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Effect of a structured plaque control on MMP-1 and MMP-9 crevicular levels in patients with desquamative gingivitis associated with oral lichen planus.

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ABSTRACT

Objectives: No data are available in the literature on the extent to which the immune host-response and bacterial-elicited inflammation separately contributes to the increase in gingival crevicular fluid (GCF) levels of inflammatory biomarkers in patients affected by desquamative gingivitis (DG) secondary to oral lichen planus (OLP). The aim of this study was to investigate the effect of a structured plaque control intervention on GCF levels of MMP-1 and MMP-9 in OLP patients with DG and to compare them with those of non-OLP patients.

Materials and Methods: The study population consisted of 18 unrelated Caucasian patients with DG, while 18 periodontally healthy subjects were recruited for the control group. Periodontal parameters and GCF biomarker amounts were evaluated at baseline and 2 months after a structured plaque control intervention, comprising professional oral hygiene sessions, manual toothbrushing and interdental cleaning advice, only for DG patients. Determination of MMP-1 and MMP-9 levels was carried out by means of an enzyme-linked immunosorbent assay.

Results: Plaque control program led to improvement in all examined clinical parameters and resulted in significant decrease in GCF total amount and concentration of MMP-1 and MMP-9 in comparison to baseline ($p < 0.001$). However, MMP-1 and MMP-9 levels in DG patients were still significantly higher than those in the healthy control group ($p < 0.01$).

Conclusions: These findings would seem to support an intrinsic up-regulated expression of MMPs in DG patients that is exacerbated by bacterial plaque.

Clinical relevance: The present outcomes provide further scientific grounds for the importance of strict professional oral hygiene sessions in DG patients.

Keywords: desquamative gingivitis; MMP-1; MMP-9; oral lichen planus; plaque control.

Introduction

Chronic desquamative gingivitis (DG) manifests mainly as erythema, epithelial desquamation, atrophy, painful erosions or ulceration of the marginal and attached gingiva, unrelated to, but aggravated by, local plaque accumulation [1, 2]. The majority of cases of DG are expression of mucocutaneous conditions, in particular oral lichen planus (OLP) and mucous membrane pemphigoid (MMP) [2, 3]. OLP is a relatively common T-cell mediated inflammatory disease of unknown aetiology and has several morphological forms including reticular, erosive, papular, vesiculo-bullous, and atrophic/erythematous [4, 5]. The erosive and atrophic forms of OLP present often gingival involvement giving the classical appearance of DG. Histologically, the alterations in the basal cell layer of the epithelium and the disruption of the underlying basal membrane are central to lesion development [6]. Basal membrane degeneration may be mediated by matrix metalloproteinases (MMP), a family of zinc-dependent endopeptidases responsible for tissue remodelling and degradation of connective tissue matrix proteins in normal and pathological inflammatory processes [7-9]. It has been reported that expression of MMP-1 and MMP-9 is up-regulated in gingival tissues from OLP patients compared to healthy controls suggesting a role in OLP pathogenesis [10-13]. MMP-1 degrades fibrillar collagen in the extracellular space and MMP-9 acts on type IV collagen (gelatin), which is the major structural component of basement membrane [14]. They are released by activated T-lymphocytes within the gingival epithelium but also by gingival fibroblasts and neutrophils in response to bacterial infection [15,16]. In sites with plaque-induced gingivitis as well as during active and progressing phases of periodontitis, MMP-1 and MMP-9 levels in the

GCF are significantly elevated [17-19].

It is well known that painful gingival lesions may impede OLP patients to maintain proper oral hygiene, favouring dental plaque accumulation and onset of typical gingival inflammation in the areas closer to the DG lesions [20-22]. To the best of our knowledge, it is unclear to what extent the immune host response and the bacterial-elicited inflammation separately contributes to the increase in gingival crevicular fluid (GCF) levels of inflammatory biomarkers in OLP patients with DG [12].

Therefore, the primary aim of this study was to investigate the effect of a structured plaque control intervention on the GCF levels of MMP-1 and MMP-9 in OLP patients with DG, and to compare them with those of non-OLP patients with clinically healthy periodontium; the secondary aim was to analyse the impact on the clinical outcomes.

Material and methods

Study design

This longitudinal study was conducted at the University of Turin, C.I.R. Dental School, Department of Surgical Sciences, from January to July 2017. The protocol complied with the rules of the Declaration of Helsinki of 1975, as revised in 2002, and was approved by the Institutional Ethics Committee of the "A.O.U. Città della Salute e della Scienza", Turin, Italy (no. Approval 0058273).

Participants were recruited consecutively from the general clinic population and from patients attending the Oral Medicine Section of the C.I.R. Dental School for the management of their dental or oral conditions. Written informed consent was obtained from each participant before enrolment.

Participants in the DG group had clinical and histological diagnosis of OLP according to the WHO criteria [23], with the presence of the following microscopic features: hyperkeratosis, varying thickness of the epithelium, a subepithelial lymphocytic band-like infiltrate, and vacuolar degeneration of the basal layer. Patients with histological signs of dysplasia or receiving treatment with corticosteroids (topical or systemic) in the 3 months

prior to the study were excluded.

Participants in the healthy control group (H) had good general health with no history or signs of DG, and no other oral signs related to OLP or to any other autoimmune mucocutaneous disease. Since OLP most commonly occurs in middle-aged adults, they were required to be > 40 years old [24]. In addition, they had to present bleeding on probing (BoP) at <20% of sites, probing depth (PD) ≤ 3 mm and no attachment loss caused by periodontal destruction [25].

Exclusion criteria for the both groups were as follows: <15 teeth, pregnancy, lactation, previous or current smoking, periapical pathology, continuous use of any mouthrinse for plaque control, intake of cyclosporine or calcium channel blockers, periodontal treatment or/and the use of antibiotics or anti-inflammatory medications within the previous 3 months.

Clinical Examination

All the study participants received at baseline (T0) an oral examination together with a comprehensive periodontal examination performed by two calibrated and experienced clinicians (P.G.A, F.R.) as previously described [3].

A total of 8 *non-study* subjects were recruited for the calibration of the examiners. The examiners were judged to be reproducible after meeting a percentage of agreement within 1 mm between repeated measurements of at least 95%. There was a 95.4% of concordance within 1 mm for measurements of PD and a 96.1% for clinical attachment level (CAL) between the examiners.

Clinical parameters assessed were number of teeth, presence of plaque (PI), presence of BoP, gingival index (GI) [26], PD, gingival recession and CAL. Finally, the full-mouth percentage of sites with PI (full-mouth plaque score, FMPS) and BoP (full-mouth bleeding score, FMBS) was calculated. The periodontal parameters were assessed at six sites per tooth, excluding third molars, by means of 1-mm marked periodontal probe (PCP UNC15, Hu-Friedy, Chicago, IL, USA).

The desquamative gingivitis clinical score (DGCS) [27], including the extent and the

severity of the gingival lesion, were also detailed for DG patients. They also detailed their pain perception by means of a 10-cm horizontal visual analog scale (VAS) and answered a questionnaire evaluating the impact of the disease on their quality of life. The 14-item Italian version of the Oral Health Impact Profile (OHIP-14) was used [28], in a version modified according to Salgado and co-workers [22] in which the words 'mouth' and 'teeth' were replaced by 'gingiva'. Participants were asked to rate each of the responses on a 5-point Likert scale. Responses were coded 0 (never), 1 (hardly ever), 2 (occasionally), 3 (fairly often) and 4 (very often). The OHIP was self-administered but checked for completeness.

Eight weeks after baseline (T1) clinical and patient-related outcomes were recorded again only for DG patients.

Clinical protocol

After baseline examination, all DG participants were submitted to a careful session of supragingival scaling in order to avoid injuries to the gingival tissue and received structured oral hygiene instructions by one experienced dental hygienist (E.C.). Patients were instructed to perform carefully the modified Bass technique procedures using soft-bristle toothbrush (GUM Technique PRO Soft 525, Sunstar, Saronno, VA, Italy) and fluoridated toothpaste without sodium lauryl sulphate, and inter-proximal cleaning with either appropriately sized extra soft inter-dental brushes (TePe Munhygienprodukter, Malmo, Sweden) or dental floss (Oral-B, Procter & Gamble, Weybridge, UK), according to the individual needs. The brushing time was set for 3 min, and frequency was twice a day. The interdental devices had to be used once daily. All products were freely provided for the complete duration of the study. During the study period, the use of mouthrinse was prohibited. Patients were recalled at 1, 2, 3, 4 and 8 weeks for reinforcement in oral hygiene instructions to obtain the most appropriate and non-traumatic daily plaque control procedures and polishing. No periodontal intervention or brushing instructions were carried out in the healthy subjects, which was included just as a control group for biochemical comparisons.

GCF sampling

In DG patients GCF samples were collected from two inflamed sites with evidence of DG lesions (sites with redness or BoP, PD of ≤ 3 mm) and two periodontally healthy sites on the mesial aspect of anterior teeth. The same sites were sampled at baseline and T1. In the H group two sites with no bleeding and plaque and PD ≤ 3 mm (control sites) were sampled on the mesiobuccal aspect of anterior teeth as a control for biochemical comparison. Sites to be sampled were isolated with cotton after removing the supragingival plaque and the crevicular area was gently dried with air syringe. GCF samples were collected by inserting paper strips (PerioPaper Strips, Oraflow Inc., Plainview, NY, USA) into the sulcus for 30''. Strips contaminated by bleeding were discarded. The amount of collected GCF was measured using an electronic device Periotron 8000 (Oraflow Inc., Plainview, NY, USA), which was calibrated based on a protocol previously reported [29]. The strips were placed into coded sealed Eppendorf tubes containing 350 µl of sterile phosphate-buffered saline (PBS). After 1 h at room temperature, the strips were removed, and the eluates centrifuged at 6000 g for 5'. The supernatant was stored at -80°C until further analysis.

MMP-1 and MMP-9 assay

Biochemical analysis was performed by a blinded examiner (M.M.) at the Department of Clinical and Biological Sciences, University of Turin (Italy). The GCF samples were assayed for MMP-1 and MMP-9 levels using commercially available ELISA kits (Thermo Fisher Scientific, Frederick, MD, USA and Invitrogen CA, USA) according to the manufacturer's instructions. Standards in the commercial kit were diluted according to the manufacturer's directions, and GCF samples were added to wells coated with MMP-1-, and MMP-9- specific antibodies. Stop solution was added to each well, and the absorbance values were determined by a spectrophotometric ELISA-Reader (Microplate Reader, Biorad) at an optical density of 450 nm. MMP-1 and MMP-9 determinations were carried out in duplicate for each sample from the standard curve and expressed as total amount (pg) and concentration (pg/ml).

Statistical analysis

A statistical software program (SAS, USA) was used for data analysis. Data were first examined for normality by the Kolmogorov-Smirnov test, and if the data did not achieve normality, analyses were performed using non-parametric methods. The Wilcoxon test or the paired t test was employed to detect statistically significant clinical and MMP differences within DG group before and after the plaque control program.

Differences between DG and H groups were tested using the unpaired t test or Mann-Whitney U test for quantitative variables, Chi-square or Fisher exact test for qualitative variables, as appropriate. The comparisons of MMP levels between H and DG groups at baseline or at 2 months post-therapy were performed using the Kruskal-Wallis test, followed by post-hoc Dunn test. The significance level for all analyses was set at 5%.

Results

Thirty-two OLP patients with DG and twenty-nine healthy control subjects were consecutively screened for enrolment. Twenty patients did not meet the inclusion criteria and five patients did not attend the baseline examination. Finally, 18 DG subjects (five males and thirteen females, mean age 61.5 ± 11.5 yrs) were enrolled and completed the trial. The control group included seven males and eleven females with a mean age of 56.5 ± 5.5 yrs. The two groups were balanced for age ($p = 0.11$) and gender distribution ($p = 0.48$).

Determination of GCF mediator levels

At baseline the total amounts and concentrations of MMP-1 and MMP-9 were increased in DG patients in both diseased ($p < 0.001$) and healthy sites ($p < 0.01$), compared to H individuals. As reported in Figures 1 and 2, at the end of the oral hygiene protocol the MMP-1 and MMP-9 activity in diseases sites (total amount and concentration) was no longer statistically different from healthy sites in DG patients, but it was still significantly higher than in the H group ($p < 0.01$).

Clinical parameters

Clinical parameters of sites selected for GCF sampling in both H and DG groups are presented in Table 1. According to the inclusion criteria, there was no PI or BOP at healthy sites in DG patients and in control sites in H individuals, while higher levels of PI and BOP were found in diseased sites in DG patients at baseline. Means of PD did not differ from each other. At T1, diseased sites exhibited a statistically significant decrease in all clinical parameters when compared to baseline. The GI scores and GCF volumes were comparable to those in healthy sites, while a statistically mild significant difference was found in PI values ($p = 0.015$).

At subject level, as summarized in Table 2, the plaque control program led to a statistically significant decrease in the overall mean examined clinical parameters in the DG group. Reduction in FMBS (mean change: 39.2; CI: 28.5 to 29.2; $p < 0.001$), FMPS (mean change: 46.3; CI: 37.9 to 54.7; $p < 0.001$) and DGCS (mean change: 1.9; CI: 0.6 to 3.2; $p = 0.004$) was observed compared to baseline values. Moreover, DG patients experienced a statistically significant improvement in VAS scores ($p = 0.001$) and OHIP-14 sum scores ($p < 0.001$), as summarized in Table 3.

Discussion

The current body of literature lacks controlled studies that have investigated the influence of plaque accumulation on the severity of gingival manifestations and GCF levels of MMP-1 and MMP-9 in patients affected by DG secondary to OLP. To the best of the authors' knowledge, this is the first study addressing this issue. The control of plaque-induced inflammation may help to clarify the effects of immunologic mechanisms on gingival tissues in OLP.

In the present study, patients were submitted to a weekly plaque control in the first month and then monthly for a period of 2 months, and they were also instructed to use the modified Bass technique and a soft-bristle toothbrush with individual-tailored interdental devices, that would cause minimal injury to the marginal tissue. The establishment of an intensive plaque control program led to a statistically significant change in biomarker levels and degree of clinical inflammation in OLP lesions.

It has been widely demonstrated that the pathogenesis of plaque-related periodontal disease involves a local inflammatory reaction and the activation of the immune system stimulated by bacterial factors [18]. Current evidence supports that immune-inflammatory mechanisms are also critical for the pathogenesis of OLP-associated disorders, which involves common cytokine networks and inflammatory mediators with MMP-1 and MMP-9 being key molecules [1, 13, 14].

In the current study we selected, in the same patient, sites where DG lesions were present and unaffected sites, and compared them each other and with periodontally healthy sites of a control group without OLP. This makes it possible to characterize the subject-based biochemical inflammatory response to plaque accumulation [30]. The MMP comparison was based on total amount and concentration in GCF. As stated by Duarte and co-workers [31], data based only on total amount of biomarkers should be interpreted with caution, as the total amount of cytokine/MMP may be an obvious consequence of the GCF volume sampled.

We found that, compared with healthy control group, DG patients demonstrated at baseline significantly higher GCF total amount and concentration of MMP-1 and MMP-9 in both affected and unaffected sites. After the plaque control program, along with improvement in clinical parameters, the levels of both inflammatory mediators decreased in DG sites and were not significantly different from sites where DG lesions were absent. Anyway, they remained higher than those of healthy subjects. These findings would seem to support an intrinsic up-regulated expression of MMPs in OLP patients. As reported in the literature, epithelial basal membrane degradation during OLP progression may be mediated by activated MMPs. In particular, MMP-1 and MMP-9 cleave fibrillar collagen and type IV collagen, which is the major component of the basal membrane [10-14]. Zhou and co-workers reported that T-cells from OLP lesions secreted more MMP-9 than control T-cells, suggesting a central role for MMP-9 in OLP pathogenesis [15]. T-lymphocytes produced MMP-9 may start the basal membrane degradation, afterward epithelial cells keeping contact with a structurally incomplete basal membrane may produce MMP-1,

which further amplifies MMP-9 activity [8].

Recently, it has been observed an increased level of MMP-1 and MMP-9 and a decreased level of the enzyme inhibitor TIMP-1 in the GCF and in gingival tissue samples of OLP patients with gingivitis and periodontitis when compared with non-OLP controls [13]. They questioned the role of OLP itself as the primary cause of increased MMP levels [13].

In the present study, MMPs were found to rise in response to plaque accumulation, suggesting that bacterial plaque may enhance extracellular matrix degradation and basal membrane disruption in OLP. Previous studies demonstrated that increased TNF-alpha and IL-1beta in GCF of inflamed gingiva stimulate activated T-cells to produce higher amount of MMP-1 and MMP-9, without altering TIMP levels [32, 33].

The structured plaque control program led also to a statistically and clinically significantly improvement in the plaque and gingival bleeding index and in the clinical symptoms of the OLP lesions, as demonstrated by the reduction in the DGCS values in terms of extension and severity of the gingival lesions. In a Cochrane systematic review and meta-analysis, Sambunjak and co-workers (2011) estimated that dental flossing when combined with toothbrushing could significantly reduce mean gingival bleeding scores at 1 month [34]. The present data are also in line with those of previous studies, demonstrating the central role of a meticulous and not traumatic bacterial plaque removal in controlling the severity and the painful symptoms of the gingival manifestations of OLP [20, 22, 35-37]. However, in these studies, mechanical plaque control was supplemented with chlorhexidine 0.12% mouthrinse [22] or topical corticosteroid therapy [36] or both [35], and patients were recalled weekly for 1-month period [22], monthly for 2-3 months [35, 36], or every 3 months for 1 year [20]. According to the above-mentioned studies, patients also reported improvement in oral health-related quality of life [22, 37]. The present findings provide additional support to the importance of strict professional oral hygiene sessions together with proper home oral hygiene measures reinforced constantly in OLP patients with DG.

Limitation of the present study could be the restricted sample size. However, it is important to point out that OLP is a quite rare inflammatory mucocutaneous disease, affecting about

1% of the population, and DG manifestations occur only in less than half of those patients [38]. Advantage is the study design, which allowed for a comparison of the host inflammatory reaction between different sites within the same subjects.

Conclusions

In this longitudinal study, a structured plaque control regimen was effective in improving clinically observed manifestations of OLP and the oral-health quality of life and resulted in significant decrease in the GCF levels of MMP-1 and MMP-9. However, MMP-1 and MMP-9 levels were found still higher in the OLP patients compared to healthy controls. This study may suggest an overexpression of such destructive enzymes in DG sites and provides further evidence that bacterial plaque stimulates MMPs secretion and may contribute to extracellular matrix degradation. Intensive plaque control should, therefore, become an important phase of treatment of OLP. It is known that the oral mucosa and gingiva may be the only site of involvement of this immune disease. Nevertheless, future larger prospective studies could possibly give more valuable information.

Compliance with ethical standards

Conflicts of interest. The authors declare that they have no conflict of interest.

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Ethical approval. All procedures involving humans were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent. Informed consent was obtained from all individual participants included in the study.

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Table 1. Clinical parameters [mean ± SD (median; interquartile range)] of GCF sampling sites over the experimental period in OLP patients with DG and healthy controls.

	Healthy controls (*n* = 18)	DG patients (*n* = 18)		
	Healthy sites	Healthy sites	Diseased sites	
		Baseline	Baseline	2 months
PI	0 (0.0; 0.0)	0 (0.0; 0.0)	2.1 ± 0.4 (2.0; 0.6)[a]	0.3 ± 0.3 (0.0; 0.5)[b]
GI	0 (0.0; 0.0)	0.0 ± 0.1 (0.0; 0.02)	1.9 ± 0.6 (2.0; 0.5)[a]	0.2 ± 0.2 (0.0; 0.5)[b]
PD (mm)	1.8 ± 0.5 (2.0; 0.63)	1.9 ± 0.6 (2.0; 0.5)	2.2 ± 0.8 (2.0; 1.5)	2.0 ± 0.5 (2.0; 1.0)[b]
GCF (µl)	0.18 ± 0.05 (0.17; 0.06)	0.25 ± 0.12 (0.24; 0.16)	0.54 ± 0.34 (0.49; 0.66)[a]	0.28 ± 0.14 (0.25; 0.22)[b]

GCF gingival crevicular fluid volume, *PI* presence of plaque, *GI* gingival index, *PD* probing depth, *SD* standard deviation.
[a]Significantly different from healthy controls, p < 0.001 (Kruskal-Wallis test and post-hoc Dunn test).
[b]Significantly different from baseline, p < 0.001 (Wilcoxon test).

Table 2. Clinical parameters (mean ± SD) at subject level (full-mouth data) in OLP patients with DG and healthy controls.

	Healthy controls (*n* = 18)	DG patients *(n* = 18)		
		Baseline (T0)	2 months (T1)	P* value T0 vs T1
FMPS (%)	13.9 ± 3.5	69.4 ± 15.7[a]	23.1 ± 9.9	< 0.001
FMBS (%)	12.0 ± 4.8	57.1 ± 24.9[a]	17.8 ± 7.7	< 0.001
PD (mm)	2.4 ± 0.4	2.9 ± 0.5[a]	2.6 ± 0.4	0.001
DGCS	-	6.8 ± 3.4	4.9 ± 2.7	0.004

FMPS full-mouth plaques score, *FMBS* full-mouth bleeding score, *PD* probing depth, *DGCS* desquamative gingivitis clinical score, *SD* standard deviation.
[a] Significantly different from healthy controls, p < 0.001 (unpaired t-test).
*Paired t test.

Table 3. OLP patients' experience in terms of pain and quality of life [mean ± SD (median; interquartile range)] before and after the structured plaque control.

	Baseline (T0)	2 months (T1)	
	Mean ± SD	**Mean ± SD**	**P* value T0 vs T1**
PAIN (VAS score)	5.5 ± 1.8 (5.0; 2.5)	2.6 ± 1.9 (2.0; 3.25)	0.001
OHIP-14	12.4 ± 7.5 (12.5; 10.75)	8.4 ± 5.5 (8.5; 6.5)	< 0.001

*Wilcoxon test

Figure Legends

Figure 1. Box-and-whisker plots showing the concentration (A) and total amount (B) of MMP-1 in gingival crevicular fluid of periodontally healthy controls and desquamative gingivitis subjects prior and following the plaque control program in sites with (DS) and without (HS) desquamative gingivitis lesions. The box represents median, 25% and 75% percentiles; the whiskers represent data within 10% and 90% percentiles. ***P < 0.001 *versus* HS and *versus* healthy controls sites. **P < 0.01 *versus* healthy controls sites. *P < 0.05 *versus* healthy controls sites.

Figure 2. Box-and-whisker plots showing the concentration (A) and total amount (B) of MMP-9 in gingival crevicular fluid of periodontally healthy controls and subjects with desquamative gingivitis prior and following the plaque control program in sites with (DG) and without (HS) desquamative gingivitis lesions. The box represents median, 25% and

75% percentiles; the whiskers represent data within 10% and 90% percentiles. ***P < 0.001 *versus* HS and *versus* healthy controls sites. **P < 0.01 *versus* healthy controls sites.

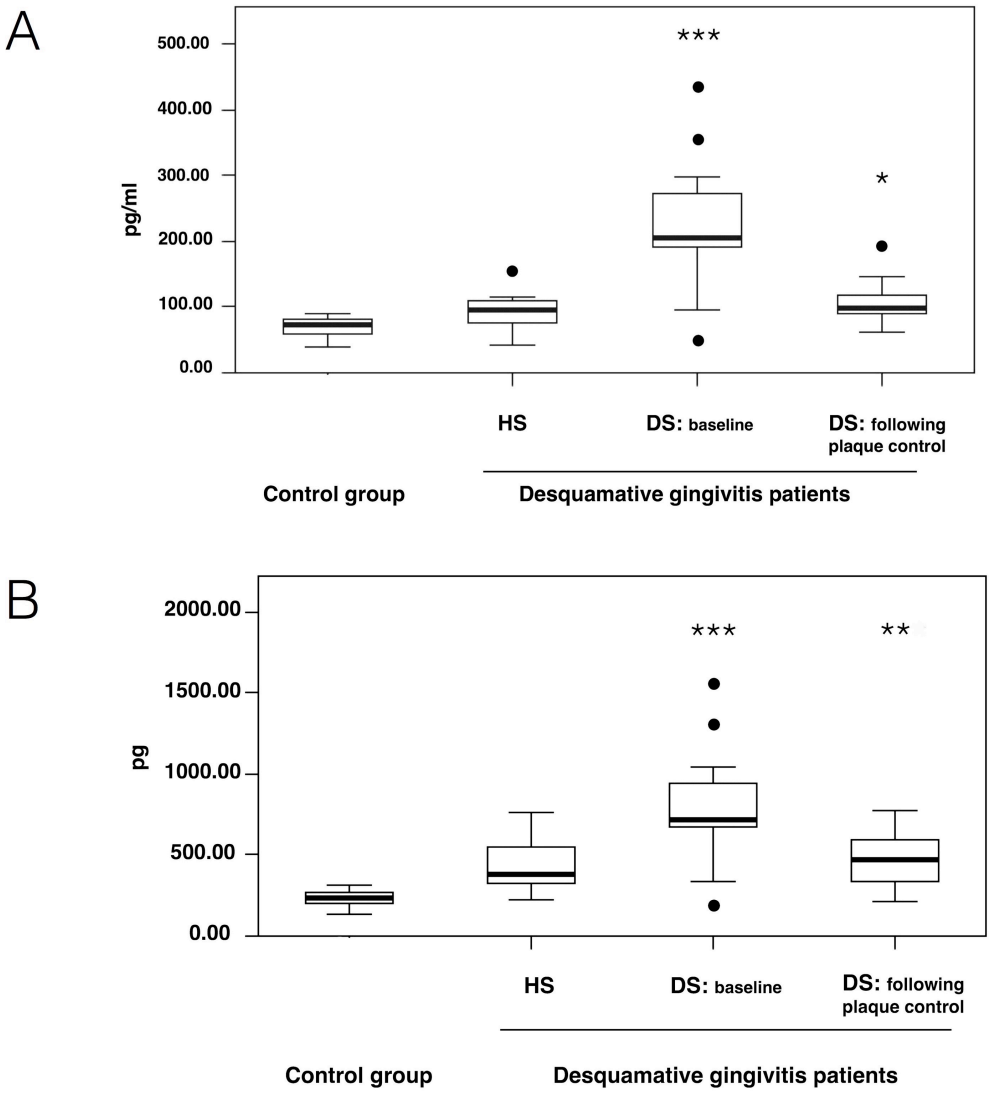
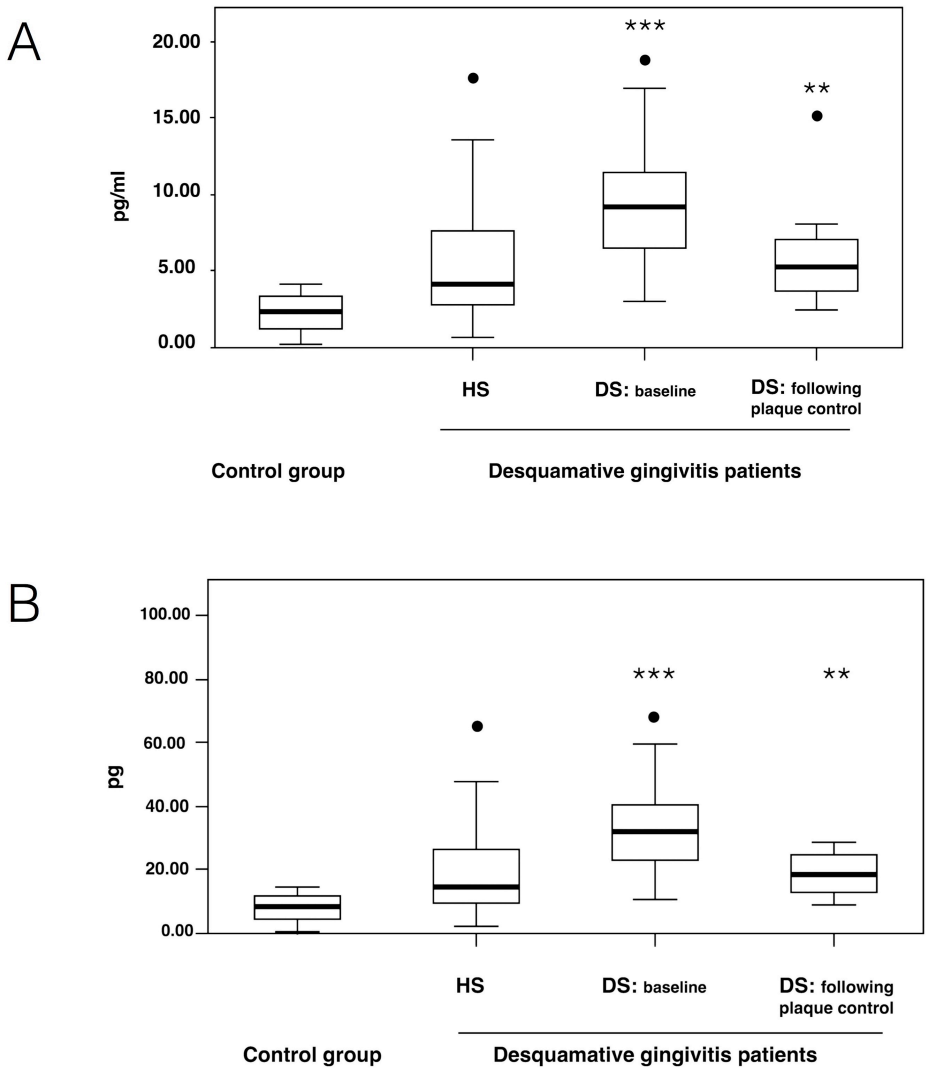


Figure 1.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**
HS
DS: baseline
DS: following plaque control
Control group
Desquamative gingivitis patients
B
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**
HS
DS: baseline
DS: following plaque control
Control group
Desquamative gingivitis patients

Figure 2.