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Gingival crevicular fluid EMAP-II, MIP-1α and MIP-1β levels of patients with periodontal disease

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Abstract

Background: Periodontal diseases may differ, which could be attributed to the factors that might modify the host response to microbial pathogens. The aim of this study was to examine gingival crevicular fluid (GCF) levels of EMAP-II, MIP-1α and MIP-1β in patients with different periodontal diseases (EMAP-II, endothelial-monocyte activating polypeptide; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β).

Methods: Eighty-two subjects were included in this study. GCF samples were collected from 26 patients with generalized aggressive periodontitis (G-AgP), 26 patients with chronic periodontitis (CP), 15 with gingivitis and 15 periodontally healthy subjects. Clinical periodontal parameters were recorded. GCF EMAP-II, MIP-1α and MIP-1β levels were quantified by enzyme immunoassay.

Results: GCF EMAP-II levels of G-AgP group were higher than those of gingivitis and healthy groups ($p < 0.008$). G-AgP group showed a trend for higher GCF EMAP-II levels compared with CP group ($p > 0.008$). G-AgP, CP, gingivitis and healthy groups had comparable GCF MIP-1α and MIP-1β levels.

Conclusions: Our results suggest that elevated GCF EMAP-II could contribute to the pathogenesis of G-AgP. Alternatively, EMAP-II reflects the extent of the inflammatory activity in the periodontal tissues. At this point, MIP-1α and MIP-1β levels in GCF do not seem to play a discriminatory role in periodontitis. Our data document for the first time the essential role of EMAP-II in the pathogenesis of different periodontal diseases.

Key words: endothelial-monocyte activating polypeptide-II; gingival crevicular fluid/analysis; macrophage inflammatory protein-1α; macrophage inflammatory protein-1β; periodontal disease/pathogenesis

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Current knowledge about the pathogenesis of periodontal disease suggests that interactions between host inflammatory response and the pathogenic bacteria involve a complex series of events not completely understood (Kornman et al. 1997). The local host response to these bacteria involves the recruitment of leucocytes and the subsequent release of inflammatory mediators and cytokines that appear to play crucial roles in destroying host tissues (Page 1991).

Endothelial-monocyte activating polypeptide (EMAP)-II, a novel tumour-derived mediator, was originally purified from the supernatant of cultured murine fibrosarcoma cells based on its ability to induce tissue factor (the initiator of coagulation) on the surface of endothelial cells in vitro (Kao et al. 1992). Cloning of its cDNA has shown that it is a unique molecule and that it is distinct from any of the known cytokines (Tas et al. 1997). EMAP-II modulates a wide range of activities resembling those of proinflammatory mediators (Kao et al. 1994). It acts on endothelial cells directly by inducing procoagulant activity and release of von Willebrand factor and can induce apoptosis in endothelial cells (Berger et al. 2000). EMAP-II also enhances the production of adhesion molecules such as E-selectin and P-selectin which mediate selective migration of T-helper type 1 (Th1) cells to the site of inflammation (Kao et al. 1994, Berger et al. 2000). This novel cytokine-like molecule has also a wide range of biological properties on the activity and function of mononuclear and polymorphonuclear phagocytes, which are key components of the host defenses (Kornman et al. 1997). EMAP-II stimulates monocyte and neutrophils chemotaxis. It also causes myeloperoxidase release from neutrophil (Berger et al. 2000). Stimulation of monocytes in culture with

EMAP-II resulted in the production of interleukin 8, tumour necrosis factor-α and tissue factor by monocytes (Tas & Murray 1996).

Cell migration is an essential part of several physiological and pathogenic processes including chronic inflammatory conditions, tumour invasion and wound healing. Chemokines are chemotactic cytokines that direct the recruitment and subsequent activation of specific types of leucocyte populations into inflamed periodontal tissues (Graves 1999, Baggiolini 2001). They are a complex superfamily of about 40 small distinct, but structurally and functionally related secretory proteins (Zlotnik & Yoshie 2000). Chemokines play well-established roles in the inflammatory process as well as in other physiological and pathological activities, such as lymphoid trafficking, Th1/Th2 development and wound healing (Ward & Westwick 1998, Luster 2002). They can be divided into C, CC, CXC and CX_3C subfamilies based on the structural, functional and genetic criteria (Zlotnik & Yoshie 2000). Macrophage inflammatory protein-1α (MIP-1α) and macrophage inflammatory protein-1β (MIP-1β) are both acidic proteins belonging to the CC subgroup of chemokines (Baggiolini 2001). They are secreted by a range of inflammatory cells, including neutrophils, monocytes and lymphocytes as well as non-inflammatory cell types at sites of inflammation (Ward & Westwick 1998). MIP-1α and MIP-1β have regulatory roles during cell-mediated immune responses (Siveke & Hamann 1998). They cause the selective migration of human monocytes and lymphocytes (Schall et al. 1993). While MIP-1α is preferentially chemotactic for the $CD8^+$ T-cell subset, MIP-1β is involved in the migration of $CD4^+$ T-cell subset (Schall et al. 1993).

EMAP-II was shown to participate in a variety of pathogenic immune processes in chronic bacterial and viral diseases (Zheng et al. 2001). Owing to the potential chemotactic and proinflammatory activities, EMAP-II might be a potential mediator of inflammatory responses in periodontal disease. We hypothesize therefore that it may also contribute to the chronic inflammatory conditions in which B cells are the predominating cells. However, EMAP-II levels have not been studied so far in periodontal disease. We now have investigated whether EMAP-II is also involved in periodontal disease and have evaluated gingival crevicular fluid (GCF) EMAP-II levels of patients with different periodontal diseases. MIP-1α and MIP-1β are specifically increased in several inflammatory diseases such as rheumatoid arthritis (Koch et al. 1994). These molecules were previously shown in the inflamed gingival tissues of patients with different periodontal diseases (Gemmell et al. 2001, Garlet et al. 2003). Thus, MIP-1α and MIP-1β might play an important role in the host response by recruiting inflammatory cells into the foci of active inflammation and by inducing the release of other cell mediators. However, little is known about the involvement of MIP-1α and MIP-1β chemokines in different periodontal diseases.

Periodontal diseases may differ in their aetiological 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. The aim of the present study was to examine EMAP-II, MIP-1α and MIP-1β levels in GCF of patients with gingivitis, chronic periodontitis (CP) and generalized aggressive periodontitis (G-AgP) and to test whether EMAP-II, MIP-1α and MIP-1β levels are correlated with clinical parameters.

Materials and Methods

Study population

A total of 82 subjects was included in this study. All subjects were recruited from the Department of Periodontology, School of Dentistry, Ege University, İzmir. Informed consent was obtained from each subject. Complete medical and dental histories were taken from all subjects. None of the subjects had a history of systemic disease and none had received antibiotics or other medicines or periodontal treatment within the past 4 months. The subjects were classified as follows (Armitage 1999).

CP group

The CP group included nine females and 17 males ranging in age from 39 to 63 years with a mean age of 49.9 ± 6.2 years. They had moderate-to-severe alveolar bone loss and clinical attachment loss of $\geqslant 5$ mm and probing pocket depth (PPD) of $\geqslant 6$ mm in multiple sites of all four quadrants of the mouth, but with no evidence of rapid progression.

G-AgP group

The G-AgP group consisted of 12 females and 14 males between the ages of 18 and 39 years (mean of 28.9 ± 6.8 years). These patients demonstrated a generalized pattern of severe destruction and clinical attachment loss of $\geqslant 5$ mm on eight or more teeth; at least three of those were other than central incisors or first molars.

Gingivitis group

The gingivitis group included seven females and eight males with varying degrees of gingival inflammation, but no signs of attachment loss were observed. These patients ranged in age from 17 to 49 years (mean age 31.5 ± 10.2 years).

Healthy group

The healthy group consisted of seven females and eight males who exhibited PPD < 3 mm and no clinical attachment loss, clinical inflammation, sulcular bleeding and radiographic evidence of bone loss (mean age 40.0 ± 14.4 years; range 23–72 years). These individuals were healthy volunteers from the Department of Periodontology.

Determination of periodontal status

To determine the clinical periodontal status, all subjects had a clinical periodontal examination including the measurement of probing depth and clinical attachment loss. Dichotomous measurement of supragingival plaque accumulation and bleeding on probing were also recorded. Measurements were performed at six sites per tooth for whole mouth.

Collection of GCF samples

In the CP and G-AgP groups, GCF samples were collected from one approximal site of a tooth with $\geqslant 6$ mm probing depth. In the gingivitis group, GCF sampling was done from one approximal site of a tooth with bleeding and $\geqslant 2$ mm probing depth. In the healthy group, GCF samples were collected from one approximal site of a tooth with $\leqslant 2$ mm probing depth. 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. Paper strips were carefully inserted into the crevice until mild resistance was felt and left there for 30 s (Lamster et al. 1985). Care was taken to avoid mechanical injury. Strips contaminated with blood were discarded (Cimasoni 1983). The absorbed GCF volume of each strip was determined by electronic impedance (Periotron 8000, ProFlow Inc., Amityville, NY, USA) and placed into sterile eppendorff vials and kept at -40°C until analysed. The readings from the Periotron 8000 were converted to an actual volume (μl) by reference to the standard curve.

Analysis of EMAP-II, MIP-1α and MIP-1β

Eighty-two GCF samples were analysed. Two hundred and fifty microliters of phosphate-buffered solution with 0.05% Tween-20 was added into each vial. GCF was extracted from the paper strips by centrifugation three times at 10,000 $\times$ *g* for 5 min. at 4°C. Aliquots of each GCF sample were assayed by an enzyme immunoassay (CytImmune Science Inc., Rockville, MD, USA) to determine the levels of MIP-1α, MIP-1β and EMAP-II. Procedures were performed according to the instructions in the kit. Results for EMAP-II are converted as total EMAP-II (ng/sample), for MIP-1α as total MIP-1α (ng/sample) and as total MIP-1β (pg/sample) for MIP-1β in the GCF sample. Calculation of the concentration data for each mediator was performed by dividing the amount of each mediator by the volume of the sample.

Statistical analysis

Statistical analysis was performed using nonparametrical techniques. Comparisons were performed using the Kruskal–Wallis test. When there were significant differences ($p<0.05$), post hoc two-group comparisons were assessed with Bonferroni-corrected Mann–Whitney *U*-tests, and *p*-values <0.008 were considered to be statistically significant. In order to analyse the correlations between GCF EMAP-II, MIP-1α and MIP-1β levels and clinical parameters, Spearman's rank correlation analysis was used and $p<0.05$ was considered as significant. All data analyses were performed using a statistical package (Abacus Concepts Inc., Berkeley, CA, USA).

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

	CP	G-AgP	Gingivitis	Healthy
PPD (mm)	7.5 $\pm$ 1.3‡	8.0 $\pm$ 1.4‡	2.7 $\pm$ 0.5*	1.5 $\pm$ 0.5
AL (mm)	8.8 $\pm$ 1.6‡	8.7 $\pm$ 2‡	–	–
% of sites with bleeding on probing	88.5 $\pm$ 32.6*	92.3 $\pm$ 27.2*	100*	0
% of sites with plaque	92.3 $\pm$ 27.2*	80.8 $\pm$ 40.2*	100*	0

*Significant difference from healthy group (Kruskal–Wallis test, $p<0.0001$, Mann–Whitney *U*-test, $p<0.008$). ‡Significant difference from gingivitis and healthy groups (Kruskal–Wallis test, $p<0.0001$, Mann–Whitney *U*-test, $p<0.008$). PPD, probing pocket depth; AL, attachment loss; CP, chronic periodontitis; G-AgP, generalized aggressive periodontitis.

Results

Clinical findings

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

Pocket depth and clinical attachment loss

As expected, the mean probing depth scores of sampling sites in all patient groups were significantly higher than the healthy group ($p<0.008$). CP and G-AgP groups had similar probing depth scores, and they had higher scores compared with the gingivitis group ($p<0.008$). The mean clinical attachment loss of sampling sites in G-AgP and CP groups was significantly higher than in the gingivitis and healthy groups ($p<0.008$). CP and G-AgP groups had similar clinical attachment loss scores ($p>0.008$).

Percentage of sites with bleeding on probing and plaque

All patient groups had significantly higher percentage of sites with bleeding on probing and plaque compared with the healthy group ($p<0.008$). G-AgP, CP and gingivitis groups had similar percentage of sites with bleeding and plaque ($p>0.008$).

Laboratory findings

GCF EMAP II levels

Distribution of the total amount of GCF EMAP-II is shown in Fig. 1. Significant differences were found between study groups ($p=0.0008$). Among the groups, G-AgP group had the highest GCF EMAP-II levels compared with gingivitis and healthy groups (31.6, 9.0 and 8.4 ng/sample; $p=0.0007$ and 0.0017, respectively). GCF EMAP-II levels of G-AgP group were also higher compared with those of CP group, but this did not reach significance (31.6 and 13.3 ng/sample, respectively; $p=0.0158$). CP, gingivitis and healthy groups had a similar total amount of GCF EMAP-II levels ($p>0.008$) (Fig. 1).

When the data were expressed as concentration, again significant differences were found between study groups ($p<0.0008$). The CP and gingivitis groups had lower EMAP-II concentrations compared with that of the healthy group ($p=0.0003$ and 0.0013, respectively). EMAP-II concentration of G-AgP group was comparable with that of the healthy group ($p=0.04$). On the other hand, G-AgP group had higher EMAP-II concentration than the gingivitis group ($p=0.0020$). No significant differences were found between CP and gingivitis groups ($p>0.008$) (Table 2).

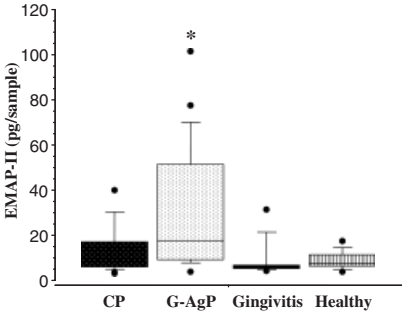

Fig. 1. The mean endothelial-monocyte activating polypeptide (EMAP)-II levels ($\pm$ SD) in gingival crevicular fluid for patients in the chronic periodontitis (CP), generalized aggressive periodontitis (G-AgP), gingivitis and healthy groups. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as dots. Significantly different from gingivitis and healthy groups (Kruskal–Wallis test, $p<0.001$, Mann–Whitney *U*-test, $p<0.008$).

Table 2. GCF, EMAP-II, MIP-1α and MIP-1β concentration of CP, G-AgP, gingivitis and healthy groups (mean ± SD)

	CP	G-AgP	Gingivitis	Healthy
GCF (μl)	0.5 ± 0.2‡,*	0.6 ± 0.2‡,*	0.3 ± 0.1‡	0.2 ± 0.1
EMAP-II (ng/μl)	26.5 ± 16.7‡	58.0 ± 63.6*	30.9 ± 21.3‡	95.5 ± 86.7
MIP-1α (ng/μl)	3.5 ± 2.6‡	3.1 ± 4.2‡	5.5 ± 3.0‡	17.5 ± 13.4
MIP-1β (pg/μl)	234.0 ± 199.5‡	185.5 ± 142.0‡,*	364.3 ± 121.6	1103.9 ± 1008.7

*Significant difference from gingivitis group (Kruskal–Wallis test, $p<0.001$, Mann–Whitney U-test, $p<0.008$) ‡Significant difference from healthy group (Kruskal–Wallis test, $p<0.001$, Mann–Whitney U-test, $p<0.008$). GCF, gingival crevicular fluid; EMAP-II, endothelial-monocyte activating polypeptide; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; CP, chronic periodontitis; G-AgP, generalized aggressive periodontitis.

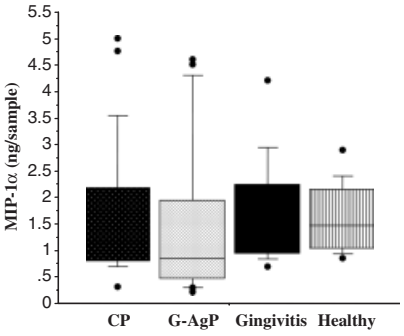

Fig. 2. The mean macrophage inflammatory protein-1α (MIP-1α) levels (± SD) in gingival crevicular fluid for patients in chronic periodontitis (CP), generalized aggressive periodontitis (G-AgP), gingivitis and healthy groups. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as dots.

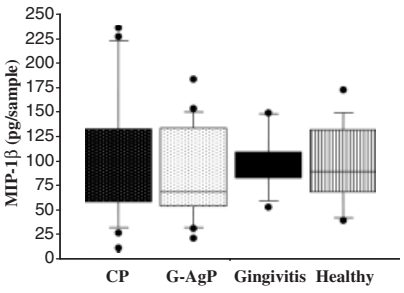

Fig. 3. The mean macrophage inflammatory protein-1β (MIP-1β) levels (± SD) in gingival crevicular fluid for patients in chronic periodontitis (CP), generalized aggressive periodontitis (G-AgP), gingivitis and healthy groups. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as dots.

GCF MIP-1α levels

GCF MIP-1α total amount of the groups is given in Fig. 2. G-AgP, CP, gingivitis and healthy groups had similar GCF MIP-1α total amount (1.48, 1.69, 1.53 and 1.59 ng/sample, respectively) ($p = 0.1787$) (Fig. 2). When the data were expressed as concentration, GCF MIP-1α levels of the G-AgP, CP and gingivitis groups were significantly lower compared with those of the healthy group ($p<0.0008$) (Table 2).

GCF MIP-1β levels

The G-AgP, CP, gingivitis and healthy groups had a similar total amount of GCF MIP-1β (88.1, 102.4, 97.6 and 96.3 pg/sample, respectively, $p = 0.1787$) (Fig. 3). When the data were expressed as concentration, GCF MIP-1β levels of G-AgP and CP groups were significantly lower than those of the healthy group ($p<0.0001$ and $p<0.0001$, respectively) (Table 2). G-AgP group had also significantly decreased GCF MIP-1β levels compared with the gingivitis group ($p = 0.0005$).

There were significant correlations between PPD, CAL and GCF EMAP-II levels ($R = 0.373$, $p = 0.0019$ and $R = 0.433$, $p = 0.0004$, respectively). GCF MIP-1α and EMAP-II levels were also positively correlated ($R = 0.264$, $p = 0.0274$).

Discussion

In the present study, we investigated EMAP-II, MIP-1α and MIP-1β levels in GCF of patients with different periodontal diseases. To our knowledge, this is the first study that examines these mediators in GCF of patients with different periodontal diseases. The result of the present study has shown that GCF EMAP-II levels were significantly elevated in patients with G-AgP compared with other study groups. On the other hand, GCF MIP-1α and MIP-1β levels were similar between study groups. Our present study has provided the first evidence about the presence of EMAP-II in periodontal disease, which is involved in the recruitment of phagocytic cells to sites of programmed cell death in tissues.

Expression of GCF data as total amount per standardized sampling time is a more sensitive way than reporting them as concentration (Lamster et al. 1986). Since collecting a standard amount of GCF is essential to express the results as concentration and also since GCF volume is very small and exhibits wide variations, expressing GCF data as total activity is a more appropriate way, rather than reporting them as concentration (Lamster et al. 1986, Hanioka et al. 2000). In the present study, we collected GCF samples for the same length of time and reported the data as total amount per sample as well as concentration (Lamster et al. 1986, Emingil et al. 2004). Our findings showed that the total amount of EMAP-II, MIP-1α and MIP-1β levels in GCF samples was decisive enough to reveal the difference between study groups.

Leucocyte recruitment and influx into and through the periodontal tissues is dependent on the expression of adhesion molecules on endothelial cells and on the chemotactic factors that are synthesized and released into the inflammatory area (Kornman et al. 1997). It has been previously demonstrated that MIP-1α and MIP-1β were expressed in gingival tissues of patients with mild-to-moderate periodontitis and these levels were correlated with the degree of inflammation (Gemmell et al. 2001). Kabashima et al. (2002) detected MIP-1α- and MIP-1β-producing cells in inflamed gingival samples of patients with chronic periodontitis. It was also shown that MIP-1α+ cells increased as related to the severity of periodontal disease. Garlet et al. (2003) detected higher expression of MIP-1α in inflamed gingival tissue of patients with aggressive periodontitis. On the other hand, in lipopolysaccharide-stimulated white blood cell cultures, the levels of MIP-1α and MIP-1β were found to be similar between periodontitis and healthy subjects (Fokkema et al. 2003). The present study revealed that G-AgP and CP patients have similar MIP-1α and MIP-1β levels in GCF samples when compared with gingivitis and periodontal healthy subjects. We suggest that MIP-1α and MIP-β are expressed in the gingival tissue, but they might not be increasing in GCF

as the intensity of inflammatory response increases in periodontitis. The low MIP-1α and MIP-1β levels in periodontitis groups could also be because of the lack of macrophages as well as subsets of lymphocytes with specific receptors for MIP-1α and MIP-1β (Chapple et al. 1998, Sallusto et al. 2000, Baggiolini 2001). Therefore, our data suggest that MIP-1α and MIP-1β might not be responsible for the selective recruitment of macrophages and T cells in periodontal lesions in which B cells are the predominating cells. On the other hand, in a recent study we have shown elevated GCF MCP-1 and RANTES levels that are also involved in recruitment of these specific cell types into inflamed tissues in G-AgP patients (Emingil et al. 2004).

The interplay between chemokines, pathogenic microorganisms and host inflammatory cells is likely to be the result of the type of the periodontitis. For example, the composition of the inflammatory infiltrate differs in various forms of periodontitis (Lappin et al. 1999). Recent studies have improved our knowledge on the pathophysiology of aggressive periodontitis including aspects of the innate, inflammatory and immune defense systems (Kornman et al. 1997, Tonetti & Mombelli 1999). Although rapid and severe periodontal destruction occurs in aggressive periodontitis compared with CP, we could not demonstrate any difference in GCF MIP-1α and MIP-1β levels between aggressive and CP patients.

There is a continuous recruitment of monocyte/macrophages to the sites of inflammation in periodontal disease. These cells play significant roles in the host defense against pathogenic bacteria through release of different mediators or because of their function as antigen presenting cells and as effector cells with phagocytic function (Kornman et al. 1997). Monocytes from AgP patients were previously found to show differences in mediator release in response to lypopolysaccharide, and it was suggested that monocyte function can predispose individuals to periodontal breakdown (Garrison & Nichols 1989, Shapira et al. 1994). Therefore, a hyperinflammatory monocyte phenotype might represent a risk factor for the aggressive form of periodontal disease (Garrison & Nichols 1989, Shapira et al. 1994). In the present study, G-AgP patients while having low GCF MIP-α and MIP-1β levels, had significantly elevated GCF EMAP-II levels compared with other study groups. Moreover, GCF EMAP-II levels were positively correlated with the severity of periodontal disease. Thus, elevated GCF EMAP-II levels in spite of low GCF MIP-1α and MIP-1β levels in G-AgP patients might indicate the presence of different macrophage phenotypes in inflamed tissues. This might allow effective recruitment of monocyte/macrophages to the sites of inflammation in this form of disease. This proinflammatory and antiangiogenic cytokine is associated with key events of inflammation including monocyte and endothelial activation, expression of adhesion molecules and apoptosis (Kao et al. 1992). Considering the role of EMAP-II, in both activation and recruitment of inflammatory cells, it can be speculated that rapid and severe periodontal destruction in G-AgP patients could be a result of the elevated production of EMAP-II by mononuclear cells in periodontal environment. EMAP-II releasing from activated monocyte at sites of inflammation could indirectly amplify monocyte functions by recruiting additional cells to the inflammatory site and could contribute to the severe periodontal destruction in G-AgP. Alternatively, EMAP-II levels reflect the extent of the inflammatory stage, which may be high in G-AgP because of other as yet unidentified factors.

In conclusion, our data show that patient groups differ considerably in their capacity to release inflammatory mediators in GCF and these results are suggestive that EMAP-II could reflect the extent of the inflammatory activity in the periodontal tissues of G-AaP. Our data document for the first time the essential role of EMAP-II in the pathogenesis of different periodontal diseases.

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