. Assessment of inflammation around the dental implant surface and around natural tooth was done based on the readings of these parameters. For the measurement of the MPO levels, spectrophotometric MPO assay was used. All the results were analyzed by Statistical Package for the Social Sciences (SPSS) software.

Results: The mean plaque index values were 1.56 and 0.97 in periodontitis cases of natural teeth and inflamed cases of dental implants respectively. While comparing mean plaque index, mean probing depth, and mean gingival bleeding index in between the two groups, significant difference was obtained. Mean MPO concentration in periodontitis and gingivitis cases in natural teeth were 0.683 and 0.875 U/µL, while in inflamed dental implant cases, the mean value was 0.622 U/µL. While comparing the total MPO levels, total nitrite levels, and total nitrite concentration in between two study groups, significant difference was obtained. On comparing the healthy and periodontitis cases in natural teeth, significant difference was obtained.

Conclusion: In the inflammatory processes occurring around dental implant and natural teeth, MPO and NO make some amount of significant contribution.

Clinical significance: The present study enforces on the role of MPO and nitrite as diagnostic and prognostic marker.

Keywords: Implants, Inflammation, Myeloperoxidase, Nitrite.

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INTRODUCTION

Rehabilitation of edentulous areas in most of the cases in today's modern world is preferred by dental implants.

Although being one of the ideal lines of treatment, a significant number of failure cases of dental implants have also been reported in the past literature. Success of dental implants is dependent upon the skills of the surgeon and the amount and quality of the bone remaining at the edentulous area where dental implant has to be placed. Apart from these factors, another factor is also significantly responsible for the success for dental implants. This factor is maintenance of postoperative oral hygiene by the patient.[1] Therefore, for the proper functioning and good prognosis of the dental implants, periimplant pathologies should be recognized at the earliest so that supportive treatment measures can be taken for its correction.[2] Inflammation-related diagnostic tools are required for the early detection of the inflammation-related changes occurring in the periimplant areas.[3] Various enzymes and molecules are hypothesized to be predictor of inflammation occurring in the periodontium. Myeloperoxidase (MPO) and nitrites are such enzyme and molecules which are said to be altered in inflammation. However, the exact role they play in the inflammation around natural tooth and dental implant is unclear.[4,5] Hence, we comparatively evaluated the levels of MPO and nitrites in the areas around the dental implants and natural teeth.

MATERIALS AND METHODS

The present study was conducted at the Department of Oral Surgery and Oral Implantology of the institute and included all the patients that underwent prosthetic rehabilitation by dental implants from 2011 to 2014. A total of 42 patients were enrolled into the study that fulfilled the inclusion and exclusion criteria. Only those subjects were included that underwent rehabilitation by dental implant-supported porcelain-fixed partial denture for at least 8 months in the recent past and also had some number of natural teeth. Ethical approval was taken from the Ethical Committee of Institute in written, and also written consent was obtained from all the patients after explaining them the entire research protocol. The exclusion criteria included:

- Patients with history of any systemic illness
- Patients who underwent any type of antibiotic therapy in the recent past
- Patients with any known drug allergy
- Completely edentulous patients
- Patients in which implant-supported fixed prosthesis was given less than 8 months ago
- Patients more than 50 years of age
- Patients who underwent any type of major or minor surgical procedure in the past 6 months.

Within the same study design, the present cross-sectional study was designed to compare and evaluate clinical sites of dental implant and natural tooth along with the presence or absence of inflammation.

## Clinical Soft Tissue Assessment

For the assessment of periimplant soft tissue changes, analysis of various periodontal and clinical parameters was done. These parameters included: Depth of probing value (DP), score of plaque index (SPI), gingival index (GI), and index of gingival bleeding time (GBT).[6-9] Assessment of inflammation around the dental implant surface and around natural tooth was done based on the readings of these parameters. All the four surfaces of the tooth and the implant-supported prosthetic framework were assessed to measure values of all the above-mentioned parameters. All the parameters were clinically assessed by the same clinician in order to avoid variability in measurement values. After assessment of the clinical parameters, all the cases were divided into three main subgroups based on the assessment of inflammatory parameter. Subgroup 1: GI = 0 (clinically non-inflamed), subgroup 2: GI = 1 (slightly inflamed), and subgroup 3: GI = 2 (highly inflamed). All the four clinical sites were analyzed for collecting samples of collection of periimplant sulcus fluid (PF) of implants and gingival crevicular fluid (GC) around the dental implant and natural tooth. 10 mmol/L phosphate buffer was added to the tube containing 300 μL of PF/GC sample, followed by vigorous mixing so that nitrite and MPO can be extracted into the buffer.[10] For the estimation of concentration of nitrite in PF/GC sample, preparation of a standard curve was done. Spectrophotometric MPO assay was used for the measurement of the MPO levels of PF/GC samples. This method represented the modification of a previous method used in various other studies.[11] 50 mmol/L phosphate buffer was mixed to the 50 μL of the GC sample, followed by addition of hydrogen peroxide. The quantity of enzyme that produced the change of 1 absorbance under the conditions in which assay was performed was characterized as the MPO activity. All the results were analyzed by Statistical Package for the Social Sciences (SPSS) software. Kruskal–Wallis test and Mann-Whitney test were used for the assessment of clinical parameters and chi-square test was used to compare the various groups. The p-value of < 0.05 was taken as significant.

RESULTS

Graph 1 shows the mean clinical values of the periodontal parameters in tooth and dental implant cases. The value of mean plaque index in periodontitis cases of natural teeth was 1.56, while mean plaque index value in inflamed cases of dental implants was 0.97. In natural teeth, gingivitis and periodontitis cases showed
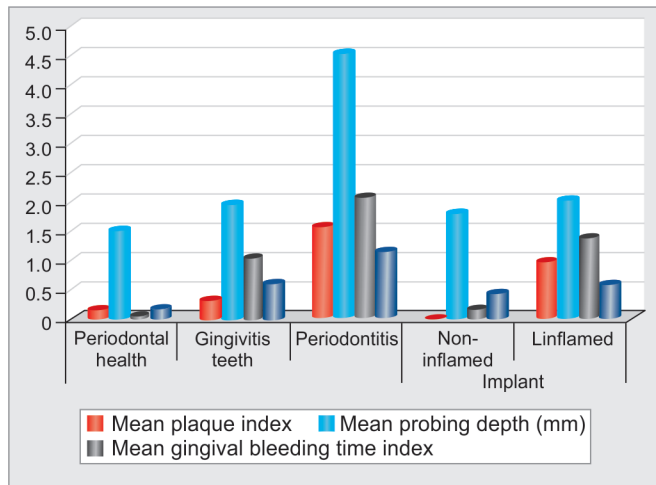
Graph 1: Mean values for various clinical parameters among patients in natural tooth and dental implant group
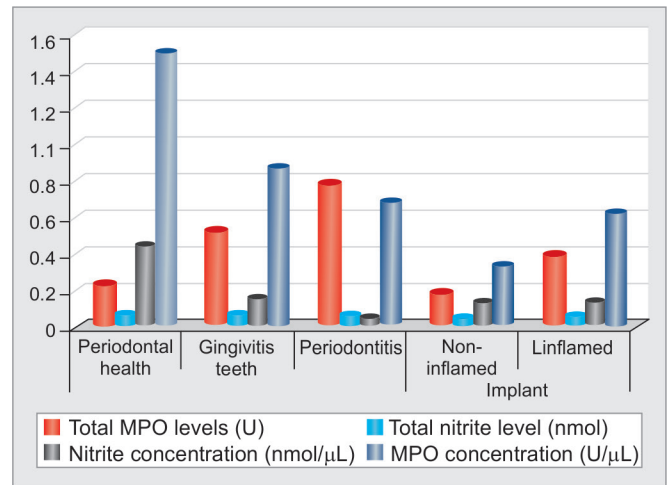
Graph 2: Mean values for various inflammatory parameters among patients in natural tooth and dental implant group

Table 1: The p-value for various clinical parameters among both the study groups

Parameters	p-value
Mean plaque index	0.003*
Mean probing depth (mm)	0.002*
Mean gingival bleeding time index	0.004*
Mean gingival crevicular fluid/periimplant sulcus fluid volume	0.001*

*Significant

Table 2: The p-value for various inflammatory parameters among both the study groups

Parameters	p-value
Total MPO levels (U)	0.010*
Total nitrite level (nmol)	0.018*
Nitrite concentration (nmol/μL)	0.020*
MPO concentration (U/μL)	0.069

*Significant

periodontal probing depth of 1.95 and 4.52 mm. Mean GBT index in periodontitis and gingivitis cases was 2.10 and 1.09, while its value in inflamed dental implant cases was 1.43. Table 1 shows the p value for the mean values of various clinical and periodontal parameters in-between dental implant cases and natural teeth. Significant difference was obtained while comparing mean plaque index, mean probing depth, and mean gingival bleeding index in-between the two groups. Graph 2 shows the total MPO and nitrite levels in patients of both the groups. Total nitrite levels in periodontitis cases and inflamed dental implant cases were 0.055 and 0.140 nmol/μL.

Mean MPO concentration in periodontitis and gingivitis cases in natural teeth was 0.683 and 0.875 U/μL, while in inflamed dental implant cases, the mean value was 0.622 U/μL. Table 2 shows the p value of comparison of mean values of various inflammatory parameters in-between the different study groups. Significant difference was obtained while comparing total MPO levels, total nitrite levels, and total nitrite concentration in-between two study groups. However, on comparing the mean MPO concentrations, nonsignificant difference was obtained. Table 3 shows the p value for comparison of various clinical and inflammatory parameters in-between the various

Table 3: The p-value for comparison of clinical and inflammatory parameters between both the study groups and within the same groups

		p-value for mean plaque index	p-value for mean probing depth (mm)	p-value for mean gingival bleeding time index	p-value for mean gingival crevicular fluid/ periimplant sulcus fluid volume	Total MPO levels (U)	Total nitrite level (nmol)	Nitrite conc. (nmol/μL)	MPO conc. (U/μL)
Teeth	Healthy *vs* gingivitis	0.002*	0.001*	0.041*	0.001*	0.211	0.842	0.003*	1.020
	Healthy *vs* periodontitis	0.010*	0.002*	0.031*	0.007*	0.030*	0.145	0.001*	1.320
	Gingivitis *vs* periodontitis	0.030*	0.010*	0.021*	0.007*	0.013*	0.685	0.002*	1.812
Implant	Noninflamed *vs* inflamed	0.002*	0.471	0.013*	0.612	0.012*	0.020*	0.630	0.985
Noninflamed teeth *vs* noninflamed implant		0.612	0.098	0.452	0.008*	0.975	0.010*	0.002*	0.152
Inflamed teeth *vs* inflamed implant		0.845	0.414	0.314	0.812	1.002	0.497	0.152	1.003

*Significant

groups and within the groups. On comparing the healthy and periodontitis cases in natural teeth, significant difference was obtained.

DISCUSSION

Dental implants have become one of the mainline treatment in the today's present world as a routine option for the prosthetic rehabilitation of missing teeth. Apart from operator's skills, prognosis of dental implants is largely dependent upon osseo-integration and healing at the periphery of the dental implant surfaces.[12] Reversible changes associated with the soft tissues around the dental implants are referred to as periimplant mucositis. On the contrary, inflammatory process resulting in significant resorption of the bone around the dental implant, i.e., periimplant area is referred to as periimplantitis. For the initiation of the inflammation around the dental implant surface, subgingival biofilm has been shown to play an important etiologic role resulting in subsequent bone loss.[13-15] Literature quotes studies that highlight a minute link between the generation of reactive nitrogen species in response to inflammation and inflammatory mediators. Data also clearly indicate the conversion of MPO into nitrite and peroxide under the effects of nitrogen species.[16-19] Hence, we analyzed and compared the nitrite and MPO levels in PF and GC of dental implants and natural teeth respectively, in relation to the severity of inflammation.

In the present study, no significant difference was observed on comparing the volume of PF and GC in-between inflamed and noninflamed sites (Graph 1, Table 1). Complex changes and modifications occur in the periphery of the areas of dental implants and natural teeth, although in-between the molecular pathology and the severity and presence or absence of inflammation, a close correlation has been observed in the past.[20,21] Serious alterations occurring in the volumes of PC and GF in response to inflammation can be attributed to the above-mentioned factors.[22-24] When inflammatory parameters were compared with the clinical parameters, a nonsignificant difference was obtained, which attributes to the importance of time of profiling of GC and measurement of clinical parameters (Graph 2, Table 2).[25,26] In the present study, we observed elevation in the production of GC MPO in both the periodontitis cases and in cases of inflammation around dental implants which is in correlation with the results of the previous studies (Graph 2, Table 2).[26] We also observed that in comparison with the noninflamed sites in dental implant cases, patients with inflammation around dental implants exhibited significant elevation in the total MPO levels (Table 3). In comparison with the healthy gingival tissue cases of natural teeth group, an increase in the levels of nitrites has

been observed in the cases of high inflammation, which is also in correlation with the results of previous studies (Table 3).[24] Tözüm et al analyzed the various molecular parameters of inflammation in the PG and GC of dental implant and natural tooth respectively. They evaluated the 109 sites of tooth/dental implants and observed a gradual increase in the various periodontal parameters with the advancement of inflammation. They also observed higher content of nitrites in PF samples from inflamed sites. From the results, they concluded that some amount of diagnostic potential is presented by changes in parameters and findings of PF.[27] Güncü et al analyzed the effect of inflammatory changes on MPO levels of PF and GC and comparatively evaluated them. They assessed more than 200 sites of the dental implants and natural tooth and observed an increase in the values of the clinical parameters with the advancement in the inflammatory process. From the results, they concluded that a strong correlation exists between the production of the MPO and the severity of inflammation.[28] Durrani et al evaluated the levels of MPO observed in the PF and GC of dental implants and natural tooth respectively. They evaluated more than 100 sites of dental implants and assessed their level of inflammation in terms of various inflammatory parameters and observed that with the advancement in the levels and severity of gingival inflammation, a gradual increase in the levels of PF and GC is also associated. From the results, they concluded their study by emphasizing on the importance of the diagnostic potential of PF and GC.[5] Yamalik et al comparatively evaluated the levels of cathepsin-K enzyme in PF and GC of dental implant surfaces and natural tooth surfaces respectively. They evaluated the clinical parameters of the periodontium in patients with dental implants and patients with natural tooth and observed a higher activity of cathepsin-K in periodontitis patients in comparison with gingivitis patients. From the results, they concluded that to some extent, cathepsin-K is involved in the metabolism of bone around the teeth and dental implants.[29] Yamalik et al assessed the GC/PF cathepsin-K levels in sites around natural tooth and dental implants and compared them. They measured the activity of cathepsin-K levels of 42 GC and 54 PF samples and observed no significant difference on comparing various clinical periodontal parameters in-between the two study groups. From the results, they concluded that for the monitoring of the bone loss around the dental implants and the natural teeth, cathepsin-K activity levels can be used as a diagnostic parameter.[30]

CONCLUSION

From the above results, the authors concluded that MPO and NO make some amount of significant contribution in the process of inflammation occurring at periimplant and

periradicular areas. However, this field requires further research for exploring the diagnostic and prognostic significance of these markers.

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