

# Gingival crevicular fluid calprotectin, osteocalcin and cross-linked N-terminal telopeptid levels in health and different periodontal diseases

Sema Becerik<sup>a</sup>, Beral Afacan<sup>a</sup>, Veli Özgen Öztürk<sup>a</sup>, Harika Atmaca<sup>b</sup> and Gülnur Emingil<sup>a,\*</sup>

<sup>a</sup>Ege University, School of Dentistry, Department of Periodontology, Izmir, Turkey

<sup>b</sup>Celal Bayar University, School of Science, Department of Biology, Manisa, Turkey

**Abstract.** *Aim:* The aim of the present study was to investigate gingival crevicular fluid (GCF) calprotectin, osteocalcin and cross-linked N-terminal telopeptide (NTx) levels in health along with different periodontal diseases.

*Material and methods:* Twenty chronic periodontitis (CP), 20 generalized aggressive periodontitis (G-AgP), 20 gingivitis and 20 healthy subjects were included. Probing depth, clinical attachment level, plaque index and papillary bleeding index was recorded. GCF calprotectin, osteocalcin and NTx levels were analyzed by enzyme-linked immunosorbent assay (ELISA).

*Results:* CP, G-AgP and gingivitis groups had higher GCF calprotectin total amount compared to healthy subjects ( $p < 0.008$ ). CP and G-AgP groups had similar, but higher levels compared to gingivitis groups ( $p < 0.008$ ). CP and G-AgP groups had lower GCF osteocalcin total amount compared to gingivitis and healthy groups ( $p < 0.008$ ). CP group had higher GCF NTx but lower osteocalcin total amount and osteocalcin/NTx ratio than the G-AgP group ( $p < 0.008$ ).

*Conclusions:* Our results suggest that elevated GCF calprotectin levels play a role as a reliable inflammatory marker in the pathogenesis of periodontal disease. Fluctuating GCF levels of osteocalcin and NTx might point out to the abnormal bone turnover in periodontitis. Our data document for the first time the role of NTx in the pathogenesis of different periodontal diseases.

**Keywords:** Calprotectin, osteocalcin and NTx in periodontitis

## 1. Introduction

Current knowledge about the pathogenesis of periodontal disease suggests that the central cause of periodontal disease is the loss of a healthy balance between microbial virulence agents and host inflammatory response [1,2]. The immune system while protecting the host against microbial dental plaque, also participates in attacking the host. Inflammation and tissue destruction are early and continuing events during host-mediated process in response to the bacterial infection [3].

Periodontal diseases may differ in their etiological factors and pattern of progression. This variability can be attributed to differences in the presence of factors that might modify the host response to microbial pathogens. Chronic periodontitis (CP) and aggressive periodontitis, two forms of inflammatory periodontal disease, differ from each other in terms of the magnitude, sequel and control of the response [4].

The destruction of soft and hard tissues seen in periodontitis is caused by a large number of cytokines as well as due to the presence of other effector molecules released by resident and migrating cells [2,5]. Calprotectin is a 36.5-kDa calcium and zinc binding protein consisting of one light (8 kDa, MRP8) and one heavy (14 kDa, MRP14) subunit that belong to the S100 protein family [6]. Calprotectin is constitutively expressed in the cytosol of neutrophils, monocytes,

\*Corresponding author: Gülnur Emingil, DDS, PhD, Ege University, School of Dentistry, Department of Periodontology, Bornova-35100, Izmir-Turkey. Tel.: +90 232 388 1105; Fax: +90 232 388 0325; E-mail: gemingil@yahoo.com.

activated macrophages, and keratinocytes and released during activation or death of these cells. Calprotectin complex, also known as L1 antigen, calgranulin A and B, macrophage migration inhibitory factor-related protein 8 and 14 (MRP8 and MRP14), S100A8/S100A9, and cystic fibrosis antigen, has several functions in inflammatory reactions [7]. It acts as a chemotactic factor and regulates adhesion and migration of neutrophils or monocytes and is therefore considered a pro-inflammatory marker [6]. It also shows *in vitro* antimicrobial and antifungal activities against the periodontopathic bacteria as well as *Candida* strains [8, 9]. It has been previously shown that calprotectin was present in human dental calculus and suggested that it might be derived from gingival crevicular fluid (GCF), saliva and dental plaque [10]. Studies have shown that calprotectin level in GCF from periodontitis patients is higher than that from healthy subjects and positively correlated with clinical and biochemical markers of periodontal inflammation [11–14].

Bone homeostasis maintains by a coupled process of resorption followed by formation which reflect a change in bone turnover [15]. Markers of bone formation are proteins revealing osteoblast activity and are byproducts of collagen synthesis, matrix proteins or osteoblastic enzymes [16,17]. Osteocalcin is a small (5.4 kDa), calcium-binding protein of bone accounting for 10–20% of the non-collagenous protein in bone matrix. It has three residues of a calcium-binding amino acid, gamma-carboxyglutamic acid (Gla), that allow specific conformational changes enabling its binding to hydroxyapatite and later accumulation in bone matrix [18,19]. This vitamin K- and D-dependent protein produced by mature osteoblasts, osteocytes and odontoblasts, is found in the extracellular mineralized matrix of bone and in the serum of circulating blood [18, 20]. It may be involved in regulation of osteoblast function, regulation of bone turnover and/or mineralization. Markers of bone resorption, which reflect osteoclastic activity are mostly the breakdown products of type I bone collagen, the main component of the organic bone matrix [16,17]. N-terminal cross-linked telopeptide of type I collagen (NTx) is the aminoterminal peptides of mature type I collagen with the cross-links attached and is released during bone resorption [21,22]. The NTx molecule, a reliable marker for subtle changes in bone turnover, can be measured in either serum or urine and used as a marker of bone resorption in several systemic diseases [23,24].

It is well known that abnormal activation of the immune system leads to bone destruction in periodon-

tal diseases. The increased recognition of the interactions between the cells of the immune and skeletal system has led to the seeking of a variety of regulatory molecules that play a role in the progression of periodontal disease [25–27]. In previous studies, the best-known markers of the bone turnover have been widely investigated in GCF samples of patients with different periodontal diseases and implicated to be associated with the progression of periodontal diseases [28–30]. Several investigations provided contradictory evidence about the role of osteocalcin in GCF of patients with periodontitis [31–33]. Little is known about the role and presence of the novel NTx in periodontal diseases. Moreover, there is lack of information about the GCF calprotectin, osteocalcin and NTx levels of patients with generalized aggressive periodontitis (G-AgP) and CP and gingivitis. Therefore, the aim of the present study was to examine how the GCF calprotectin, osteocalcin and NTx levels changes in the presence of gingival health, gingival inflammation (in gingivitis) and/or periodontal tissue destruction (in G-AgP and CP) and to test whether calprotectin, osteocalcin and NTx levels are correlated with clinical parameters.

## 2. Materials and methods

### 2.1. Study population

A total of 80 subjects were included in this study. All consecutive subjects were recruited from the Department of Periodontology, School of Dentistry, Ege University, Izmir, Turkey. The purpose and procedures were explained to all subjects prior to participation, and all participants gave written informed consent in accordance with Helsinki declaration. The study protocol was approved by the Ethics Committee of the Ege University School of Medicine. The purpose of the study was completely explained to each subject before entering the study and informed consent was obtained from each subject. Complete medical and dental histories were taken from all subjects. All of the patients were non-smokers. None of the subjects had a history of systemic disease and had received antibiotics or other medications or periodontal treatment within the past 4 months. Patients with severe medical disorders including diabetes mellitus and immunological disorders as well as alcoholics were excluded from the study. Post-menopausal, pregnant and lactating females and those taking oral contraceptive drugs were also excluded from the study. The selection of the patients was

made according to the clinical and radiographic criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions [34].

#### 2.1.1. Generalized aggressive periodontitis group (G-AgP)

The G-AgP group included 11 females and 9 males ranged in age from 19 and 39 with a mean age of  $31.4 \pm 6.8$  years. These patients demonstrated a generalized pattern of severe destruction and clinical attachment loss (CAL) of  $\geq 5$  mm and probing depth (PD)  $\geq 6$  mm on 8 or more teeth; at least 3 of those were other than central incisors or first molars. Additionally, CAL was not consistent with the amount of plaque accumulation or local contributing factors. All subjects had at least 16 teeth.

#### 2.1.2. Chronic periodontitis group (CP)

The CP group consisted of 9 females and 11 males between the ages of 35 to 50 (mean of  $43.1 \pm 4.2$  years). They had moderate to severe alveolar bone loss and CAL of  $\geq 5$  mm and PD of  $\geq 6$  mm in multiple sites of all four quadrants of the mouth, but with no evidence of rapid progression. Diagnosis of CP was made if the CAL was commensurate with the amount of plaque accumulation of the patients and all had at least 16 teeth in their mouth.

#### 2.1.3. Gingivitis group

The gingivitis group, ranged in age from 22 to 54 (mean age  $38.0 \pm 9.7$  years), included 8 females and 12 males. They had varying degrees of gingival inflammation, but no CAL  $> 2$  mm, no sites with alveolar bone loss present in radiography (i.e., distance between the cemento-enamel junction and bone crest at  $> 95\%$  of the proximal tooth sites  $\leq 3$  mm).

#### 2.1.4. Healthy group

The healthy group consisted of 11 females and 9 males who exhibited PD  $< 3$  mm and no CAL, clinical inflammation and sulcular bleeding (mean age  $43.6 \pm 9.9$  years; range 30 to 61 years). These individuals were healthy volunteers from the Department of Periodontology. No radiographic evidence of alveolar bone loss was observed in these patients (i.e., distance between the cemento-enamel junction and bone crest  $\leq 3$  mm at  $> 95\%$  of the proximal tooth sites).

## 2.2. Determination of periodontal status

At the screening stage, to determine the clinical periodontal status, all subjects had a clinical periodontal examination including PD, CAL, papilla bleeding index (PBI) [35] and plaque index (PI) [36] by one examiner (S.B). PD measurements were performed using manual Williams probe. All measurements were performed at 6 sites per tooth for whole mouth.

## 2.3. Collection of GCF samples

After being selected for the study, subjects were recalled for GCF sampling. In the G-AgP and CP groups, GCF samples were collected from two approximal sites of anterior teeth with  $\geq 6$  mm PD. In the gingivitis group, GCF sampling was done from two approximal sites of anterior teeth with bleeding on probing and PD  $\geq 2$  mm. In the healthy group, GCF samples were collected from two approximal sites of two teeth with PD  $\leq 2$  mm. Prior to GCF sampling, the supragingival plaque was removed from the interproximal surfaces with a sterile curette; these surfaces were dried gently by an air syringe and were isolated by cotton rolls. GCF was sampled with filter paper (Periopaper, ProFlow, Inc., Amityville, NY, USA). Paper strips were carefully inserted into the crevice until mild resistance was felt and left there for 30 seconds [37]. Care was taken to avoid mechanical injury. Strips contaminated with blood were discarded [38]. The absorbed GCF volume of each strip was determined by electronic impedance (Periotron 8000, ProFlow, Inc., and Amityville, NY, USA), pooled and placed into a sterile eppendorf vials and kept at  $-40^{\circ}\text{C}$  until being analyzed. The readings from the Periotron 8000 were converted to an actual volume ( $\mu\text{l}$ ) by reference to the standard curve.

## 2.4. Analysis of calprotectin, osteocalcin and NTx

GCF samples were eluted from the strips by placing them in  $300 \mu\text{l}$  of PBS. GCF calprotectin (Immundiagnostik AG, Bensheim, Germany), osteocalcin (Bender Med Systems, Vienna, Austria) and NTx (Wampole Laboratories, Princeton, NJ, USA) levels were assayed by using commercially available ELISA kits. Procedures were performed according to the instructions in the kit. The minimum detectable limits of calprotectin, osteocalcin and NTx were  $1.9 \text{ pg/ml}$ ,  $0.2 \text{ ng/ml}$  and  $3.2 \text{ nM}$  bone collagen equivalent (BCE), respectively. The amounts of calprotectin, osteocalcin and NTx in each sample were calculated based on the dilutions

Table 1  
Clinical parameters of the sampling areas in study groups (mean  $\pm$  SD)

	G-AgP	CP	Gingivitis	Healthy
PD (mm)	5.8 $\pm$ 0.8 <sup>†‡</sup>	7.2 $\pm$ 0.5 <sup>‡</sup>	2.5 $\pm$ 0.5*	1.5 $\pm$ 0.4
CAL (mm)	6.8 $\pm$ 1.5 <sup>†‡</sup>	8.2 $\pm$ 1.2 <sup>‡</sup>	–	–
PBI	2.2 $\pm$ 1.1*	3.1 $\pm$ 0.5 <sup>‡</sup>	2.2 $\pm$ 0.9*	0.35 $\pm$ 0.4
PI	3.9 $\pm$ 0.9*	4.2 $\pm$ 0.5*	3.75 $\pm$ 0.8*	1.25 $\pm$ 0.5
GCF ( $\mu$ l)	0.76 $\pm$ 0.2*	0.77 $\pm$ 0.1 <sup>‡</sup>	0.60 $\pm$ 0.2*	0.19 $\pm$ 0.1

PD: Probing depth, CAL: Clinical attachment loss, PBI: Papillary bleeding index, PI: Plaque index, GCF: Gingival crevicular fluid, G-AgP: Generalized aggressive periodontitis, CP: Chronic periodontitis.

\*Significant difference from healthy group (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ).

<sup>†</sup>Significant difference from chronic periodontitis group (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ).

<sup>‡</sup>Significant difference from gingivitis and healthy groups (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ).

and the results were expressed as total amount in the 30 second of the two GCF sample. Calculation of the concentration data for each mediator was performed by dividing the amount of each mediator by the GCF volume.

### 2.5. Statistical analysis

Considering a difference of 50% in mean GCF levels of the biochemical markers and assuming standard deviations to be maximum 80% of the mean values and accepting a power of 90%, P-value of 5% in healthy and diseased groups, minimum sample size was calculated. Power calculation analysis revealed that the minimum required sample size was nine subjects for each group. Statistical analysis was performed using non-parametrical techniques. Comparisons between the study groups were performed using the Kruskal-Wallis test. When there were significant differences ( $p < 0.05$ ), post-hoc 2-group comparisons were assessed with Bonferroni-corrected Mann-Whitney U tests, and P-values  $< 0.008$  were considered to be statistically significant. Spearman rank correlation analysis was used to analyze the correlations between GCF calprotectin, osteocalcin and NTx levels and clinical parameters and  $p < 0.05$  was considered as significant. All data analysis was performed using a statistical package Abacus Concepts, Inc., Berkeley, CA, USA.

## 3. Results

### 3.1. Clinical findings

The mean clinical data for the sampling areas are shown in Table 1.

#### 3.1.1. PD and CAL

The mean PD scores of sampling sites in G-AgP, CP and gingivitis groups were significantly higher than the healthy group ( $p < 0.008$ ). CP group had higher scores compared to G-AgP group and both groups had higher PD scores than the gingivitis group ( $p < 0.008$ ). The mean CAL of sampling sites in G-AgP and CP groups were significantly higher than that of the gingivitis and healthy group ( $p < 0.008$ ). CP groups had elevated CAL scores compared to G-AgP group ( $p < 0.008$ ).

#### 3.1.2. PBI and PI scores

All patient groups had significantly higher PBI and PI scores compared to the healthy group ( $p < 0.008$ ). CP group had significantly elevated PBI scores compared to the gingivitis group ( $p < 0.008$ ). G-AgP and gingivitis groups had similar PBI and PI scores ( $p > 0.008$ ).

#### 3.1.3. GCF scores

All patient groups had significantly higher GCF scores compared to the healthy group ( $p < 0.008$ ). CP group had significantly elevated GCF scores compared to the gingivitis group ( $p < 0.008$ ). G-AgP and gingivitis groups had similar GCF scores ( $p > 0.008$ ).

### 3.2. Biochemical findings

#### 3.2.1. GCF calprotectin levels

Distribution of the total amount of GCF calprotectin is shown in Fig. 1. Significant differences were found between study groups ( $p = 0.008$ ). CP and G-AgP groups had higher GCF calprotectin total amount compared to gingivitis and healthy groups. GCF calprotectin total amount of G-AgP group was similar to that of CP group ( $p > 0.008$ ). Gingivitis group had signif-

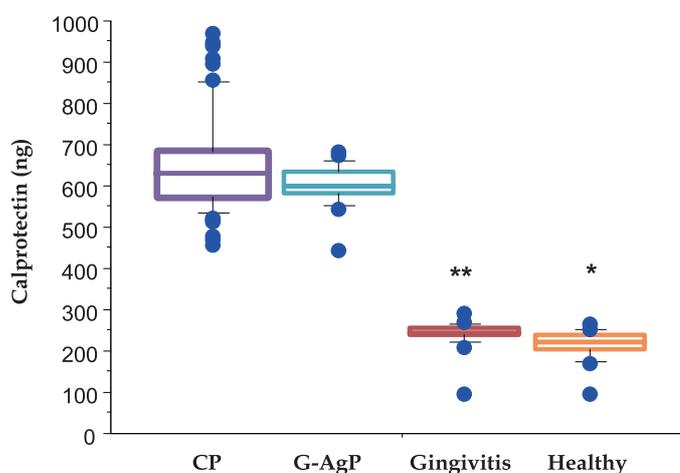


Fig. 1. GCF calprotectin levels (sample pooled from two sites) of CP, G-AgP, gingivitis and healthy groups. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as blue circles. \*Significantly different from G-AgP, CP, and gingivitis groups. (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ). \*\*Significantly different from G-AgP and CP groups. (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ).

ificantly higher GCF calprotectin total amount than the healthy group ( $p < 0.008$ ) (Fig. 1).

When the data were expressed as concentration, patient groups had lower GCF calprotectin concentration compared to the healthy group ( $p < 0.008$ ). Gingivitis group had also lower GCF calprotectin concentration than the CP and G-AgP group ( $p < 0.008$ ), while both CP and G-AgP groups had similar GCF calprotectin concentration ( $p > 0.008$ ) (data not shown).

### 3.2.2. GCF osteocalcin levels

CP and G-AgP groups had lower GCF osteocalcin total amount compared to gingivitis and healthy groups. GCF osteocalcin total amount of CP group was significantly lower compared to those of G-AgP group ( $p < 0.008$ ). Gingivitis group had similar GCF osteocalcin total amount to the healthy group ( $p > 0.008$ ) (Fig. 2).

When the data were expressed as concentration, patient groups had lower GCF osteocalcin concentration compared to the healthy group ( $p < 0.008$ ). Gingivitis group had higher GCF osteocalcin concentration than the CP and G-AgP group ( $p < 0.008$ ), while both CP and G-AgP groups had similar GCF osteocalcin concentration ( $p > 0.008$ ) (data not shown).

### 3.2.3. GCF NTx levels

CP and G-AgP groups had similar GCF NTx total amount to gingivitis and healthy groups ( $p > 0.008$ ). G-AgP group had significantly lower GCF NTx total amount than the CP group ( $p < 0.008$ ). Gingivitis group had similar GCF NTx total amount to the healthy group ( $p > 0.008$ ) (Fig. 3).

When the data were expressed as concentration, patient groups had lower GCF NTx concentration compared to the healthy group ( $p < 0.008$ ). Gingivitis group had higher GCF osteocalcin concentration than the CP and G-AgP group ( $p < 0.008$ ). G-AgP group had lower GCF NTx concentration compared to CP group ( $p < 0.008$ ) (data not shown).

### 3.2.4. GCF osteocalcin/NTx ratio

Among the study groups CP group had the lowest osteocalcin/NTx ratio compared to the other groups ( $p < 0.008$ ). G-AgP, gingivitis and healthy groups had similar GCF osteocalcin/NTx ratio ( $p > 0.008$ ) (Fig. 4).

The correlation between GCF calprotectin, osteocalcin, NTx and osteocalcin/NTx ratio and clinical parameters of G-AgP, CP, gingivitis and healthy groups is presented in Table 2. All clinical parameters were positively correlated with GCF calprotectin total amount ( $p < 0.05$ ). GCF osteocalcin total amount was negatively correlated with all clinical parameters ( $p < 0.05$ ). There was no correlation between GCF NTx total amount and clinical parameters ( $p > 0.05$ ). GCF osteocalcin/NTx ratio was negatively correlated with all clinical periodontal parameters ( $p < 0.05$ ).

## 4. Discussion

In the present study, we investigated calprotectin, osteocalcin and NTx levels in GCF of patients with different periodontal diseases. The result of the present

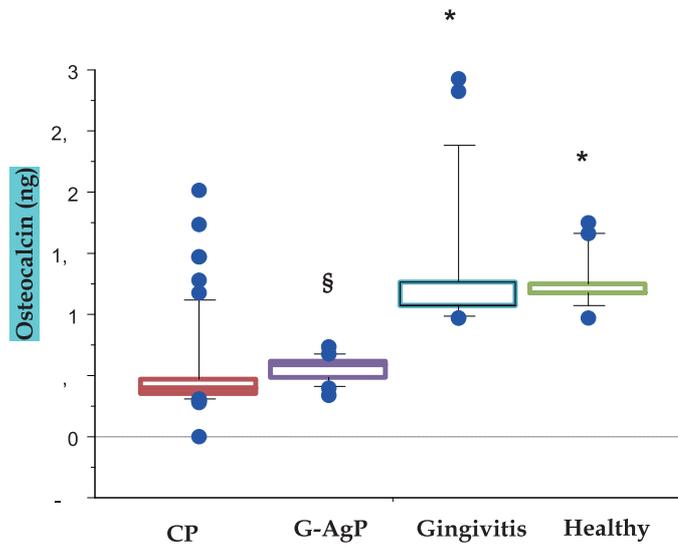


Fig. 2. GCF osteocalcin levels (sample pooled from two sites) of CP, G-AgP, gingivitis and healthy groups. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as blue circles. \*Significantly different from G-AgP and CP groups. (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ). §Significantly different from CP group. (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ).

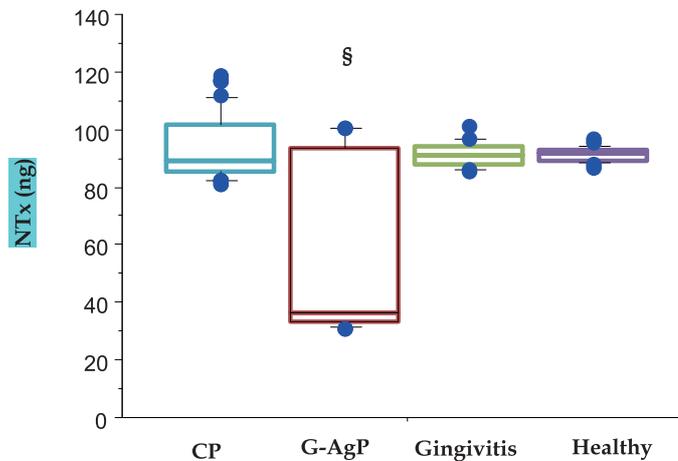


Fig. 3. GCF NTx levels (sample pooled from two sites) of CP, G-AgP, gingivitis and healthy groups. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as blue circles. §Significantly different from CP group. (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ).

study has shown that GCF calprotectin total amount is enhanced with the severity of periodontal disease and significantly elevated in G-AgP and CP compared to other study groups. In relation to bone turnover markers, CP group had lower osteocalcin/NTx ratio while G-AgP, gingivitis and healthy groups had similar ratio. The role of these biomarkers in the pathogenesis of cyclosporine-induced gingival overgrowth was recently investigated by the same study group [39]. Our data has provided the first evidence about the presence

of NTx in GCF of patients with different periodontal diseases.

It is known that various sampling protocols are present regarding GCF collection [37,40], still no general agreement was currently present for regarding sampling method. In the present study, paper strips were carefully inserted into the crevice until mild resistance was felt and left there for 30 seconds [37]. Although this technique provides larger GCF volumes compared to superficial intracrevicular technique [40], in accor-

Table 2  
Correlations between calprotectin, osteocalcin, NTx and osteocalcin/NTx and clinical parameters of G-AgP, CP, gingivitis and healthy groups

Clinical parameters	Calprotectin	Osteocalcin	NTx	Osteocalcin/NTx
PD (mm)	0.767*	-0.643*	0.067	-0.605*
CAL (mm)	0.736*	-0.559*	0.078	-0.511*
PBI	0.577*	-0.406*	-0.141	-0.330*
PI	0.451*	-0.403*	-0.030	-0.340*
GCF ( $\mu$ l)	0.401*	-0.370*	-0.187	-0.273*

Spearman correlation (\* $p < 0.05$ ). PD: Probing depth, CAL: Clinical attachment loss, PBI: Papillary bleeding index, PI: Plaque index, GCF: Gingival crevicular fluid. G-AgP: Generalized aggressive periodontitis, CP: Chronic periodontitis.

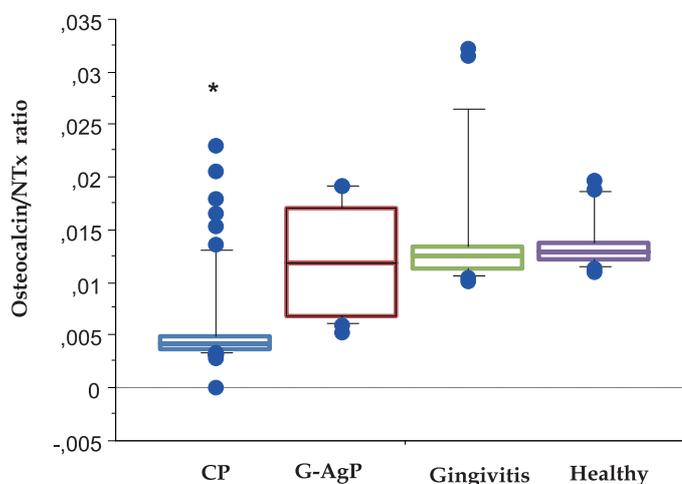


Fig. 4. GCF osteocalcin/NTx ratio of CP, G-AgP, gingivitis and healthy groups. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as blue circles. \*Significantly different from G-AgP, gingivitis and healthy groups. (Kruskal Wallis test,  $p < 0.05$ , Mann-Whitney U test,  $p < 0.008$ ).

dance with the suggestions of others our discussion of the data as well as studying relationship with clinical data was based on the total amount rather than concentration of the data which consider the amount of GCF collected [37,41].

The relationship between GCF calprotectin levels and several other inflammatory markers has been previously investigated and calprotectin levels were shown to be a significant contributor to host defense against infection [8,9,42,43]. The present data indicated that GCF calprotectin levels increased as the periodontal disease progresses from health to disease, but similar in G-AgP and CP. Our findings are consistent with the previous data showing the role of calprotectin as an inflammatory marker of periodontal disease [11,12,42,43]. Kaner et al. [44] have demonstrated that GCF calprotectin levels are significantly correlated with the levels of periodontal inflammation and decreased after periodontal therapy in G-AgP patients. Elevated GCF calprotectin levels in diseased groups might be due to the increased activation of inflammatory cells by bac-

terial components as well as greater release of intracellular material in gingivitis and periodontitis [45–47]. As a result, calprotectin could contribute to the host inflammatory immune defense against bacteria in periodontal disease [8,9,48,49]. In an *in vitro* study, epithelial calprotectin was shown to promote resistance to *Porphyromonas gingivalis* invasion and thereby reduce bacterial invasion [8]. Based on the present data we suggest that calprotectin could be a reliable GCF biomarker of gingivitis and periodontitis. On the other hand, similar GCF calprotectin levels in both G-AgP and CP groups might show that magnitude of the inflammatory component of the host response is not different in these clinically distinct periodontal diseases as has been stated [50].

It is well known that the homeostasis in bone turnover is perturbed during the progression of periodontal disease and leads to the irreversible bone resorption where immune cells and cytokines released play an important role [5]. It is of importance to investigate both bone formation and resorption markers to understand abnor-

mal bone turnover rate in periodontitis. In the present cross-sectional study, both osteocalcin as a marker of bone formation and NTx as a marker of bone resorption were investigated to have an idea about the change in bone turnover in different periodontal disease, in other words how the levels of these biochemical markers changes in GCF while the disease progress from gingivitis to periodontitis. Osteocalcin/NTx ratio has been suggested to be a marker of bone turnover normalized with respect to resorption [33]. In the present study, this ratio was also calculated in order to evaluate how bone turnover changes in different periodontal diseases. The presence of measurable amount of GCF osteocalcin and NTx in gingivitis and healthy groups might be due to the normal bone homeostasis.

Serum osteocalcin levels increase in several diseases such as osteoporosis where rapid bone turnover are seen and accepted as a valid marker of bone turnover when resorption and formation are coupled [22,51]. It has been previously shown that bone turnover profiles from periodontal bone surfaces and GCF differed from systemic bone turnover profiles [33]. In periodontitis osteocalcin has been suggested to be a marker of bone formation where bone resorption is greater than formation, and GCF osteocalcin levels are more revealing than serum or saliva levels regarding bone turnover in periodontium [52]. On the other hand, there is a controversy about the GCF osteocalcin levels in periodontal diseases. Kunimatsu et al. [53] did not find osteocalcin in GCF of patients with gingivitis, while in periodontitis GCF osteocalcin was positively correlated with clinical parameters. Lee et al. [32] demonstrated similar GCF osteocalcin levels in diseased and healthy sites in patients with CP. Wilson et al. [33] could not detect osteocalcin in GCF of untreated periodontitis patients. On the other hand, Nakashima et al. [54] found elevated osteocalcin total amount in GCF from periodontitis sites compared to those found in healthy and gingivitis sites. In the present study both periodontitis groups (G-AgP and CP) had lower osteocalcin levels compared to gingivitis and healthy groups. Furthermore, GCF osteocalcin levels were negatively correlated with periodontal parameters. These findings might point out to the abnormal bone turnover in periodontitis. Moreover, variations in osteocalcin levels among different studies as well as the current study might reflect inability to differentiate between sites undergoing attachment loss and others in a "bone loss arrest" state, where clinical signs of PD (CAL, increased PPD, bleeding on probing) are present, but no activity in present.

The present data show that NTx, originally identified in urine as a product of osteoclastic bone resorp-

tion [21], can also be measured in GCF of patients with different periodontal diseases. CP and G-AgP groups had similar GCF NTx levels with gingivitis and healthy but G-AgP group had lower levels compared to the CP group. The presence of GCF NTx in untreated periodontitis has been previously reported and suggested that NTx may be a useful marker of active periodontal bone loss [33]. Friedman et al. [54] evaluated the NTx levels in GCF and peri-implant crevicular fluid (PCF) and speculated that increased NTx levels may predict extensive bone destruction earlier than the GCF and PCF calprotectin levels. To the best of our knowledge this is the first study investigating GCF NTx levels in different forms of periodontal diseases. Therefore, we were not able to compare our results with others. Increased levels of NTx as a reliable and sensitive biochemical marker of bone resorption have been shown in several systemic diseases associated with bone loss [23, 24,51].

It is known that the quality of the host immune-inflammatory response against bacterial challenge determines the severity and extent of disease. CP and G-AgP are different disease entities with different etiology and pathogenesis [4,56]. In the present study, G-AgP patients had significantly lower GCF NTx levels compared to CP patients. This is an interesting finding of the present study since rapid and severe periodontal destruction occurs in G-AgP compared to CP [4]. Considering the role of NTx as a resorption marker in bone turnover one could expect higher GCF NTx and lower osteocalcin/NTx ratio in G-AgP. The diversity in bone-specific markers of tissue breakdown in G-AgP and CP groups might indicate high (or abnormal) bone turnover rate in both G-AgP and CP. Alternatively, GCF NTx levels might reflect the catabolic stage of the bone metabolic activity that may be low in G-AgP due to other as yet unidentified factors. As aggressive form of periodontitis seems to have a rapid periodontal destruction as well as higher rate of bone loss compared to the chronic form of periodontitis, whether or not these markers play a role on this rapid progression seen in G-AgP deserves further investigation.

In conclusion, our data show that patient groups differ considerably in their capacity to release osteocalcin and NTx as well as calprotectin in GCF. Decreased or unchanged GCF levels of bone turnover markers as well as calprotectin levels in periodontal diseases amend and further extend the understanding of the pathogenesis of periodontal diseases. Our data document for the first time the presence of NTx in GCF of different periodontal diseases. The cross-sectional nature of the

present study limits its ability to make causal relationship and the findings should be confirmed by longitudinal studies investigating the levels of these biomarkers after periodontal treatment. Additional studies are necessary to clear the role, regulation and function of these molecules in the pathogenesis of periodontal disease as well as if these play a role in distinct forms of periodontitis.

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