Increased Levels of Serum and Gingival Crevicular Fluid Monocyte Chemoattractant Protein-1 in Smokers With Periodontitis

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Background: Smoking alters the host response, including vascular function, neutrophil/monocyte activities, adhesion molecule expression, antibody production, and cytokine and inflammatory mediator release. Monocyte chemoattractant protein-1 (MCP-1) is involved in the activation and recruitment of inflammatory and immune cells to infected sites, thereby mediating a variety of pathophysiologic conditions. Estimation of serum and gingival crevicular fluid (GCF) MCP levels could be a reliable indicator of periodontal disease activity. Hence, the objective of this study is to analyze the serum and GCF MCP-1 levels of smokers and never-smokers with periodontitis and compare them with those in periodontally healthy individuals.

Methods: A total of 90 participants (30 periodontally healthy individuals, 30 non-smoking individuals with periodontitis, and 30 smokers with periodontitis) formed the study group. Serum and GCF samples were collected, and MCP-1 levels were estimated using enzyme-linked immunosorbent assay.

Results: Mean MCP-1 levels in serum and GCF were found to be highest in smokers with periodontitis, followed by the periodontitis group, and then by the healthy controls. The values were statistically significant ($P < 0.001$).

Conclusions: It can be concluded that the high levels of both serum and GCF MCP-1 found in smokers could explain the severity of periodontitis in smokers. More longitudinal, prospective studies will help to verify the observations of the present study. Further research in this direction could reveal reliable markers to forecast the progression of periodontitis in high-risk groups. *J Periodontol 2013;84:■■■-■■■.*

KEY WORDS

Chemokine CCL2; gingival crevicular fluid; periodontal attachment loss; periodontitis; serum; smoking.

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Smoking is a major risk factor in the development and progression of periodontal diseases. Smokers have a greater risk than non-smokers in exhibiting more extensive and more severe alveolar bone loss.[1,2] Smoking can mask the early signs of periodontal disease by suppressing the inflammatory response, which can result in diagnostic problems, especially in young people with early periodontitis. Gingival pockets tend to be greater in anterior segments and maxillary lingual sites. There is often recession in the maxillary and mandibular anterior segments. The marginal gingiva tends to be thickened and fibrotic, with a rolled margin.[3]

Nicotine can cause vasoconstriction of the peripheral blood vessels and thus reduce the clinical signs of gingivitis. This induced vasoconstriction could contribute to impaired gingival blood flow and decrease the amount of oxygen and blood constituents that reach the gingiva.[4] The capacity to remove tissue waste products would also be reduced, leading to tissue damage or to a compromised immune response. Smokers are more susceptible to developing severe periodontal disease at a young age. Periodontitis progresses rapidly in smokers and responds poorly to treatment. Treated smokers tend to exhibit attachment loss with time while

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undergoing maintenance therapy. These findings are particularly interesting in light of analyses that have shown that 85% to 90% of patients with refractory periodontitis are current smokers.[5]

It is well documented that smoking can impair various components of the host response and immune system.[6-12] These effects include inhibition of neutrophil chemotaxis and phagocytosis,[6] inhibition of cellular immunity, and suppression of local antibody production. Cigarette smoke products, such as nicotine, can also incorporate into root surfaces and into fibroblasts, which can inhibit the reattachment of fibroblasts to the root surface.[7] Smoking not only can impair the normal host response but also can stimulate the destructive arm of the host response.[8,9] Cigarette smoke can stimulate neutrophil oxidative bursts, with the release of oxygen species that are potentially destructive to tissue, such as superoxide, hydrogen peroxide, and hydroxyl radicals.[10] Not only do these products of the oxidative burst have direct cytotoxic effects on the cells of the periodontium, but they can also alter the tissue protease/antiprotease balance in favor of tissue-destructive activity.[13]

During periodontal disease, host inflammatory cells are recruited and inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), are released from fibroblasts, macrophages, connective tissue, and junctional epithelial cells. As a result, host-derived enzymes, such as matrix metalloproteinase (MMP)-8, MMP-9, and calprotectin, are released by polymorphonuclear leukocytes (PMNs) and osteoclasts, leading to connective tissue and alveolar bone degradation.

Chemokines are able to recruit and activate phagocytes.[14] Monocyte chemoattractant protein-1 (MCP-1), a potent mediator of both monocyte recruitment and activation, differs from most other chemokines in its high degree of cell-type specificity. MCP-1 is a significant chemoattractant for specific subsets of lymphocytes, such as monocytes and macrophages.[15] MCP-1 is expressed by monocytes, endothelial cells, fibroblasts, and T cells, primarily on the basal layer of epithelial tissues. MCP-1 has been implicated in the pathogenesis of various systemic diseases, such as granulomatous disease, rheumatoid arthritis, heart disease, bone trauma, and asthma.[16,17] MCP-1 is related to the stages of oral infection by means of its monocyte chemotactic ability, which has been known to increase with increasing inflammation.[18]

Tonetti et al.[19] showed marked MCP-1 expression in gingival biopsies and in the inflammatory infiltrate from diseased periodontal sites. High levels of MCP-1 have been reported in the gingival crevicular fluid (GCF) of patients with both aggressive periodontitis (AgP) and chronic periodontitis (CP).[20,21] The MCP activity of the crevicular fluids of adult patients with periodontitis increases with an increase in severity of this disease, also suggesting that MCP-1, a chemoattractant specific for monocytes produced by the gingival tissues, could be involved in the mechanism of monocyte recruitment from the circulating pool into periodontal tissues.[22]

Recent studies estimating the serum and GCF levels of MCP-1 showed a proportionate increase with increasing severity of periodontitis.[23,24] So far, no studies have been conducted in smokers with periodontitis. Traditional clinical measurements used for periodontal diagnosis, such as probing depth (PD), bleeding on probing (BOP), and clinical attachment level (CAL), are often of only limited usefulness because they are indicators of previous periodontal disease rather than present disease activity. Knowing disease activity might help in early intervention in patients with the disease. Although several epidemiologic cross-sectional studies have found an association between periodontitis and smoking, evidence on the mechanism involved in the severity of periodontitis in smokers is scarce. Hence, in the present study, the serum and GCF MCP levels are estimated to assess periodontal disease activity in smokers and never smokers with periodontitis. The results were compared with periodontally healthy individuals.

MATERIALS AND METHODS

Study Population

A total of ninety (30 participants in each group) systemically healthy smoking and non-smoking males (aged 25 to 55 years) were enrolled in this study during September 2010 to May 2011. The individuals included in the test groups were selected from patients who were referred to periodontology clinics for the diagnosis and treatment of periodontitis. The control group was derived from individuals who attended restorative dental clinics and from the staff and graduate students of the College of Dentistry. The periodontal status of the patients and control group was assessed according to the classification of the American Academy of Periodontology.[25] Smoking status was determined based on daily consumption.[26] Approval of the ethics committee was obtained from the College of Dentistry Research Center, King Saud University, Riyadh, Saudi Arabia.

Sixty patients with PD ≥4 mm and CAL ≥2 mm in at least 30% of their teeth were designated as the study group. Individuals who smoked a minimum of 20 cigarettes per day for ≥2 years were included in the smoker periodontitis group (n = 30). Individuals who used other forms of smoking along with

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cigarettes were excluded from the study. The remainder of the patients, who never smoked, were assigned to the never-smoker periodontitis group (n = 30). A total of 30 individuals who had clinically healthy gingiva and no CAL (≤3 mm periodontal PD) were also included as the healthy group.

An informed written consent form was obtained from all the participants enrolled in the study. The participants were screened clinically, biochemically, and biophysically to exclude individuals with any systemic illnesses. The following exclusion criteria were also used: 1) age <25 or >55 years; 2) <22 permanent teeth; 3) chronic use or use in the last 2 weeks of any type of medication; 4) presence of any chronic medical condition, including diabetes and viral, fungal, or bacterial infections; 5) presence of any medical condition within the previous 2 weeks, including flu, upper respiratory infection, allergy, skin disorder, or sinus problem; 6) any form of physical trauma experienced within the previous 2 weeks; 7) presence of AgP, periodontal abscess, or necrotizing ulcerative gingivitis or periodontitis; 8) periodontal treatment and/or antibiotic therapy received within the preceding 3 months; 9) any type of dental work or tooth extraction performed in the last 2 weeks; 10) active carious lesions; 11) former smokers (had quit smoking); and (12) refusal to sign the consent form.

Clinical Periodontal Examination

An extensive medical history was recorded, based on a written questionnaire and on interviews of 20 to 30 minutes in duration. For each patient, a set of complete examinations of extra-oral and intra-oral full-mouth clinical parameters and the individual number of teeth present, excluding the third molars, were documented. One clinical examiner (SA) performed all the clinical measurements. Calibration exercises for probing measurements were performed in five patients before the actual study. The intra-examiner agreement was good, with a κ value of 0.82. PD and CAL were measured at the mesial, distal, buccal, and lingual aspects of each tooth. Smoking history was assessed according to a standardized interview and a self-reported questionnaire. Smoking exposure was expressed in terms of consumption (number of cigarettes per day) and duration (years).

Venous Blood Samples

Ten milliliters of venous blood were collected from each patient by venipuncture in the antecubital fossa without excessive venous stasis. Blood samples were collected into blood collection tubes[§] containing no anticoagulant. The samples were then centrifuged at 1200 × *g* for 10 minutes. The serum samples were collected and stored in plastic vials at –70°C.

Collection of GCF Samples

For GCF sampling, tooth numbers 3, 9, 19, and 25 were chosen for both the healthy and periodontitis groups. If one of the participants was missing one of these teeth, then the nearest tooth was used for sampling. Before GCF sampling, supragingival plaque was removed from the interproximal surfaces with a sterile curet, and these surfaces were dried gently using an air syringe. The area was carefully isolated to prevent samples from being contaminated by saliva. Care was taken to avoid mechanical injury of the gingival tissues. The GCF samples were collected by placing the microcapillary pipette at the entrance of the gingival sulcus, gently touching the gingival margin.[24] The collected GCF samples were immediately transferred to airtight plastic vials and stored at –70°C until assayed. From each group, a standardized volume of 1 μL was collected, using the calibration on white, color-coded, 1- to 5-μL calibrated, volumetric microcapillary pipettes.[‖]

Estimation of Serum MCP-1

The MCP-1 level in serum was measured by means of a sandwich enzyme-linked immunosorbent assay using a kit.[¶] Briefly, the serum samples and standards (recombinant human MCP-1) were incubated (2 hours at room temperature) in wells precoated with primary anti-human MCP-1 antibody. After incubation, the wells were washed three times, and horseradish peroxidase–conjugated polyclonal antibodies against MCP-1 were added and again incubated for 2 hours at room temperature. Finally, tetramethylbenzidine substrate solution was added. After 30 minutes, the reaction was stopped with stop solution (2 M sulfuric acid). The absorbance was measured at 450 nm as the primary wavelength, and optical density values were obtained for both the standards (provided with the kit) and the samples. Duplicate measurements were performed for each sample to minimize errors. The concentration of MCP-1 in the tested samples was estimated using the reference calibrated standard curve, obtained by plotting the optical density values of the standards against their concentrations.[23]

The GCF samples were expelled from the microcapillary pipettes with a jet of air, using the blower provided with the pipettes, and by further flushing them with a fixed amount of the diluent. After appropriate dilution of the GCF samples, the samples and standards (provided with the kit) were added to the appropriate wells in a microtiter plate.

§ Vacutainer, Becton Dickinson, Mountain View, CA.
‖ Sigma-Aldrich, St. Louis, MO.
¶ MCP-1 ELISA, R&D Systems, Minneapolis, MN.

First Preview

Table 1.

Characteristics of Study Population

Group	Age (years)	CAL (mm)	PD (mm)	Serum MCP-1 (pg/mL)	GCF MCP-1 (pg/μL)
Healthy	34.53 ± 6.19	0	0	153.75 ± 38.26	19.97 ± 2.93
Non-smokers with periodontitis	34.57 ± 7.76	3.78 ± 0.41	5.64 ± 0.42	451.07 ± 59.80	71.20 ± 6.34
Smokers with periodontitis	34.8 ± 8.19	3.79 ± 0.42	5.66 ± 0.41	527.47 ± 60.75	96.43 ± 5.77

Data are mean ± SD; n = 30 in each group. PD = probing depth; MCP-1 = monocyte chemoattractant protein-1.

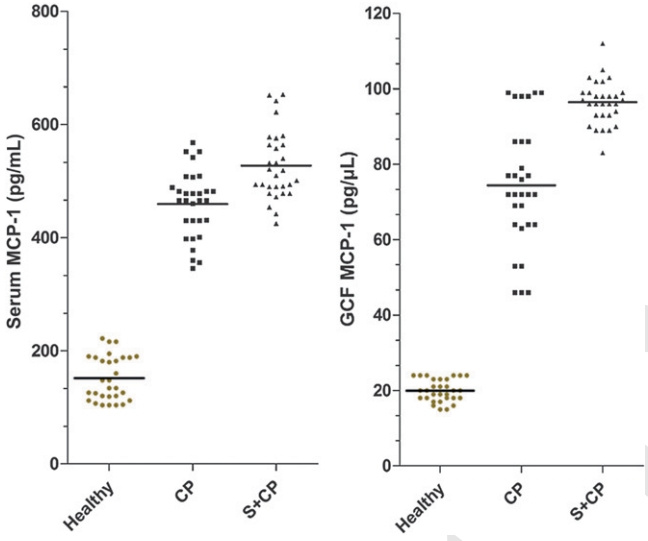

Figure 1.
Serum and GCF MCP-1 levels in periodontally healthy individuals, non-smoking patients with CP, and smokers with CP (S+CP).

Statistical Analyses

Statistical analysis of the data were performed with software.# Means and standard deviations for age, number of teeth, plaque index, BOP, PD, CAL, and serum and GCF MCP-1 levels of the participants (healthy, never-smoker periodontitis, and smoker periodontitis) were analyzed. Differences among the three study groups for all of the variables were determined with one-way analysis of variance (ANOVA). When overall ANOVA showed statistical significance, post hoc testing (Tukey-Kramer multiple comparisons test) was performed to explore the differences between any two groups. *P* values <0.05 were considered significant. Student *t* test was used to analyze the mean differences of PD and CAL between the two periodontitis groups.

RESULTS

The distribution of individuals according to age is presented in Table 1. PD and CAL were measured at six sites, and the mean values were calculated for each individual in the smoker and never-smoker groups with periodontitis (Table 1). The smoker group had slightly higher PD and CAL than the periodontitis group, but this difference was not statistically significant.

Serum and GCF MCP-1 levels are shown in Table 1 and Figure 1. The mean MCP-1 concentration in serum was found to be highest in the smokers with periodontitis (527.47 ± 60.75), compared to 451.07 ± 59.80 in the non-smoker periodontitis group. The difference was found to be statistically significant. Both groups had relatively higher MCP-1 levels than the healthy controls. Similarly, mean GCF MCP-1 levels were also significantly higher in smokers (96.43 ± 5.77) than in the never-smoker periodontitis group (71.20 ± 6.34) and controls (*P* <0.001).

DISCUSSION

Cigarette smoking is a significant risk factor for the initiation and progression of periodontal disease. Studies have consistently demonstrated negative periodontal effects and greater probabilities of established periodontal disease among smokers compared with non-smokers.[27] Inhibition of the inflammatory destructive effects of smoke on periodontal tissues, coupled with the possible vasoconstrictive effects of tobacco products, might explain the attachment loss, bone loss, and tooth loss in former smokers, but paradoxically, there are fewer signs of inflammation and greater reduction of BOP.[28,29] Clinical trials have also shown poorer responses to non-surgical and surgical periodontal treatment in smokers.[30] Although smoking is accepted as a strong modifying factor for periodontal diseases, there is a lack of consensus regarding its precise mechanism in the pathogenesis of these diseases. Several studies have shown that smoking impairs various aspects of the innate and adaptive immune responses, including altered neutrophil function, antibody production, altered fibroblast activity, vascular factors, and inflammatory mediator production.[7-12,31] Smoking affects many aspects

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of the host's immune response; therefore, it is probable that these effects on the immune system could be the primary contributing factors of smoking to the pathogenesis of periodontal disease.[8]

Cytokines, such as IL-1, IL-6, IL-8, and TNF-α, are considered to be involved in the host response to periodontal disease as mediators of tissue breakdown. Increased levels of these cytokines have been observed in the GCF of patients with periodontal disease.[32,33] The chemokines are a family of structurally related glycoproteins with potent leukocyte activation and/or chemotactic activity. MCP-1 is chemotactic for monocytes and is known to regulate the expression of proinflammatory cytokines, such as IL-1 and IL-6. MCP-1 is also a potent activator of human basophils, inducing degranulation and the release of histamines and, thus, likely contributing to the inflammatory responses observed in periodontitis.

In the present study, a significant increase is found in the serum and GCF levels of MCP-1 in smokers and non-smokers with periodontitis. This observation is in agreement with earlier studies that showed higher serum[23] and GCF MCP-1 levels in patients with periodontitis compared to healthy controls.[20,21,24] However, a study by Tymkiw et al.[34] showed a relatively lower amount of MCP-1 in the GCF of selected sites with periodontitis in smokers. The site selection among smokers might have influenced their results. In the present study, smokers are grouped, and GCF is collected from selected teeth and pooled to estimate the MCP-1 in smokers and non-smokers with periodontitis and in healthy individuals.

Although a fourth group of smokers without periodontitis may have been useful as a positive control group, no individuals fit these criteria (20 cigarettes per day and 2 years duration). This is one limitation of the study.

CONCLUSIONS

From the observations of the present study, it can be concluded that both serum and GCF MCP-1 levels are significantly increased in smokers with periodontitis compared to non-smoking patients with periodontitis and healthy controls. More longitudinal, prospective studies are necessary to confirm the observations of the present study. Further research in this direction could reveal reliable markers to forecast the risk for diseases, such as periodontitis, that lack any sensitive prognostic indicators.

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