Analysis of the Inflammatory Process Around Endosseous Dental Implants and Natural Teeth: Myeloperoxidase Level and Nitric Oxide Metabolism

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Purpose: *The aim of the present study was to analyze the 2 molecular measures of inflammation: (1) the nitrite, an end metabolite of nitric oxide (NO) oxidation and (2) myeloperoxidase (MPO). Both are found in peri-implant sulcus fluid (PISF) of implants and gingival crevicular fluid (GCF) of natural teeth in healthy or diseased states.* ***Materials and Methods:*** *A total of 109 tooth or dental implant sites, either healthy/noninflamed, inflamed (Gingival Index [GI] > 0), or affected by periodontitis, were classified, and GCF/PISF samples were obtained. GCF/PISF MPO and nitrite levels were spectrophotometrically determined. For comparison of clinical parameters and PISF/GCF nitrite and MPO levels, Kruskal-Wallis analysis followed by Mann-Whitney test with Bonferroni correction was performed. Healthy/noninflamed, slightly inflamed, moderate/severely inflamed sites were also analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test with Bonferroni correction. The correlation between nitrite and MPO levels and clinical inflammatory status were analyzed with Spearman's correlation coefficient.* ***Results:*** *Clinical parameters, including both the GCF and PISF volumes, demonstrated gradual increases with the presence of gingival/peri-implant inflammation (*$P < .05$*). Despite the higher PISF than GCF volume at healthy sites (*$P = .001$*), there were no volumetric differences at inflamed sites (*$P = .771$*). PISF from inflamed sites (*$P = .025$*) and GCF from gingivitis and periodontitis sites presented higher total MPO levels (*$P < .05$*) than samples from noninflamed sites. Despite the relatively stable GCF nitrite levels at healthy and diseased sites, PISF from inflamed sites had higher nitrite content than noninflamed sites (*$P < .05$*).* ***Conclusions:*** *The present study demonstrated the volumetric similarities of PISF and GCF in terms of response to inflammation. However, some differences between the 2 biochemical measures of inflammation and their presence in PISF and GCF were also observed. PISF is likely to have a considerable diagnostic potential for reflecting the biologic changes around load-bearing endosseous dental implants.* (Cohort Study) (More than 50 references.) Int J Oral Maxillofac Implants 2007;22:969–979

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Among a variety of other factors, the close monitoring of the clinical health of peri-implant tissues and efficient control of the etiologic agents that cause onset of peri-implant tissue inflammation play a critical role in the long-term success of endosseous dental implants.[1–4] The reason for this is that the period following such initial peri-implant inflammation, which generally resembles the reversible plaque-related tissue reactions around a natural tooth, can include irreversible destruction of the peri-implant tissues.[2,5,6] Dental-implant studies have demonstrated that any loss of the integrity of the biological seal of the peri-implant tissues is an important factor that may trigger peri-implant soft tissue inflammation and alveolar bone loss (peri-implantitis), which may result in implant failure.[2,5–7] As early recognition of any peri-implant pathology, including peri-implant soft tissue inflammation, is vital for long-term proper functioning

of dental implants, the development of simple and reliable diagnostic tools for early detection of initial peri-implant inflammatory processes and prevention of any irreversible host reactions, such as destructive peri-implant disease, may be of particular importance.

According to the dental literature, various clinical measures (eg, pocket probing, Gingival Index, and Plaque Index) are used for this purpose.[2,5,7,8] However, currently no measure is suggested to be valid and sensitive for monitoring of peri-implant conditions at the desired level. In addition to the evaluation of clinical measures, recent research has also focused on the features of the molecular mechanisms of the inflammatory process of peri-implant tissues.[1,2,9–12] There is increased interest in peri-implant sulcus fluid (PISF), an osmotically mediated transudate/inflammatory exudate around dental implants. Both the volumetric features and the constituents of PISF are of particular concern. Such studies provide considerable evidence for a better understanding of the inflammatory process around dental implants.[1,2,9–11] On the other hand, analysis of gingival crevicular fluid (GCF) samples is an accepted method for evaluation of the clinical periodontal status of the natural dentition and for a better understanding of the pathogenesis of periodontal diseases. Thus, studies on the potential similarities or discrepancies between PISF from dental implants and GCF may also contribute to our understanding of the peri-implant inflammatory process and thus to the success or failure of dental implants.[1,9,10,12,13]

Nitric oxide (NO) is a diatomic free radical produced by activated phagocytic leukocytes; it has both harmful and beneficial effects on the pathophysiology of the tissues.[14–16] In gingival and periodontal diseases, it has been reported that macrophages and endothelial cells contribute to NO production.[17,18] It has been suggested that the expression of Inducible NO synthase (iNOS) from macrophages in high levels may damage the periodontal tissues of patients with localized aggressive periodontitis (LAP) and iNOS activity in macrophages was reported to have the potential to inhibit leukocyte recruitment by acting on leukocytes that increase the inflammatory state in LAP patients.[19] It has also been suggested that patients with chronic periodontitis have increased amounts of iNOS and arginase activity in periodontal tissues and that periodontal treatment, including scaling and root planing, or periodontal flap surgery may decrease the formation of iNOS and arginase activity in these subjects.[20] In the peri-implant region as well as the natural dentition, it has also been demonstrated that NO metabolism is closely related to the status and degree of peri-implant gingival inflammation.[10] Moreover, myeloperoxidase (MPO) is an antimicrobial leukocyte-derived enzyme found in high concentrations in the primary granules of leukocytes that catalyzes the formation of a number of reactive oxidant species.[21,22] In addition to being an integral component of the immune response, MPO-derived oxidants contribute to tissue damage during inflammation.[21,23] The increased amount of MPO at sites with gingival inflammation and alveolar bone destruction in chronic and aggressive periodontitis suggests that MPO has a role in destructive periodontal disease.[24] MPO is also a good indicator of neutrophil activity in failed peri-implant sites compared to successful endosseous dental implant sites.[1,9] The evidence for a link involves the generation of reactive nitrogen species by neutrophils that respond to cytokines and enzymes at inflammatory sites. It is clear that NO can influence MPO and that MPO can convert nitrite and peroxides into a nitrating agent for proteins and lipids.[21,25] At low levels of NO, the rate of MPO-catalyzed peroxidation of substrates is increased. MPO can also generate nitrating capacity for lipids and proteins.[21,25] The mechanism of nitration involves electron oxidation by nitrite followed by recombination. The medical literature also provides some evidence about the interaction of NO metabolism and MPO in inflammation.[21,25]

The aim of the present study was to comparatively and quantitatively analyze the 2 molecular measures of inflammation, nitrite, an end metabolite of NO oxidation, and MPO, both in PISF of endosseous dental implants and GCF of natural teeth in inflammatory or destructive periodontal states.

MATERIALS AND METHODS

Inclusion Criteria and Selection of the Participants

Patients were required to have an unremarkable medical history, with no known allergies and/or no metabolic bone diseases. They could have no history of antibiotic treatment for the prior 3 months. In spite of the study design, to minimize the interpatient variation, patients presenting with both dental implants and natural teeth were preferred. However, subjects with only dental implants or natural teeth were not excluded. In addition, care was taken to ensure that all participants in the natural tooth group had at least 1 site that would allow them to be integrated into an appropriate subgroup (clinical periodontal health, gingivitis, or periodontitis). With respect to the dental implant group, in addition to meeting the criteria for general health, patients were required to have dental implant-supported fixed porcelain restorations. Den-

tal implants were required to have been in function for at least 6 months.

Since the study design did not consider the effect of race, gender, or age such variables were not among the exclusion criteria. However, all participants were white. Patients of the dental faculty being seen for periodontal care and/or dental implant therapy served as the source of the study population. No further randomization was attempted. The study was planned as a cross-sectional study to comparatively analyze dental implant sites and natural tooth sites with and without apparent clinical inflammation within the same study design. The sample size was planned with the intention of including enough natural tooth and dental implant sites to achieve statistical relevance. This cross-sectional study was conducted on dental implant or natural tooth sites from 21 subjects who were selected according to the aforementioned criteria.

Determination of the Clinical Status of the Soft Tissue

Clinical status of peri-implant soft tissues and clinical periodontal status of natural tooth sites were evaluated by assessing the probing depth (PD),[26] Plaque Index (PI) score,[27] Gingival Index (GI) score,[28] and the Gingival Bleeding Time Index (GBTI).[29] These measurements were used to assess the presence/extent of both periodontal and peri-implant inflammatory destruction. All measurements were performed at 4 sites around each implant and natural tooth (mesial, distal, buccal, and lingual) and were carried out to the nearest mm using a Michigan 'O' probe. To avoid any volumetric disturbance, all of the clinical measurements were recorded after PISF and GCF sampling. All clinical parameters were measured by the same periodontist.

Determination of Experimental Groups

A GI score of 0 was considered to represent the state of clinical health (noninflamed); a GI score of > 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 3 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. PISF/GCF samples were obtained according to the method described by Rüdin et al[30] using standardized paper strips (Periopaper, no. 593525; Ora Flow, Amityville, NY). 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.[31] 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 seconds. Samples with evidence of gingival bleeding were not included. To eliminate the risk of evaporation,[32] paper strips were immediately transported to a previously calibrated Periotron 8000 (Ora Flow, Amityville, NY) located chairside for electronic volume determination.

Prior to 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 previously described.[32,34] The PISF/GCF was measured electronically in Periotron units, which were converted to microliters (μL) by MLCONVRT.EXE software (Ora Flow).[32,34] The PISF/GCF samples were then placed in sterile, wrapped Eppendorf tubes and stored at –20°C until the day of laboratory analysis. To reduce interexaminer variability, all PISF and GCF samplings were performed by the same periodontist.

Determination of Nitrite Level of PISF/GCF

To each PISF/GCF sample in the Eppendorf tube, 300 μL extraction buffer (10 mmol/L phosphate buffer containing 0.5% (10 mmol/L phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide [HETAB], pH 6.0), pH 6.0) was added, and the samples were vigorously mixed for the extraction of nitrite and MPO into the buffer. For the determination of nitrite levels, 150 μL of the extract was mixed with 150 μL of freshly prepared Greiss reagent using a microplate. After 10 minutes of incubation at room temperature, the absorbance of each sample in microplate wells was determined at 540 nm.[35] A standard curve was prepared using sodium nitrite to calculate nitrite concentration in PISF/GCF.

Determination of MPO Level of PISF/GCF

The MPO level of the PISF/GCF was measured using a spectrophometric MPO assay, a modification of the method reported by Suzuki et al.[36] 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, 1mmol/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 PISF/GCF samples was calculated and expressed as both enzyme concentration and the total enzyme activity.

Statistical Analysis

SPSS 11.5.0 software for Windows (SPSS, Chicago, IL) was used for all statistical analyses. For clinical parameters and PISF/GCF nitrite and MPO levels in healthy/noninflamed sites, inflamed sites, and sites with periodontitis, the Shapiro-Wilk test was used to test the normality of the distribution.[37] Since data were not normally distributed, the Kruskal-Wallis analysis followed by Mann-Whitney test with Bonferroni correction was performed for the comparison of healthy/noninflamed and inflamed/gingivitis sites.[38,39] Moreover, healthy/noninflamed, slightly inflamed, and moderate/severely inflamed sites were also analyzed using the Kruskal-Wallis test, which was followed by the Mann-Whitney test with Bonferroni correction for bilateral comparisons. The correlation between nitrite and MPO levels and clinical inflammatory status was analyzed with Spearman's correlation coefficient.[40] P less than .05 was considered statistically significant.

RESULTS

Analysis of Clinical Parameters of Natural Teeth and Dental Implants Grouped by State of Inflammation

A total of 109 sites in 21 subjects were included in the present cross-sectional study. Of these, 21 patients (with a mean age of 44 years), 10 men and 11 women, had been treated with screw-type endosseous dental implants. Ten of 21 patients had both dental implants and natural teeth; 51 periimplant and periodontal sites in these patients were measured. Eleven subjects had only either dental implants or natural teeth; 58 sites in these patients were measured. Of the 109 sites, 42 were dental implant sites, while 67 were natural tooth sites with clinical health or some state of inflammation.

The descriptive statistical analysis and actual P values are provided in Table 1. When natural tooth sites were concerned, clinical parameters, including PI, GBTI, and GCF volume, were higher in periodontally diseased sites than clinically healthy sites (periodontal health < gingivitis < periodontitis). Inflamed dental implant sites had higher PI and GBTI scores than noninflamed implant sites ($P = .0001$), while PD and PISF volume did not present any significant difference. Comparison of clinically healthy natural tooth and noninflamed dental implant sites revealed no significant difference, except the higher PISF than GCF volume ($P = .0001$). All clinical parameters from natural teeth with gingivitis and inflamed dental implant sites were similar ($P > .05$), and no difference was observed between PISF and GCF volume at these sites ($P = .771$).

The impact of severity of inflammation on clinical measures, together with descriptive statistical analysis and actual P values, is shown in Table 2. In general, all of the clinical parameters demonstrated a trend of increase with the severity of gingival inflammation, and PI and GBTI of moderately/severely inflamed sites had higher scores than healthy sites ($P < .005$). No statistical significance could be observed when natural tooth sites and dental implant sites were compared with respect to clinical parameters either at slightly inflamed and moderately/severely inflamed sites ($P > .05$).

Analysis of Nitrite and MPO Levels of Natural Teeth and Dental Implants Grouped by State of Inflammation

Descriptive statistical analysis and actual P values are provided in Table 1. For both of the laboratory measures, total and concentration modes of data presentation did not match and presented different trends. While GCF total nitrite levels stayed quite stable in all clinical circumstances (periodontal health: 0.050 nmol; gingivitis: 0.053 nmol; periodontitis: 0.052 nmol); $P > .05$), GCF nitrite concentration clearly presented a different pattern (periodontal health: 0.433 nmol/µL; gingivitis: 0.159 nmol/µL; periodontitis: 0.051 nmol/µL; $P = .0001$). Despite the higher GCF total MPO levels at periodontitis sites compared to healthy sites ($P = .0001$), such a difference was not observed for GCF MPO concentration. Where dental implant sites were concerned, PISF total nitrite ($P = .001$) was significantly higher, where 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

Table 1 Analysis of Clinical Parameters and Nitrite and MPO Levels of Natural Teeth and Dental Implants Grouped by State of Inflammation

	PI			PD			GBTI			GCF/PISF volume		
	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max
Tooth												
Periodontal health (n = 16)	0.13 ± 0.342	0.00	0.00–1.00	1.50 ± 0.632	1.00	1.00–3.00	0.06 ± 0.250	0.00	0.00–1.00	0.179 ± 0.112	0.14	0.03–0.44
Gingivitis (n = 27)	0.89 ± 0.801	1.00	0.00–2.00	1.93 ± 0.675	2.00	1.00–3.00	1.07 ± 0.730	1.00	0.00–2.00	0.616 ± 0.402	0.47	0.05–145
Periodontitis (n = 24)	1.54 ± 0.588	1.50	1.00–3.00	4.50 ± 0.722	4.00	3.00–6.00	2.08 ± 0.504	2.00	1.00–3.00	1.130 ± 0.328	1.15	0.47–1.86
Implant												
Noninflamed (n = 20)	0.00 ± 0.00	0.00	0.00–0.00	1.80 ± 0.768	2.00	0.00–3.00	0.20 ± 0.410	0.00	0.00–1.00	0.440 ± 0.264	0.35	0.11–0.96
Inflamed (n = 22)	0.95 ± 0.785	1.00	0.00–2.00	2.00 ± 0.617	2.00	1.00–3.00	1.41 ± 0.908	1.50	0.00–3.00	0.582 ± 0.400	0.49	0.13–1.54
		χ^2 = 53.806			χ^2 = 64.719			χ^2 = 64.833			χ^2 = 52.049	
		P = .0001			*P* = .0001			*P* = .0001			*P* = .0001	

*Statistically significant (*P* < .05). NS = not significant. Since data were not normally distributed, the Kruskal-Wallis test followed by the Mann-Whitney test with the Bonferroni correction was performed for comparison of healthy/noninflamed and inflamed sites.

Table 1 continued Analysis of Clinical Parameters and Nitrite and MPO Levels of Natural Teeth and Dental Implants Grouped by State of Inflammation

	Total nitrite level (nmol)			Total MPO level (U)			Nitrite concentration (nmol/µL)			MPO concentration (U/µL)		
	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max
Tooth												
Periodontal health (n = 16)	0.050 ± 0.010	0.050	0.034–0.440	0.217 ± 0.229	0.113	0.007–0.720	0.433 ± 0.386	0.331	0.09–1.68	1.469 ± 1.639	0.916	0.060–4.800
Gingivitis (n = 27)	0.053 ± 0.008	0.054	0.038–0.080	0.510 ± 0.602	0.346	0.005–2.676	0.159 ± 0.199	0.102	0.027–1.080	0.861 ± 1.140	0.584	0.010–5.694
Periodontitis (n = 24)	0.052 ± 0.011	0.055	0.030–0.070	0.773 ± 0.521	0.623	0.121–1.905	0.051 ± 0.025	0.049	0.021–0.126	0.678 ± 0.408	0.541	0.162–1.372
Implant												
Noninflamed (n = 20)	0.039 ± 0.008	0.037	0.030–0.056	0.167 ± 0.209	0.067	0.016–0.607	0.128 ± 0.080	0.101	0.03–0.320	0.323 ± 0.317	0.203	0.017–1.017
Inflamed (n = 22)	0.054 ± 0.017	0.053	0.024–0.106	0.371 ±0.329	0.307	0.028–1.052	0.135 ± 0.093	0.110	0.035–0.382	0.615 ± 0.392	0.549	0.083–1.189
		χ^2 = 20.005			χ^2 = 23.496			χ^2 = 47.668			χ^2 = 8.767	
		P = .0001			*P* = .0001			*P* = .0001			*P* = .067	

Table 1 continued Analysis of Clinical Parameters and Nitrite and MPO Levels of Natural Teeth and Dental Implants Grouped by State of Inflammation

	PI		PD		GBTI		GCF/PISF volume		Total nitrite level (nmol)		Total MPO level (U)		Nitrite concentration (nmol/µL)		MPO	
	z	*P*	*z*	*P*	*z*	*P*	*z*	*P*	*z*	*P*	*z*	*P*	*z*	*P*	*z*	*P*
Tooth																
Healthy vs gingivitis	−3.258	.001*	−2.014	.044*	−4.337	.0001*	−4.210	.0001*	−1.044	.297	−1.435	.151	−4.020	.0001*	NS	NS
Healthy vs periodontitis	−5.256	.0001*	−5.432	.0001*	−5.702	.0001*	−5.302	.0001*	−0.318	.751	−3.692	.0001*	−5.190	.0001*		
Gingivitis vs periodontitis	−2.865	.004*	−6.218	.0001*	−4.591	.0001*	−4.030	.0001*	−0.057	.955	−2.400	.0160*	−4.086	.0001*		
Implant																
Noninflamed vs inflamed	−4.446	.0001*	−0.663	.507	−4.279	.0001*	−1.045	.296	−3.200	.001*	−2.245	.0250*	−0.403	.687	NS	NS
Healthy/noninflamed																
Tooth vs implant	−1.604	.109	−1.709	.088	−1.169	.242	−3.408	.0001*	−2.787	.005*	−0.776	.4380	−4.011	.0001*	NS	NS
Gingivitis/inflamed																
Tooth vs implant	−0.300	.764	−0.411	.681	−1.359	.174	−0.292	.771	−0.141	.888	−0.367	.7130	−0.141	.888	NS	NS

Table 2 Impact of the Severity of Inflammation on Laboratory Measures

	PI			PD			GBTI			GCF/PISF volume		
	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max
Tooth												
Periodontal health (n = 16)	0.13 ± 0.342	0.00	0.00–1.00	1.50 ± 0.632	1.00	1.00–3.00	0.06 ± 0.250	0.00	0.00–1.00	0.179 ± 0.112	0.140	0.03–0.44
Slight gingivitis (n = 16)	0.75 ± 0.775	1.00	0.00–2.00	1.81 ± 0.750	2.00	1.00–3.00	0.81 ± 0.655	1.00	0.00–2.00	0.483 ± 0.329	0.325	0.05–1.15
Moderate/severe gingivitis (n = 11)	1.09 ± 0.831	1.00	0.00–2.00	2.09 ± 0.539	2.00	1.00–3.00	1.45 ± 0.688	2.00	0.00–2.00	0.809 ± 0.433	0.710	0.16–1.45
Implant												
Noninflamed (n = 20)	0.00 ± 0.00	0.00	0.00–0.00	1.80 ± 0.768	2.00	0.00–3.00	0.20 ± 0.410	0.00	0.00–1.00	0.440 ± 0.264	0.350	0.11–0.96
Slightly inflamed (n = 11)	0.55 ± 0.522	1.00	0.00–1.00	2.00 ± 0.632	2.00	1.00–3.00	0.91 ± 0.831	1.00	0.00–2.00	0.471 ± 0.407	0.420	0.13–1.54
Moderate/severely inflamed (n = 11)	1.36 ± 0.809	2.00	0.00–2.00	2.00 ± 0.632	2.00	1.00–3.00	1.91 ± 0.701	2.00	1.00–3.00	0.693 ± 0.378	0.630	0.28–1.36

*Statistically significant ($P < .05$). The relationship between healthy/noninflamed, slightly inflamed, and moderately/severely inflamed sites was analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test with the Bonferroni correction.

Table 2 continued Impact of the Severity of Inflammation on Laboratory Measures

	Total nitrite level (nmol)			Total MPO level (U)			Nitrite concentration (nmol/μL)			MPO concentration (U/μL)		
	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max	Mean ± SD	Median	Min–Max
Tooth												
Periodontal health (n = 16)	0.050 ± 0.010	0.050	0.034–0.067	0.217 ± 0.229	0.113	0.007–0.072	0.433 ± 0.386	0.331	0.090–1.683	1.469 ± 1.639	0.916	0.060–4.800
Slight gingivitis (n = 16)	0.53 ± 0.009	0.053	0.030–0.080	0.263 ± 0.321	0.162	0.005–1.206	0.199 ± 0.244	0.154	0.040–1.080	0.519 ± 0.492	0.434	0.010–1.550
Moderate/severe gingivitis (n = 11)	0.53 ± 0.007	0.050	0.030–0.060	0.825 ± 0.736	0.601	0.020–2.670	0.099 ± 0.089	0.081	0.020–2.350	1.295 ± 1.560	0.0727	0.040–5.690
Implant												
Noninflamed (n = 20)	0.039 ± 0.008	0.037	0.030–0.056	0.167 ± 0.209	0.067	0.016–0.607	0.128 ± 0.800	0.101	0.030–0.320	0.323 ± 0.317	0.203	0.017–1.017
Slightly inflamed (n = 11)	0.051 ± 0.009	0.053	0.030–0.060	0.196 ± 0.165	0.137	0.028–0.476	0.177 ± 0.113	0.141	0.030–0.380	0.474 ± 0.333	0.473	0.080–1.160
Moderate/severely inflamed (n = 11)	0.056 ± 0.023	0.052	0.020–0.100	0.529 ± 0.366	0.540	0.040–1.050	0.093 ± 0.039	0.080	0.040–0.170	0.742 ± 0.413	0.772	0.100–1.180

Table 2 continued Impact of the Severity of Inflammation on Laboratory Measures

	PI		PD		GBTI		GCF/PISF volume		Total nitrite level (nmol)		Total MPO level (U)		Nitrite concentration (nmol/μL)		MPO	
	z	*P*	*z*	*P*	*z*	*P*	*z*	*P*	*z*	*P*	*z*	*P*	*z*	*P*	*z*	*P*
Tooth																
Healthy vs slight gingivitis	−2.655	.008*	−1.221	.222	−3.583	.0001*	−3.262	.001*	−0.944	.345	0.0001 > .99		−3.241	.001*	−1.539	.124
Healthy vs moderate/severe gingivitis	−3.276	.001*	−2.386	.017*	−4.342	.0001*	−4.000	.0001*	−0.815	.415	−2.682	.007*	−3.652	.0001*	−0.109	.913
Slight gingivitis vs moderate/severe gingivitis	−1.077	.281	−1.099	.272	−2.271	.023*	−2.098	.036*	−0.692	.489	−2.682	.007*	−1.974	.048*	−1.642	.101
Slight gingivitis vs periodontitis	−3.079	.002*	−5.389	.0001*	−4.816	.0001*	−4.363	.0001*	−0.110	.912	−3.571	.0001*	−4.307	.0001*	−1.241	.215
Moderate/severe gingivitis vs periodontitis	−1.486	.137	−4.825	.0001*	−2.688	.007*	−1.973	.049*	−0.249	.803	−0.071	.943	−2.150	.032*	−1.350	.177
Implant																
Noninflamed vs slightly inflamed	−3.618	.0001*	−0.549	.583	−2.638	.008*	−0.206	.836	−2.872	.004*	−1.035	.301	−1.239	.215	−1.369	.171
Noninflamed vs moderately/severely inflamed	−4.663	.0001*	−0.549	.583	−4.673	.0001*	−1.920	.055	−2.375	.018*	−2.667	.008*	−0.578	.563	−2.233	.026
Slightly inflamed vs moderately/severely inflamed	−2.414	.016*	0.0001 > .99		−2.534	.011*	−1.576	.115	−0.033	.974	−1.960	.050	−1.806	.071	−1.470	.142
Slight gingivitis/slightly inflamed																
Tooth vs implant	−0.571	.568	−0.732	.464	−0.268	.789	−0.395	.693	−0.099	.921	−0.126	.900	−0.197	.844	−0.252	.801
Moderate/severe gingivitis/moderately/severely inflamed																
Tooth vs implant	−0.812	.417	−0.359	.719	−1.353	.176	−0.755	.450	−0.131	.895	−0.775	.439	−0.624	.533	−0.563	.573

any of the laboratory parameters between inflamed GCF and PISF samples, total nitrite level demonstrated a pattern of increase in GCF from healthy natural tooth sites compared to PISF from noninflamed implant sites. The impact of the severity of inflammation on laboratory measures, together with descriptive statistical analysis and actual *P* values, is shown in Table 2. Despite a trend of increase in clinical parameters and the severity of inflammation at moderately/severely inflamed sites, GCF total nitrite levels stayed stable and were not influenced by the severity of clinical inflammation. A similar pattern of increase was evaluated in GCF total MPO levels; sites with moderate/severe inflammation provided more GCF MPO than slightly inflamed sites. GCF nitrite concentration decreased with the increased severity of inflammation compared to healthy sites and presence of periodontal breakdown ($P < .005$), but differences in MPO concentration were not significant ($P > .05$). At dental implant sites, higher PI and GBTI scores were observed at moderately/severely inflamed sites than both the clinically noninflamed sites ($P = .0001$). Total nitrite levels presented a trend of increase in the inflamed sites compared with the clinically noninflamed dental implant sites, while slightly inflamed sites demonstrated a significantly increased total nitrite level compared to noninflamed peri-implant sites ($P = .004$). However, no significant difference was observed between slightly inflamed and moderately/severely inflamed sites ($P > .05$). Furthermore, total MPO levels tended to increase with the severity of clinical inflammation. Analysis for concentration mode of data presentation was mostly nonsignificant for both laboratory measures (MPO and nitrite level. No statistical significance could be observed when GCF from natural tooth sites and PISF from dental implant sites were compared for their nitrite and MPO content either at slightly inflamed and moderately/severely-inflamed sites ($P > .05$). Moreover, a significant correlation ($P < .05$) was found between total MPO level and MPO concentration at natural tooth and dental implant sites (healthy, inflamed, and periodontitis). However, no correlation was found for total nitrite level and nitrite concentration at natural tooth and dental implant sites. Furthermore, total MPO and nitrite levels were not significantly correlated.

DISCUSSION

In addition to the examination of clinical parameters, quantitative and qualitative analysis of GCF samples is an accepted method for the evaluation of early and destructive stages of inflammatory periodontal diseases around natural teeth, while PISF analysis may serve for further clarification of the biologic mechanisms around dental implants and the pathophysiology of peri-implant diseases.[1,2,8–10,24,41] There is the possibility that these 2 biological fluids may share much in terms of their diagnostic potential and their involvement in the pathogenesis of periodontal or peri-implant disorders.[1,2,8–10,24,41] The volumetric findings of the present study support the idea of such a similarity; both the GCF and PISF volumes exhibited clear increases at diseased sites compared to clinically healthy sites, in addition to the similarities between the sites with respect to clinical peri-implant or periodontal parameters. Further, despite the higher PISF volume at noninflamed sites, no significant difference between PISF and GCF volume could be observed at inflamed sites. Thus, it seems that that PISF and GCF share similar volumetric features in their local response to existing clinical inflammation.

It is clear that molecular changes around natural teeth and/or load-bearing dental implants are complex, and a close relationship has been demonstrated between inflammatory status and the molecular pathophysiology.[1,2,9,10] This, in fact, seems to be the main reason for the great interest in GCF and PISF and their potential changes with inflammation.[1,2,8–10,24,41,42] The lack of significant correlations between certain laboratory measures and clinical parameters has been attributed to the lack of a correlation between time of GCF profiling and clinical status.[31,43,44] The relatively "early" nature of such laboratory measures (eg, enzymatic changes in GCF profile), which precede clinically detectable changes, has also been suggested.[44,45] Thus, analysis of various laboratory-based parameters (eg, biochemical measures), rather than clinical parameters, may serve for the development of reliable diagnostic tests for early detection of periodontal inflammation.[31,43,44] In addition to these, the significant limitations of most clinical parameters (eg, having subjective elements, reflecting only past events, providing limited information on actual disease status, the potential for missing early signs of disease, lack of 100% specificity and sensitivity, having limited prognostic value) continue to give rise to laboratory-based studies that aim to overcome these limitations.[31,45]

MPO is demonstrated to be a significant ingredient of GCF and to be involved in the pathogenesis of inflammatory periodontal diseases.[2,24,46] The vast majority of previous studies demonstrate the close association of MPO activity with the clinical and microbial signs of periodontal disease.[2,24,46,47] Increased GCF MPO levels have been shown at sites with gingivitis and chronic and aggressive periodontitis.[24,46–50] A sig-

nificant decrease in GCF MPO activity following successful periodontal treatment has also been observed.[49,50] Polymorphonuclear leukocytes that accumulate at sites of gingival 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 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.[24,46–50]

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.[1,9] 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.[1] 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.[9] The present study also revealed that inflamed peri-implant sites demonstrated a pattern of increased total MPO level compared to 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.

NO has been considered an important molecular signal in a wide variety of tissues and may play a significant role as a cytotoxic mediator of the nonspecific immune response, with beneficial and harmful effects.[14–16] Inducible NO synthase (iNOS) is expressed in response to inflammatory stimuli, resulting in higher amounts of NO production, and much of the nitrite of body fluids is formed from oxidation of NO produced by iNOS.[15,16] Increased numbers of iNOS-positive cells have been demonstrated in periodontally diseased tissues.[17,19,51,52] Because of the reactivity of NO and its short life, direct measurement of NO from body fluids has been thought hard to perform.[53] Thus, nitrite, a stable end-product of NO oxidation, has been measured instead.[10,53] In the present study, using Greiss reactions, increased NO metabolism with peri-implant inflammation, represented by higher PISF nitrite levels at inflamed dental implant sites, compared to healthy ones was observed in patients with edentulous mandibles rehabilitated with overdentures with ball attachments supported by 2 implants.[10] The finding of an increase in PISF NO metabolism at inflamed sites is in line with the previously reported results of a comparative analysis of PISF nitrite levels at inflamed and noninflamed peri-implant sites in subjects with implant-supported fixed prostheses.[10]

Based on the previously-demonstrated discrepancy between "concentration" and "total activity" modes of data presentation for GCF,[24,54] in the present study it was not surprising to observe that these 2 modes of data presentation for GCF were not completely correlated. The same discrepancy was also observed for PISF samples.[10] 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 2 modes of data presentation suggests the volume-dependent nature of the concentration expression.[10,24,54] 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.[10,24,54]

Although a detectable amount of nitrite was available at all GCF and PISF samples, MPO was not detectable at 13% of 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,[22] 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 to nitrite level. Further, NO metabolism may be affected by force and loading.[10,55,56] 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 (eg, a complete mandibular prosthesis supported by a ball attachment[10]) may affect NO production at dental implant sites and the subsequent PISF nitrite levels.

In the present study, 2 biological fluids, PISF and GCF, were comparatively analyzed. Although the results may shed light on the features the 2 fluids

share and the diagnostic potential of PISF, 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 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 2 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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