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Gingival crevicular fluid monocyte chemoattractant protein-1 and RANTES levels in patients with generalized aggressive periodontitis

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Background: Local and systemic inflammatory and immune mechanisms may be implicated in the pathogenesis of the aggressive forms of periodontal disease. Chemokines, monocyte chemoattractant protein-1 (MCP-1) and regulated on activation, normal T cells expressed and secreted RANTES (regulated on activation, normal T cells expressed and secreted), are involved in the activation and recruitment of inflammatory and immune cells to the infected sites and thereby mediating a variety of pathophysiological conditions. The aim of the present study was to examine the gingival crevicular fluid (GCF) levels of MCP-1 and RANTES in patients with generalized agressive periodontitis (G-AgP).

Methods: MCP-1 and RANTES levels were investigated in GCF samples of 10 patients with G-AgP and 10 periodontally healthy subjects. Periodontal status was evaluated by measuring probing depth, clinical attachment loss, presence of bleeding on probing and plaque. In the G-AgP group, GCF samples were collected from the two approximal sites;

from one single-rooted tooth and from one first molar tooth with 6 mm probing depth. In the healthy group, GCF samples were collected from one of the single-rooted teeth. GCF MCP-1 and RANTES levels were quantified by enzyme immunoassay.

Results: The G-AgP patients had significantly higher GCF MCP-1 and RANTES levels compared to the healthy group ($p<0.05$). GCF MCP-1 and RANTES levels were positively correlated with both probing depth and clinical attachment loss ($p<0.05$). There was no correlation between GCF MCP-1 and RANTES levels and the percentage of sites with bleeding ($p>0.05$).

Conclusions: The results of the present study suggest that MCP-1 and RANTES could play key roles in both activation and recruitment of inflammatory and immune cells in periodontal environment of G-AgP patients. In conclusion, these CC chemokines may be considered in the biological mechanism underlying the pathogenesis and progression of G-AgP.

Aggressive periodontitis (AgP) comprises a heterogeneous group of periodontal diseases that affects adolescents and young adults. It is characterized by a very rapid loss of periodontal tissue in otherwise clinically healthy subjects (Armitage 1999, Tonetti & Mombelli 1999). Although AgP is comparatively rare in the general population (0.1–5%) (Albandar & Tinoco 2000), there is considerable interest in studies aimed at understanding its etiology and pathogenesis of this disorder. Increasing literature have implicated that AgP are caused by multiple potential risk factors. Previous studies have shown that the presence of pathogenic oral biofilm (Albandar et al. 1997), alteration in host defense cell functions (Altman et al. 1985, Emingil et al. 2001a, Takahashi et al. 2001), disregulated cytokine and inflammatory mediator levels (Shapira et al. 1994, Emingil et al. 2001b, c), and the presence of specific genetic risk factors (Hart 1996, Parkhill et al. 2000) could attribute to the

pathogenesis of the aggressive form of periodontal disease. However, the immunopathogenic mechanisms involved in AgP are not completely elucidated.

Inflammation and tissue destruction is early and continuing events during host-mediated process in response to the bacterial infection (Listgarten 1987). In these processes, mononuclear and polymorphonuclear phagocytes are key components of host defenses (Kornman et al. 2000). Upon stimulation by bacterial products these cells and local resident cells such as fibroblasts synthesize and secrete a wide variety of inflammatory and immune mediators (Page 1991). The appearance of specific types of leukocytes in inflammatory infiltrate might be mediated by cell-specific chemotactic cytokines called chemokines that were recently identified (Kornman et al. 2000, Seymour & Gemmell 2001). These chemoattractant cytokines are responsible for the migration and subsequent activation of specific types of leukocyte populations into inflamed periodontal tissues (Graves 1999). Chemokines are relevant in inflammatory process not only for their role in regulating leukocyte recruitment, but also for other physiological and pathological activities, such as lymphoid trafficking, T helper (Th)1/Th2 development and wound healing (Rossi & Zlotnik 2000, Baggiolini 2001). Chemokines are produced by activated monocyte/macrophage, endothelial cells and fibroblasts (Graves 1999, Rossi & Zlotnik 2000, Baggiolini 2001). They mainly exert their effects on target cells by binding to specific receptors on the surface of a variety of cell types (Sallusto et al. 2000). They consist of about 40 small distinct, but structurally and functionally related secretory proteins (Baggiolini 2001). Four subfamilies of chemokines can be distinguished on the basis of structural, functional and genetic criteria, of which there are two major subgroups. CXC and CC chemokines (Graves 1999, Rossi & Zlotnik 2000, Sallusto et al. 2000, Baggiolini 2001). Monocyte chemoattractant protein-1 (MCP-1) is one of the members of CC chemokines (Rollins 1996). It shows significant chemotactic activity to cells of the monocytes/macrophage lineage, and is different from other chemoattractants in that it is relatively specific for monocytes (Rollins 1996, Baggiolini 2001). MCP-1 expression has been implicated in several diseases with chronic inflammation such as atherosclerosis, rheumatoid arthritis and delayed-type hypersensitivity reactions (Rossi & Zlotnik 2000). It has been previously shown that MCP-1 is synthesized in inflamed gingival tissues by endothelial cells and mononuclear phagocytes of patients with chronic periodontitis (Yu et al. 1993, Yu & Graves 1995). Regulated on activation, normal T cells expressed and secreted (RANTES) also belongs to the CC chemokine subfamily, and has a unique spectrum of chemotactic activity (Graves 1999). It is a potent chemoattractant for eosinophils, monocytes, natural killer cells and Th 1 cells, while it is not effective on Th 2 cells (Siveke & Hamann 1998, Ward & Westwick 1998, Graves 1999). Thus, RANTES may 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. Gamonal et al.

(2000a, b, 2001) showed the first evidence on the presence of the RANTES in the gingival crevicular fluid (GCF) of patients with chronic periodontitis. Hence, MCP-1 and RANTES were shown to be important mediators of the host response in chronic adult periodontitis. However, to our knowledge, MCP-1 and RANTES levels of aggressive periodontitis patients have not been studied so far. The present study, therefore, aimed to examine the MCP-1 and RANTES levels in GCF of patients with generalized aggressive periodontitis and to correlate these levels with clinical parameters.

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Study population

A total of 20 subjects were 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 generalized AgP (G-AgP) patients were classified as follows (Armitage 1999).

Generalized aggressive periodontitis group

The G-AgP group consisted of eight females and two males in the age range 17–36 (mean of 28.5±7.09) years. These patients demonstrated a generalized pattern of severe destruction and attachment loss of at least 5 mm on eight or more teeth; at least three of those were other than central incisors or first molars.

Healthy group

The healthy group consisted of five females and five males with no clinical evidence of periodontal disease (mean age, 33.0±8.93 years). These individuals were healthy volunteers from the Department of Periodontology.

Clinical studies

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 G-AgP group, GCF samples were collected from the two approximal sites: from one single rooted tooth and from one first molar tooth with 6 mm probing depth. In the healthy group, GCF samples were collected from one of the single-rooted teeth. 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 6000, ProFlow, Inc., Amityville, NY, USA). and placed into a sterile Eppendorf vials (Eppendorf, Cambridge, UK) and kept at −40°C until analysis. The readings from the Periotron 6000 were converted to an actual volume (μl) by reference to the standard curve.

Analysis of MCP-1 and RANTES

One hundred and fifty microliters of phosphate-buffered solution with 0.05% Tween-20 was added to each vial. GCF was extracted from the paper strips by centrifugation three times at 10,000g for 5 min at 4°C. Aliquots of each GCF sample were assayed by an enzyme immunoassay (Cytimmune Science Inc., Collage Park, MD, USA) to determine the levels of MCP-1 and RANTES. Procedures were performed according to the instructions in the kit. Results for MCP-1 were received as total MCP-1 (pg/sample) and for RANTES as total RANTES (ng/sample) in the GCF sample. Calculation of the concentration data for each chemokine was performed by dividing the amount of each chemokine by the volume of the sample.

Statistical analysis

Patient mean values of clinical parameters for two pocket sites per patient were calculated. MCP-1 and RANTES levels obtained from both pocket sites were also averaged and the patient was used as the unit of analysis. Statistical analysis was performed using non-parametrical techniques. The Mann–Whitney U-test was used to analyse differences in clinical and biochemical parameters between G-AgP and healthy groups. The Spearman rank correlation analysis was used to analyse the correlations between GCF and MCP-1 and RANTES levels and clinical parameters. All data analysis was performed using a statistical package (Abacus Concepts Inc., Berkeley, CA, USA).

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Clinical findings

The mean clinical data for the sampling areas are shown in Table 1. As expected, mean probing depth and clinical attachment loss of sampling sites in the G-AgP group were significantly higher than those of the healthy group (p=0.0001, p<0.0001, respectively). G-AgP had significantly higher percentage of sites with bleeding and plaque compared to the healthy group (p=0.0134, 0.0223, respectively). The GCF volume was significantly higher in the G-AgP group compared to the healthy group (p=0.0005). Healthy group was similar in age to the G-AgP patients (p=0.2104).

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

	G-AgP group	Healthy group
probing depth	8.4±2.01*	1.6±0.52
clinical attachment loss	9.5±1.9*	0±0
% of sites with bleeding	75±43*	0±0
% of sites with plaque	60±51*	10±32
GCF (µl)	0.54±0.19*	0.19±0.10

*

Statistically significantly different from healthy group (p<0.05).

G-AgP=generalized aggressive periodontitis.

Laboratory findings

Distributions of the total amount and concentration of GCF MCP-1 are shown in Figs 1 and 2, respectively. The G-AgP group had significantly higher total amount of GCF MCP-1 compared to that of the healthy group (p<0.05). When the data were expressed as concentration, no significant differences were found between study groups (p>0.05).

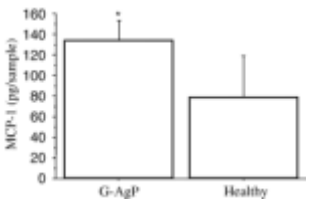

Figure 1. The mean monocyte chemoattractant protein-1 (MCP-1) levels (±standard deviation) in gingival crevicular fluid of patients with generalized aggressive periodontitis (G-AgP) and healthy groups. *Significantly different from healthy group ($p<0.05$).

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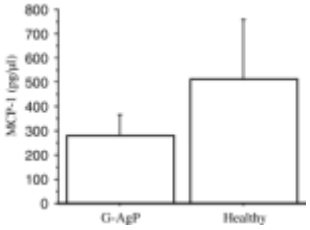

Figure 2. The mean monocyte chemoattractant protein-1 (MCP-1) concentration (±standard deviation) in gingival crevicular fluid of patients with generalized aggressive periodontitis (G-AgP) and healthy groups.

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The total amount and concentration of GCF RANTES of both G-AgP and healthy groups are given in Figs 3 and 4, respectively. The G-AgP group had significantly higher total amount of GCF RANTES compared to that of the healthy group ($p<0.05$). When the data were expressed as concentration, no significant differences were found between study groups ($p>0.05$).

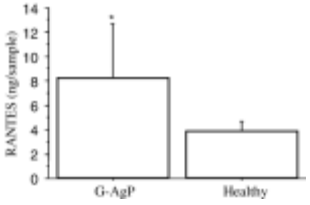

Figure 3. The mean regulated on activation, normal T cells expressed and secreted (RANTES) levels (±standard deviation) in gingival crevicular fluid of patients with generalized aggressive periodontitis (G-AgP) and healthy groups. *Significantly different from healthy group ($p<0.05$).

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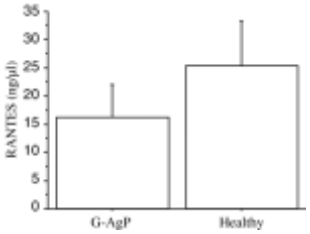

Figure 4. The mean RANTES concentration (±standard deviation) in gingival crevicular fluid of patients with generalized aggressive periodontitis (G-AgP) and healthy groups.

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The correlation between total amount of chemokines and clinical parameters is presented in Table 2. Total amount of MCP-1 was positively correlated with both probing depth and clinical attachment loss (r=0.557, 0.733; p<0.05, respectively). RANTES total amount was also positively correlated with both probing depth and clinical attachment loss (p=0.538, 0.611; p<0.05, respectively). There was no correlation between total amount of GCF MCP-1 and the percentage of sites with bleeding (r=0.317; p>0.05). However, significant correlation was found between GCF MCP-1 total amount and the percentage of sites with plaque (p=0.827; p<0.05). No correlation was found between total amount of GCF RANTES and the percentage of sites with bleeding and plaque (r=0.461, 0.369; p>0.05, respectively). Strong correlations were present between GCF volume and MCP-1 and RANTES total amount (r=0.685, 0.528; p<0.05, respectively).

Table 2. Correlation between total amount of MCP-1 and RANTES and clinical parameters

Clinical parameters	MCP-1	RANTES
probing depth	0.557*	0.538*
clinical attachment loss	0.733*	0.611*
% of sites with bleeding	0.317	0.461
% of sites with plaque	0.827*	0.369
GCF (µl)	0.685*	0.528*

*

p<0.05.

MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation, normal T cells expressed and secreted.

Recent studies have improved our knowledge of the defects in different parts of the host response in aggressive periodontitis including aspects of the innate, inflammatory and immune defense systems. However, we still do not have enough knowledge about the pathological mechanisms linking abnormalities in host response to the development of aggressive periodontitis. In the present study, the presence of elevated MCP-1 and RANTES levels in GCF from G-AgP patients suggest that these chemokines could play a role in the pathogenesis of G-AgP.

Expression of GCF data as total amount per standardized sampling time is a more sensitive way than reporting them as concentration (Lamster et al. 1985, Tsai et al. 1995). Since collecting a standard amount of GCF is essential to express the results as concentration and also 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. 1985, 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, Tsai et al. 1995, Chung et al. 1997, Emingil et al. 2001a, b). Our findings showed that total amount of MCP-1 and RANTES levels in GCF samples were decisive enough to reveal the difference between G-AgP and healthy groups.

Leukocyte recruitment and influx into and through the periodontal tissues are 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. 2000). MCP-1 plays crucial role in mediating the selective migration and recruitment of monocytes into the crevicular area (Yu & Graves 1995). Activated monocyte/macrophages themselves are able to express MCP-1, leading to the accumulation of additional monocytes. It is believed that specific macrophage phenotype is involved in the degenerative and reparative process during tissue turnover as well as inflammation (Kreutz et al. 1992). Inflammatory macrophage phenotype identified at sites of inflammation has a number of functions (Sorg 1991). Upon activation of bacterial stimuli, they secrete a wide variety of cytokines and growth factors (Page 1991, Shapira 1994, Kornman et al. 2000), they act as an antigen presenting cell, and phagocytoses the invading bacteria (Page 1991, Kornman et al. 2000). Garrison & Nichols (1989) were first to hypothesize that monocyte function can predispose individuals to periodontal breakdown. They suggested that hyperinflammatory monocyte phenotype might represent a risk factor for AgP (Garrison & Nichols 1989). It has been previously demonstrated that MCP-1 was expressed in gingival tissues of patients with mild-to-moderate periodontitis and these levels have been demonstrated to be correlated with the degree of inflammation (Yu et al. 1993, Yu & Graves 1995). Moreover, macrophages, fibroblasts and endothelial cells in the inflamed periodontal tissues have been found to be

their main source (Yu & Graves 1995). In another study, it has been demonstrated that monocyte chemotactic activity in crevicular fluid increases with severity of disease and MCP-1 was expressed as the predominant cytokine of gingival tissues (Hanazawa et al. 1993). In contrast to these results, Gemmell et al. (2001) found that only few leukocytes expressed MCP-1 in the periodontal tissues. The present study revealed that G-AgP patients have significantly elevated MCP-1 levels in GCF samples when compared to periodontal healthy subjects. It is possible that MCP-1 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. Thus, MCP-1 may function either directly or synergistically with other inflammatory mediators, thereby involving in the amplification and continuation of the inflammatory response, and in the ensuing tissue destruction.

RANTES share many of its functions with other chemokines (Graves 1999). RANTES have now been implicated in the complex interaction of the several aspects of T-lymphocyte biology that could contribute to the symptoms of periodontal disease (Ward & Westwick 1998). This CC chemokine can mediate selective recruitment of T-cell subsets in periodontal inflammation (Ward & Westwick 1998). RANTES is an efficient chemoattractant of Th1 cells that predominantly control cell-mediated immune responses. It induces Th1 cell migration in a dose-dependent manner, while Th2 cells are not attracted by this chemokine (Siveke et al. 1998). Thereby, it was stated that they could mediate the complex network of interactions within the immune system by controlling the balance between proinflammatory and anti-inflammatory T cell subsets (Gamonal et al. 2001) Recent studies have shown the presence of high levels of GCF RANTES in patients with chronic periodontitis and these levels have been shown to be related to the active attachment loss and advanced periodontal destruction (Gamonal et al. 2000a, b, 2001). In the present study, elevated RANTES levels were present in GCF of patients with G-AgP. Likewise, there was a significant positive correlation between GCF MCP and RANTES levels and the clinical signs of periodontal tissue breakdown. This might suggest that elevated GCF MCP and RANTES levels could contribute to the severity of tissue destruction in G-AgP patients.

Overall, increased GCF MCP-1 and RANTES levels in G-AgP patients suggest that these CC chemokines are important mediators in the pathogenesis of G-AgP. Considering the role of MCP-1 and RANTES levels 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 MCP-1 and RANTES by mononuclear cells in periodontal environment. Therefore, these chemokines may play a key role in local amplification of the immune response as well as in severe periodontal tissue breakdown in the pathogenesis of

aggressive periodontitis. In conclusion, hyperactivity of mononuclear cells in G-AgP patients could be related to locally produced MCP-1 and RANTES levels.

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