

Assessment of periostin levels in serum and gingival crevicular fluid of patients with periodontal disease

U. Balli¹, Z. P. Keles², B. Avci³,
S. Guler², B. O. Cetinkaya²,
G. C. Keles²

¹Department of Periodontology, Faculty of Dentistry, Bulent Ecevit University, Zonguldak, Turkey, ²Department of Periodontology, Faculty of Dentistry, Ondokuzmayis University, Samsun, Turkey and ³Department of Medical Biochemistry, Faculty of Medicine, Ondokuzmayis University, Samsun, Turkey

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Background and Objective: Periostin, a secreted adhesion molecule essential for periodontal tissue integrity, is highly expressed in the periodontal ligament and plays a critical role in tooth and bone development. The purpose of this study was to investigate periostin levels in the gingival crevicular fluid and serum of patients with periodontal disease and compare them with those of healthy individuals.

Material and Methods: Eighty individuals (41 males and 39 females; age range: 25–48 years) were enrolled in the study. Individuals were divided into three groups following clinical and radiographic examinations: the periodontal-healthy group ($n = 20$), gingivitis group ($n = 30$) and chronic periodontitis group ($n = 30$). Gingival crevicular fluid and serum samples were collected and periostin levels were determined using the enzyme-linked immunosorbent assay.

Results: The total amount and concentration of periostin decreased in gingival crevicular fluid with the progression and severity of the disease from healthy controls to gingivitis and to chronic periodontitis groups and differed significantly ($p < 0.05$). However, there was no significant difference in serum periostin concentration within all groups ($p > 0.05$). Periostin in gingival crevicular fluid negatively correlated with the gingival index in the periodontal disease groups, whereas it is inversely correlated with the clinical attachment level only in the periodontitis group ($p < 0.05$). When all the clinical groups were examined together, the periostin concentration negatively correlated with clinical attachment level and gingival index; moreover, total periostin positively correlated with periostin concentration and clinical attachment level ($p < 0.05$).

Conclusions: The periostin levels in gingival crevicular fluid decreased proportionally with the progression and severity of periodontal disease, and negatively correlated with the clinical parameters. Within the limits of the study, the periostin level in gingival crevicular fluid can be considered a reliable marker in the evaluation of periodontal disease susceptibility and activity.

Umut Balli, DDS, PhD, Department of Periodontology, Faculty of Dentistry, Bulent Ecevit University, 67600 Zonguldak, Turkey
Tel: +90 530 3255749
Fax: +90 372 2613403
e-mail: umutballi@gmail.com

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The periodontium, including epithelial tissue and soft and mineralized connective tissue, comprises the gingiva,

periodontal ligament (PDL), root cementum and alveolar bone. Periodontal disease affects the composition

and integrity of all the structures of the periodontium, leading to the destruction of the connective tissue

matrix and cells, loss of fibrous attachment and resorption of the alveolar bone (1). Gingivitis is an inflammatory condition characterized by the presence of symptoms and clinical signs of inflammation that are confined to the gingiva and associated with stable attachment levels on the periodontium (2). However, periodontitis, which is an oral infectious disease caused by specific microorganisms or groups of microorganisms, is characterized by progressive clinical attachment loss, alveolar bone resorption, periodontal pocketing and gingival inflammation (3).

The PDL is a soft, highly vascular and cellular connective tissue interposed between the cementum and alveolar bone (4). PDL has heterogeneous cell populations, including fibroblast, cementoblasts, osteoblast, osteoclastic cells, sensory cells, endothelial cells and progenitor/stem cells (1,5,6). PDL cells are considered to play important roles in not only supporting teeth, protection and provision of sensory input to the masticatory system, but also in contributing to wound healing, bone remodeling and regeneration of periodontal tissues (5,7). Clearly, PDL cells play an essential role in maintaining tissue homeostasis, repair and regeneration in the periodontium.

Periostin was initially termed as osteoblast-specific factor 2 when first isolated from the mouse MC3T3-E1 osteoblastic cell line (8). It was renamed as periostin to avoid its confusion with the transcription factor Cbfa1, and to indicate its expression in the periosteum and PDL (9). Periostin, which is induced by transforming growth factor- β (TGF- β) and bone morphogenetic protein 2, directly interacts with fibronectin and type I collagen through its EMI domain, and binds to the metalloproteinase domain of bone morphogenetic protein 1 through its fas1 domain, thereby promoting collagen cross-linking and mechanical properties of the connective tissues (9–13). In addition to the interaction with extracellular matrix proteins, periostin also interacts with the cell membrane via integrins (14). TGF- β has

effects on osteoblast recruitment and attachment through its effect on periostin expression via $\alpha v \beta 3$ and $\alpha v \beta 5$ integrin-dependent cell adhesion (15).

There is evidence that absence of periostin in mice leads to severe alveolar bone loss along with attachment loss, external root resorption and widening of the PDL (16). Similar experiments indicated that periostin knockout mice showed eruption disturbance of incisors, defective remodeling in the PDL and periodontal disease-like phenotype (10,17). Periostin potently decreases PDL cell apoptosis by regulating hypoxia-inducible factor-1 α levels via TGF- β signaling (18). It has also been shown to increase PDL cells proliferation and migration, and activation of the survival signaling pathway phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) (19). In a recent experimental study, periostin levels in rat PDL decreased after periodontitis induction, and inversely related to the extent of bone alveolar loss (20).

Although recent experimental and *in vitro* studies have been concentrated on the role of periostin in both physiological and pathological processes, to our knowledge, no clinical data are available on the comparison of periostin levels in gingival crevicular fluid and serum of healthy individuals with those of patients with periodontal disease. Therefore, the present study was designed to investigate gingival crevicular fluid and serum periostin levels in individuals with clinically healthy periodontium and in patients with gingivitis and chronic periodontitis.

Material and methods

Study population

Eighty individuals, 41 males and 39 females, in the age range of 25–48 years, admitted to the Periodontology Department of Ondokuzmayis University, Faculty of Dentistry, were enrolled in the study. All individuals gave their written informed consent and the protocol of the study was approved by the Ethics Committee of

Bulent Ecevit University (2013-94-03/09).

Clinical measurements

The probing pocket depth (PPD) and clinical attachment level were measured and plaque index (PI) and gingival index (GI) scores were recorded using a Williams' periodontal probe (Hu-Friedy, Chicago, IL, USA). All clinical examinations (at six different sites around each tooth: mesiobuccal, distobuccal, midbuccal, mesiolingual, distolingual and midlingual), group allocations, sampling site selections and gingival crevicular fluid collections were performed by the same investigator, who was blinded with respect to the study design. Before actual measurement, 10 individuals were randomly selected and used to calibrate the investigator. The investigator evaluated individuals on two separate occasions, 48 h apart. Calibration of the investigator was accepted if measurements at baseline and 48 h were > 90% similar at the millimeter level (21).

Inclusion and exclusion criteria

All individuals underwent a full mouth periodontal examination, which included PPD, clinical attachment level, PI (22) and GI (23), and orthopantomography was performed. Based on the GI, PPD and clinical attachment level, individuals were categorized into three groups: individuals with clinically healthy periodontium (group 1, $n = 20$: 10 males and 10 females, age 37.75 ± 4.8 years), and patients with gingivitis (group 2, $n = 30$: 16 males and 14 females, age 38.27 ± 5.5 years) and chronic periodontitis (group 3, $n = 30$: 15 males and 15 females, age 41.17 ± 5.37 years). Criteria for the healthy group were GI = 0, PPD and clinical attachment level ≤ 3 mm, and no signs of attachment and bone loss by clinical and radiographic examination (24). Criteria for the gingivitis group were clinical signs of inflammation, such as red color and swelling of the gingival margin, GI ≥ 2 , PPD and clinical attachment level ≤ 3 mm, and no

signs of attachment and bone loss (24). Criteria for the chronic periodontitis group were clinical signs of inflammation, such as red color and swelling of the gingival margin, GI ≥ 2 , PPD ≥ 5 mm with attachment, and bone loss by clinical and radiographic examination (24). Descriptive statistics in the study population are summarized in Table 1.

The exclusion criteria were aggressive periodontitis, systemic diseases (i.e. diabetes mellitus, cancer, human immunodeficiency virus, bone and collagen-metabolic diseases, or disorders that compromise periostin levels), chronic high-dose steroid therapy, radiation or immunosuppressive therapy, allergy or sensitivity to any drug, pregnancy, lactation and smoking. All individuals had no history of periodontal therapy or drug therapy for at least 6 mo.

Collection of samples

In all groups, only one site per individual was selected as the sampling site. Gingival crevicular fluid samples were obtained from the mesiobuccal or distobuccal surface of single-rooted teeth, for a total of 80 samples. To prevent contamination of gingival crevicular fluid with blood associated

with the probing of the inflamed sites, all clinical examinations and sampling site selections were performed the day before gingival crevicular fluid and blood samples were collected. In the healthy group, gingival crevicular fluid samples were collected from sites with no clinical inflammation, whereas the sites with the greatest clinical signs of inflammation (the highest GI score with bleeding on probing) were selected in the gingivitis group. In the chronic periodontitis group, the sites showing the greatest clinical signs of inflammation (the highest GI score with bleeding on probing) and PPD along with radiographic confirmation of alveolar bone loss were selected for gingival crevicular fluid sampling. Before gingival crevicular fluid sampling, the sites were isolated with cotton rolls, saliva was removed and the supragingival plaque, if present, was removed using a sterile curette. Gingival crevicular fluid was sampled with filter paper using the intracrevicular method (Periopaper; ProFlow Inc., Amityville, NY, USA) (25). Paper strips were placed into the crevice until mild resistance was felt, and left in position for 30 s. Strips with visible signs of saliva or blood contamination were discarded. The gingival crevicular

fluid volume of each strip was determined by electronic impedance (Periotron 8000; ProFlow Inc.). The samples were placed into a sterile polypropylene tube and stored at -70°C until analysis.

Venous blood samples (5 mL) were collected with the venipuncture method in the antecubital vein. The blood samples were centrifuged at 3000 g for 5 min at 4°C . The extracted serum samples were immediately aliquoted and frozen at -70°C until analysis (24).

Analysis of periostin

A modification of the protocol described by Curtis *et al.* (26) was used for the elution of from the periopapers. Each sampled strip was placed into a 400 μL Eppendorf centrifuge tube containing 100 μL of 2% bovine serum albumin in phosphate-buffered saline and then incubated for 60 min at 4°C . This tube was placed into a 1.5 mL microcentrifuge tube and centrifugation was carried out at 10,000 g for 5 min at 4°C after creating a hole on the bottom of the 400 μL tube to allow the elution of gingival crevicular fluid into the microcentrifuge tube. The procedure was repeated twice and 200 μL of gingival crevicular fluid samples were collected. The activity of periostin in gingival crevicular fluid and serum samples was analyzed by standard enzyme-linked immunosorbent assay procedures using a commercially available Periostin kit (Uscn Life Science Inc., Wuhan, China). The enzymatic reactions were quantified in an automatic microplate photometer.

The microtiter plate provided in this kit was precoated with an antibody specific to periostin. Standards or samples were then added to the appropriate microtiter plate wells containing a biotin-conjugated antibody preparation specific for periostin. Next, avidin conjugated to horseradish peroxidase was added to each microplate well and incubated. After tetramethylbenzidine substrate solution was added, only those wells containing periostin, biotin-conjugated

Table 1. Descriptive statistics in the study population

Parameters*	Group	Mean \pm SD	p Value
PPD (mm)	Healthy	1.87 \pm 0.39	< 0.05
	Gingivitis	2.64 \pm 0.15	
	Periodontitis	6.23 \pm 0.79	
CAL (mm)	Healthy	1.87 \pm 0.39	< 0.05
	Gingivitis	2.64 \pm 0.15	
	Periodontitis	7.15 \pm 0.89	
GI	Healthy	0.26 \pm 0.04	< 0.016
	Gingivitis	2.45 \pm 0.21	
	Periodontitis	2.58 \pm 0.24	
PI	Healthy	0.49 \pm 0.09	< 0.016
	Gingivitis	1.57 \pm 0.30	
	Periodontitis	2.30 \pm 0.34	
GCF volume (μL)	Healthy	0.34 \pm 0.08	< 0.05
	Gingivitis	0.68 \pm 0.08	
	Periodontitis	0.87 \pm 0.13	

CAL, clinical attachment level; GCF, gingival crevicular fluid; GI, gingival index; PI, plaque index; PPD, probing pocket depth. Data are expressed as mean \pm SD.

Welch/Tamhane's T2.

Kruskal-Wallis/Bonferroni-adjusted Mann-Whitney U.

Bonferroni correction $\alpha = 0.05/3 = 0.0166666666666667$.

*Significant difference between groups.

antibody and enzyme-conjugated avidin exhibited a change in color. The enzyme–substrate reaction was terminated by addition of sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. Then, the periostin levels in the samples were determined by comparing the optic density of the samples to the standard curve. The total periostin levels were measured in gingival crevicular fluid samples that were collected for 30 s. The concentrations of periostin in gingival crevicular fluid were calculated by dividing total periostin by gingival crevicular fluid volume.

The mean interassay coefficient of variation (CV) % and intra-assay CV % for periostin were 12% and 10%, respectively. The samples showing the highest concentrations were diluted and measured in duplicate. All assays were conducted according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using a commercially available software (SPSS 15.0; SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to investigate whether the data were normally distributed. When equal variances were assumed in serum periostin concentration, variables were compared using one-way analysis of variance (ANOVA). When equal variances were not assumed, variables were compared by means of the Welch and Tamhane's T2 *post-hoc* tests for total gingival crevicular fluid periostin levels, gingival crevicular fluid volume, PPD and clinical attachment level. Comparisons of the concentration of periostin in gingival crevicular fluid, age, GI and PI were analyzed using the Kruskal–Wallis non-parametric test followed by *post-hoc* group comparisons with the Bonferroni-adjusted Mann–Whitney *U* test. The Spearman's rank correlation test was also used to detect the relationship between gingival crevicular fluid and serum periostin with GI and clinical attachment level. $p < 0.05$ was considered statistically significant.

Results

All gingival crevicular fluid and serum samples showed the presence of periostin. The values of total amount and concentration of periostin in gingival crevicular fluid and serum periostin concentration are summarized in Table 2. Levels of total periostin in gingival crevicular fluid were significantly lower in the gingivitis and chronic periodontitis groups than in the healthy controls ($p < 0.05$). Total gingival crevicular fluid periostin levels were significantly lower in patients with chronic periodontitis than in those having gingivitis ($p < 0.05$). There was no significant difference in serum periostin concentration across all groups ($p > 0.05$). The concentration of periostin in gingival crevicular fluid significantly decreased with the progression and severity of disease from healthy control to gingivitis to chronic periodontitis groups ($p < 0.05$).

The correlation coefficients are shown in Table 3. A statistically significant positive correlation was found between total amount, concentration in gingival crevicular fluid and serum periostin concentration in all groups ($p < 0.05$). Total amount and concentration of periostin negatively correlated with GI in the periodontal disease group ($p < 0.05$). When all clinical groups were examined together, statistically significant nega-

tive correlations were found between periostin concentration and clinical attachment level, total periostin and GI, and periostin concentration and GI; positive correlations were found between total periostin and periostin concentration, and total periostin and clinical attachment level ($p < 0.05$).

Discussion

In the present study, gingival crevicular fluid (total amount and concentration) and serum periostin concentration were investigated in individuals with clinically healthy periodontium and in patients with gingivitis and chronic periodontitis. To the best of our knowledge, this is the first clinical study examining the relationship between periostin levels and periodontal disease.

In this study, the influence of the age of the individuals on periostin levels was minimized by selecting their age range as 25–48 years. Furthermore, this study consisted of three groups, including healthy control, gingivitis group and periodontitis group to evaluate the potential effect of periodontal disease progression on periostin levels. The gingival crevicular fluid was sampled with filter paper strips using the intracrevicular method (25). The advantages of this technique are that it is quick and easy to use, can be applied to individual sites and is possibly the least traumatic when

Table 2. Total GCF periostin, GCF periostin concentration and serum periostin concentration in the study population

Parameters	Group	Mean \pm SD	<i>p</i> Value
Total GCF periostin (pg)*	Healthy	113.00 \pm 36.41	< 0.05
	Gingivitis	72.32 \pm 20.92	
	Periodontitis	43.10 \pm 8.24	
GCF periostin concentration [mean rank] (pg/ μ L)*	Healthy	346.93 \pm 118.22 [70.50]	< 0.016
	Gingivitis	108.86 \pm 34.88 [44.10]	
	Periodontitis	51.64 \pm 15.99 [16.90]	
Serum periostin (pg/mL)	Healthy	7213.55 \pm 2178.50	0.792
	Gingivitis	7726.61 \pm 2510.02	
	Periodontitis	8104.71 \pm 2293.02	

GCF, gingival crevicular fluid. Data are expressed as mean \pm SD.

Welch/Tamhane's T2.

Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney *U*.

One-way ANOVA.

Bonferroni correction $\alpha = 0.05/3 = 0.0166666666666667$.

*Significant difference between groups.

Table 3. The Spearman's rank correlation (*r*) among groups with respect to GCF, serum, CAL and GI

	TA-P to CO-P	TA-P to serum	CO-P to serum	TA-P to CAL	CO-P to CAL	Serum to CAL	TA-P to GI	CO-P to GI	Serum to GI
Group 1 (healthy)									
<i>r</i>	0.697*	0.602*	0.549*	-0.077	-0.268	-0.199	-0.434	-0.580*	-0.454*
<i>p</i>	0.001*	0.005*	0.012*	0.747	0.253	0.401	0.056	0.007*	0.044*
Group 2 (gingivitis)									
<i>r</i>	0.921*	0.484*	0.543*	-0.119	-0.128	-0.238	-0.631*	-0.504*	-0.346
<i>p</i>	0.000*	0.007*	0.002*	0.532	0.500	0.205	0.000*	0.005*	0.061
Group 3 (periodontitis)									
<i>r</i>	0.915*	0.541*	0.446*	-0.680*	-0.712*	-0.463*	-0.430*	-0.460*	-0.451*
<i>p</i>	0.000*	0.002*	0.014*	0.000*	0.000*	0.010*	0.018*	0.010*	0.012*
All groups									
<i>r</i>	0.955*	0.186	0.088	0.841*	-0.907*	0.015	-0.779*	-0.807*	-0.133
<i>p</i>	0.000*	0.098	0.439	0.000*	0.000*	0.897	0.000*	0.000*	0.241

CAL, clinical attachment level; CO-P, concentration of periostin in gingival crevicular fluid; GCF, gingival crevicular fluid; GI, gingival index; TA-P, total amount of periostin in gingival crevicular fluid.

*Statistically significant (*p* < 0.05).

correctly used (25). Data presented as the total amount of a constituent in gingival crevicular fluid instead of its concentration are more appropriate when examining the relationship between gingival crevicular fluid constituents and periodontal diseases (27–29). Increasing volumes in diseased sites would dilute the concentration of the gingival crevicular fluid constituent when it is primarily locally secreted in gingival crevicular fluid (25,27). Moreover, the volume of gingival crevicular fluid by which concentration is directly affected is a dependent variance; hence, data presented as concentration become a dependent variable in the quantification of a constituent of the gingival crevicular fluid (27). Therefore, the reporting of amounts of total biomarkers per sampling time in gingival crevicular fluid may be a better, valid and reliable indicator for diagnostic purposes. However, in the present study, results were evaluated in both total amount and concentration because of their compatibility with each other.

As it was lower in the gingival crevicular fluid from patients with chronic periodontitis than in patients with gingivitis or from healthy controls, the level of total periostin in gingival crevicular fluid decreased with the severity of the periodontal disease or with the degree of inflammation. The increase in the clinical attachment

level and the high GI score are clinical indicators of disease progression or periodontal severity and gingival inflammation from healthy to gingivitis to periodontitis (23,24). Accordingly, a significant difference in the concentration of periostin in gingival crevicular fluid was found between healthy controls and disease groups. Periostin is highly expressed in collagen-rich connective tissues (30). Therefore, as expected, we observed that the degree of inflammation and tissue damage affected the periostin levels in gingival crevicular fluid strongly and negatively. However, there was no significant difference in serum periostin concentration among all groups. Decreased level of periostin in gingival crevicular fluid in the diseased groups might suggest that this novel molecule takes part in maintaining normal periodontal tissue function. Therefore, periodontal disease, which is a low-grade local infection, may not affect serum periostin concentration.

Periostin is preferentially and highly expressed in the periosteum during embryogenesis and bone formation (15). In addition, in adults, its expression increases after fracture and mechanical pressure, including tooth movement and tooth eruption in the periosteum and PDL (16,31,32). Periostin is predominantly expressed in odontoblast and ameloblast cell layers. Periostin null mice displayed deformed and irregular enamel and

dentin structure (33). In another study conducted on periostin null mice, the cementoblasts failed to attach to the root surface, thereby compromising the attachment apparatus (16). Severe alveolar bone loss, attachment loss, external root resorption, widening of the PDL and lack of PDL fiber organization developed after tooth eruption (16,17). In a recent report, TGF-β1 induced the production of periostin in human gingival fibroblasts (34). Tumor necrosis factor-α (TNF-α) strongly inhibited periostin expression in fibroblasts under TGF-β1 stimulus; this effect, however, was not seen without TGF-β1 stimulation (34). Conversely, periostin production was suppressed by TNF-α in gingival fibroblasts and PDL cells (35). Although periostin expression was increased by stimulation with interleukin (IL)-4 and -13, *Porphyromonas gingivalis* lipopolysaccharide did not have any stimulatory effect on periostin levels. Moreover, periostin has been reported to have limited stimulatory effects on IL-6 and -8 and monocyte chemoattractant protein 1 (35).

Only one animal study has explored the role of periostin in experimental periodontitis or periodontitis-induced bone loss (20). Periodontal tissues in rats were harvested at baseline, 2 and 4 wk after experimental periodontitis induction. Similar to our biochemical findings, periostin levels proportionally decreased over time in response

to the inflammatory process (20). It was also reported that alveolar bone loss inversely correlated with the levels of periostin. In the present study, levels of periostin in gingival crevicular fluid were negatively correlated with the clinical attachment level and GI. Periostin level is decreased by TNF- α and *P. gingivalis* lipopolysaccharides in PDL fibroblasts (36), which agrees with our finding that periostin is negatively influenced by inflammatory processes.

In this study, periostin, which has a critical role on PDL cell survival, decreased under periodontal inflammation. The reduction of periostin levels directly affects repair and formation potential of the periodontal tissue (9,16,17,34). In addition to being an inflammatory marker for periodontal disease, our findings may put forward the idea that increasing periostin levels or preventing periostin reduction may result in faster tissue repair and more attachment gain.

The potential limitation of our study is the lack of periostin level results after periodontal treatment. This could be important to understand the role of periostin in the periodontal healing process.

Conclusion

The results of the present study show the presence of periostin in gingival crevicular fluid. Periostin levels in gingival crevicular fluid were gradually decreased with the progression and severity of periodontal disease. The present study also showed that periostin is associated with periodontal diseases.

When the results in the present study are considered in conjunction with those of previous reports, it might be concluded that the periostin level in gingival crevicular fluid can be considered as a reliable marker in the evaluation of periodontal disease susceptibility and activity.

Conflict of interest

The authors declare that they have no financial relationships related to any products involved in this study.

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