Journal of Oral Science, Vol. 55, No. 1, 57-62, 2013

Original

Association of stem cell factor and high-sensitivity C reactive protein concentrations in crevicular fluid and serum in patients with chronic periodontitis with and without type 2 diabetes

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(Received October 2, 2012; Accepted January 10, 2013)

Abstract: The aim of the present study was to clarify whether there is any correlation between the levels of high-sensitivity C reactive protein (hs-CRP) and stem cell factor (SCF) in serum and gingival crevicular fluid (GCF) of patients with chronic periodontitis (CP) with and without type 2 diabetes mellitus (DM). A total of 40 subjects were divided into 3 groups: 10 periodontally healthy subjects (Group 1), 15 CP patients (Group 2), and 15 type 2 DM patients with CP (Group 3). Levels of hs-CRP and SCF in GCF and serum were quantified using different techniques. The clinical outcomes evaluated were gingival index (GI), probing depth (PD) and clinical attachment level (CAL), and the correlations of the two inflammatory mediators with clinical parameters were evaluated. The levels of these inflammatory mediators increased continuously from group 1 to group 2, and to group 3. The serum levels of both hs-CRP and SCF were correlated with PD in patients with CP ($P < 0.05$). SCF levels were correlated with PD in Group 3 ($P < 0.05$). The fact that the levels of hs-CRP and SCF were highest in DM patients with CP suggests that the presence of a systemic condition has a profound effect on the levels of inflammatory mediators, both locally at sites of periodontal disease, and elsewhere.
(J Oral Sci 55, 57-62, 2013)

Keywords: chronic periodontitis; inflammation; diabetes mellitus; stem cell factor; C reactive protein.

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Introduction

Human periodontal diseases are characterized by chronic inflammatory destruction of periodontal connective tissues as a result of complex interactions between periodontal pathogens and the host defence mechanism (1). Diabetes is a metabolic disease that, due to disturbances in insulin production, leads to abnormal fat, sugar, and protein metabolism and resulting hyperglycemia that can ultimately induce multiple system pathologies (2). Diabetic patients have a 2-3-fold higher risk of developing severe periodontitis and progressive periodontal disease (3). In fact periodontitis has been referred to as the sixth most frequent complication of diabetes mellitus (DM) (4). Diabetics with periodontal infection have a greater risk of worsening glycemic control over time in comparison with diabetics without periodontitis (5).

The inflammatory host response is a fundamental feature of periodontal disorder, and is regarded as the primary cause of periodontal breakdown (6). Inflammation is significantly pronounced in the presence of diabetes, insulin resistance and hyperglycemia (7). Several inflammatory biomarkers such as cytokines, chemokines and bone-related factors have been found to play a very important role in the pathogenesis of both chronic periodontitis (CP) and type 2 DM (8,9). Studies of the links between periodontal disease and cardiovascular disease have indicated that C-reactive protein (CRP) is one the inflammatory biomarkers involved (10). CRP is an acute-phase reactant synthesized by the liver in response to the inflammatory cytokines IL-6, IL-1, and tumor necrosis factor-alpha (TNF-α). The level of circulating CRP is a marker of systemic inflammation, and is associated with periodontal disease (11). Another inflammatory mediator, stem cell factor (SCF; also

known as kit ligand, mast cell growth factor, or steel factor) is a hematopoietic cytokine that exerts its biologic effects by binding to its receptor, *c*- kit (12,13). Gingival crevicular fluid (GCF) is a dynamic fluid that emerges between the surface of the tooth and the epithelial integument. SCF levels in GCF are higher in patients with CP than in healthy controls (14). SCF levels are also elevated in tissue sections from diabetic Sprague-Dawley rats in comparison to healthy rats (15). Although the sources of hs-CRP (16) and SCF (17) production differ, both play an important role in mediating inflammation and can be good indicators of systemic as well as local inflammation resulting from CP and type 2 DM. Since CRP is a well-established, thoroughly tested gold-standard molecule for assessment of inflammatory load, and SCF has also been found to play a role in inflammation, we hypothesized that the levels of both molecules might be increased in patients with CP and DM. The aim of the present study was to evaluate and correlate the levels of the two inflammatory biomarkers, hs-CRP and SCF, in GCF and serum with periodontal clinical parameters, and also between each other. To date, no study has examined the correlation between the levels of hs-CRP and SCF in GCF and serum in patients with CP with and without type 2 DM.

Materials and Methods

A cross-sectional study of 3 months duration was designed. The study was conducted from July 2011 until September 2011 and involved 40 (20 males, 20 females) age- and gender-balanced subjects divided into three groups. Group 1 comprised 10 healthy controls, Group 2 comprised 15 patients with CP, and Group 3 comprised 15 patients with well controlled type 2 DM and CP. The subjects were selected from among patients referred to the Department of Periodontics, Government Dental College and Research Institute (GDCRI), Bangalore, India. Written informed consent was obtained from all of the subjects, who agreed to participate voluntarily. Ethical clearance was approved by Institutional Ethics Committee and Review Board, GDCRI, Bangalore.

Inclusion criteria

The inclusion criteria for the study subjects were an age of 25-45 years, presence of at least 20 natural teeth with a diagnosis of CP based on clinical parameters such as probing depth (PD), clinical attachment level (CAL) (18) and gingival index (GI) (19), a body mass index (BMI) of 18.5-22.9 kg/m^2 and a waist circumference of < 90 cm (men) or < 80 cm (women) (WHO) (20), and no periodontal therapy within the preceding six months; diabetic patients had well-controlled type 2 diabetes, classified according to the 2010 criteria of the American Diabetic Association (ADA) and the level of glycated hemoglobin (21).

Exclusion criteria

The exclusion criteria for the study subjects were consumption of tobacco or alcohol in any form, presence of chronic kidney disease, atherosclerosis, heart disease or hypertension, any other systemic disease capable of affecting the course of periodontal disease, any course of medication affecting periodontal status, or periodontal therapy within the 6 months preceding the study.

Grouping criteria

Group 1 (healthy) comprised 10 subjects with clinically healthy periodontium, GI = 0 (absence of clinical inflammation), PD ≤ 3 mm, and CAL = 0, with no evidence of bone loss on radiographs. Group 2 (patients with type 2 DM and chronic periodontitis) comprised 15 subjects who had signs of clinical inflammation, GI > 1, more than 30% of sites showing PD ≥ 5 mm, and CAL ≥ 3 mm, and HbA1c ≤ 7% with radiographic evidence of bone loss. Group 3 (CP without type 2 DM) comprised 15 subjects who had signs of clinical inflammation, GI > 1, more than 30% of sites showing PD ≥ 5 mm, and CAL ≥ 3 mm, with radiographic evidence of bone loss. Only subjects with well controlled (HbA1c ≤ 7%) type 2 DM were selected based on the ADA criteria for diagnosis of diabetes (21).

Clinical evaluation of subjects

Group allocations and sample site selection were performed by the chief coordinator (ARP). A certified examiner (NK) performed the clinical evaluation and determined the clinical parameters including PD, CAL, and GI using a periodontal probe (University of North Carolina-15 periodontal probe, Hu-friedy, Chicago, IL, USA). The same examiner (NK) also performed the radiographic evaluations and collected the GCF samples.

Site selection and GCF collection

Two test sites for GCF sample collection were selected based on the highest scored sites in the oral cavity. In Groups 2 and 3, the two sites showing the greatest CAL and signs of inflammation, along with radiographic confirmation of bone loss assessed by intraoral periapical radiographs taken by the paralleling technique, were selected for sampling. One of the two sites selected per subject was used for hs-CRP and the other for SCF analysis. In the healthy group, to standardize site selec-

tion and obtain an adequate fluid volume, sampling was predetermined to be from the mesio-buccal region of the maxillary right first molar, in the absence of which the left first molar was sampled. First, the selected site was cleaned, isolated and air-dried using sterile cotton rolls, and the supragingival plaque was removed gently using a Gracey curette (Universal Gracey curette #4R/4L, Hu-friedy, Chicago, IL, USA) to avoid contamination of the paper strips. The paper strips (periopaper, Ora Flow Inc., Amityville, NY, USA) were placed gently at the entrance of the gingival sulcus/crevice until light resistance was felt (22), taking care to avoid mechanical injury, and left in place for 60 seconds. The absorbed GCF volume of each strip was determined by electronic impedance (Periotron 8000, ProFlow Inc.). Samples that were suspected to be contaminated with blood and saliva were excluded. After collection of the gingival fluid, the two periopaper strips per site that had been used to absorb GCF from each subject were pooled and then immediately transferred to microcentrifuge tubes (premarked with the biomarker name) containing 400 µL of phosphate-buffered saline and stored frozen at -70°C for subsequent analysis. Periodontal treatment (scaling and root planing) was performed for CP subjects at the same appointment after collection of GCF by the operator (PN).

Blood collection

Two milliliters of blood was collected from the antecubital fossa by venipuncture using a 20-gauge needle with a 2-mL syringe and immediately transferred to the laboratory. The blood sample was allowed to clot at room temperature, and after 1 h serum was separated from blood by centrifuging at 3,000 × g for 5 min. The serum was immediately transferred to a plastic vial and stored at -70°C until the time of assay.

hs-CRP analysis

The samples for CRP were measured immunoturbimetrically. The microcentrifuge tubes containing the periopaper strips and plastic vials containing serum were transferred to the laboratory for immunoturbedimetric analysis. Serum was used undiluted. The measurement range for CRP was 0-220 mg/L, the normal value being < 5 mg/L (23).

SCF analysis

The samples were assayed for SCF using an enzyme-linked immunosorbent assay (ELISA) kit in accordance with the manufacturer's instructions. The GCF sample tubes were first homogenized for 30 seconds and centrifuged for 5 minutes at 1,500 × *g* to yield an eluate. The eluate was then used as a sample for ELISA estimation of SCF.

Each sample was assayed using a commercially available ELISA kit (human SCF, Assay pro, St. Charles, MO, USA) in accordance with the manufacturer's instructions. Color development was monitored using a microplate reader until an optimum optical density was reached, then a stop solution was added and the optical density was read at 450 nm. The total SCF was determined in nanograms (ng), and the calculation of the concentration in each sample was performed by dividing the amount of SCF by the volume of the sample (ng/mL).

Statistical analysis

Analysis of variance (ANOVA) was carried out for a comparison of GCF and serum hs-CRP and SCF levels between the groups. Using Pearson's correlation coefficient, the relationships between hs-CRP and SCF concentrations and the clinical parameters were analyzed using a software program. Differences at $P < 0.05$ were considered statistically significant. The intra-group correlation of serum and GCF concentrations of hs-CRP and SCF was also performed using Pearson's correlation

Table 1 Data for the study population (mean ± S.D.)

Study Group	Group 1 (*n* = 10)	Group 2 (*n* = 15)	Group 3 (*n* = 15)
Age (in years)	32.70 ± 3.68	31.47 ± 3.62	32.20 ± 3.89
Sex (M/F)	5/5	7/8	8/7
GI	-	2.12 ± 0.34	2.27 ± 0.32
PPD	2.00 ± 0.47	6.20 ± 0.78	7.80 ± 1.31
CAL	1.90 ± 0.87	6.80 ± 1.54	7.90 ± 1.10
Serum SCF (ng/mL)	3.69 ± 0.91	5.39 ± 0.78	6.95 ± 0.48
GCF SCF (ng/mL)	3.09 ± 0.82	5.52 ± 0.89	5.54 ± 0.79
Serum hs-CRP (mg/L)	2.28 ± 0.78	3.74 ± 0.63	6.25 ± 0.70
GCF hs-CRP (mg/L)	0.42 ± 0.30	0.80 ± 0.16	1.00 ± 0.19

Table 2 Results of ANOVA comparing the mean serum and GCF concentrations of SCF and hs-CRP among the three groups

Study groups	SCF				Hs-CRP			
	Serum		GCF		Serum		GCF	
	F value	*P* value	F value	*P* value	F value	*P* value	F value	*P* value
Group 1 Group 2 Group 3	46.96	< 0.001*	28.33	< 0.001*	73.73	< 0.001*	16.32	< 0.001*

*Significant at $P < 0.001$

Table 3 Correlations of serum and GCF concentrations of SCF and hs-CRP in each group using Spearman's rank correlation coefficient test

Group	Serum		GCF	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Group 1	0.334	0.344	0.123	0.733
Group 2	0.843	0.002*	0.594	0.070
Group 3	-0.001	0.996	0.534	0.111

*Significant at $P < 0.05$

Table 4 Relationship of SCF and hs-CRP levels to clinical parameters

	Parameters	Group 1	Group 2	Group 3
SCF				
Serum	GI	-	0.020*	0.896
	PD	0.005*	0.798	0.013*
	CAL	0.996	0.996	0.071
GCF	GI	-	0.071	0.371
	PD	0.009*	0.986	0.003*
	CAL	0.494	0.780	0.247
Hs-CRP				
Serum	GI	-	0.024*	0.770
	PD	0.397	0.657	0.930
	CAL	0.330	0.970	0.445
GCF	GI	-	0.318	0.316
	PD	1.000	0.633	0.546
	CAL	0.154	0.542	0.483

*Significant at $P < 0.05$

coefficient.

Results

Table 1 shows the data (mean ± SD) for the study population. The mean hs-CRP and SCF concentrations in both serum and GCF were highest in Group 3, followed by Group 2, and were lowest in Group 1. To determine the equality of means between the three groups, ANOVA was carried out (Table 2). Significant differences in the serum and GCF levels of hs-CRP and SCF were found between the three groups. The serum and GCF levels of SCF were found to be significantly correlated ($P < 0.05$) with PD in Group 3, and the serum SCF level was significantly correlated with GI in Group 2. The serum concentration of hs-CRP was significantly correlated with GI in Group 2. The Pearson correlation coefficient test was applied to evaluate the correlation between the serum level of hs-CRP and the serum level of SCF, and also the GCF values. Table 3 shows the correlation coefficients and p values. The correlations between the GCF and serum levels of the two biomarkers and clinical parameters are presented in Table 4.

Discussion

This cross-sectional study attempted to evaluate the GCF and serum levels of hs-CRP and SCF in CP patients with and without type 2 DM. The results clearly indicated increasing GCF and serum levels of the two inflammatory mediators in patients with CP and those with type 2 DM with CP, relative to healthy controls. We decided to investigate the role of SCF in the pathogenesis of CP with and without DM by comparing and correlating its levels

with a marker of inflammation (in this case, hs-CRP) that has been clearly proven to play a role in inflammation in various systemic diseases, as discussed later. It was anticipated that such a comparison and correlation would further validate the role of the new molecule (in this case, SCF) being tested. The observed increase in hs-CRP, an acute-phase reactant protein and one of the most important markers of inflammation from Group 1 to Group 2, was in accord with a previous study by Pradeep et al. in which CRP levels in GCF and serum were measured using ELISA (24), and also with a study by Noack et al. (11) The mean GCF concentration of CRP in the periodontitis group was very much higher than that in the gingivitis group and healthy group. Similarly, the mean serum concentration of CRP in the periodontitis group was higher than that in the gingivitis group, which in turn was higher than that in the healthy group. Thus, the mean concentration of CRP was elevated in GCF and serum with the simultaneous progression of periodontal disease.

The mean GCF level of hs-CRP was 0.42 mg/L and the serum concentration was 2.28 mg/L in periodontally healthy individuals. These results are within the normal range, which is 0-5 mg/L (23). The increase in values from health to CP and further in type 2 DM with CP is similar to a previous study but the levels are higher than those found in that study (25). The higher GCF and serum values in the CP group could have been due to higher mean values of periodontal parameters recorded in this series of subjects. The higher mean serum values in patients with type 2 DM and CP could have been due to the fact that the corresponding group in that study had coronary artery disease with CP and were receiving statin therapy for the same and statins lower CRP (25). The highest serum levels of hs-CRP in CP with type 2 DM could be attributed to the presence of type 2 DM in which hs-CRP levels were found to be elevated in a previous study (26).

SCF, also known as mast cell growth factor, steel factor, or c-kit ligand, is a multifunctional growth factor for hematopoietic progenitors and mast cells (27). There is mounting evidence that overexpression of stem cell factor may be implicated in proliferation, inflammation, and wound healing (28). SCF is produced constitutively by endothelial cells and fibroblasts (17). The increase in the GCF levels of SCF from Group 1 to Group 2, and to Group 3, corresponds to a previous study by Honsawek et al. in which the GCF levels of SCF were found to be highly elevated in patients with CP as compared to those with chronic gingivitis and healthy individuals (14).

The concentration of SCF in normal human serum is, on average, 3.3 ± 1.1 ng/mL (29). The serum concentration of SCF in healthy subjects in the present study was found to lie within the normal range. The GCF concentration was the lowest in periodontally healthy individuals, followed by those with CP, and was highest in patients with CP and type 2 DM. This could be related to the fact that SCF is a proliferative growth factor for mast cells. Increased production of SCF by cells residing in periodontal tissues could lead to overproduction of mast cells in those tissues, and increased mast cell density has been clearly implicated in periodontal disease severity (30). The higher production of SCF in the serum of CP patients as compared to healthy subjects could have resulted from spillover of the increased GCF SCF levels from diseased periodontal tissues, leading to a concomitant increase in serum SCF. The further increase in the levels of SCF in serum and GCF of CP patients with type 2 DM could have been due to the fact that SCF has been shown to be produced by bone marrow cells contributing to new vessel formation in DM (15,31).

The present study showed that the serum levels of hs-CRP SCF were significantly correlated with CP ($P < 0.05$). Though care was taken to exclude any subjects with known systemic illness, the higher serum levels of both molecules might indicate some element of systemic inflammation due to unknown/undetected underlying systemic disease, in addition to local periodontal inflammation in some subjects.

To our knowledge this is the first study to have evaluated and correlated hs-CRP and SCF in CP patients with and without type 2 DM. One limitation of this cross-sectional study was the small sample size evaluated. Further long-term longitudinal studies with larger sample sizes should be undertaken to validate these results.

Based on the present findings, it can be proposed that hs-CRP and SCF play roles in the pathogenesis of periodontal disease. The highest levels of the two inflammatory mediators in CP patients with type 2 DM may indicate an active inflammatory process, both locally in periodontal tissues, and also systemically.

Acknowledgments

The authors are thankful to Mr. Manjunath Sharma, Institute of Statistical Services, Bangalore, for performing the statistical analysis. The authors report no conflicts of interest.

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