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Short Communication

Gingival crevicular fluid levels of oncostatin M in periodontal conditions

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ABSTRACT

Objective: The initial study has reported the increased level of oncostatin M (OSM) in gingival crevicular fluid (GCF) of chronic periodontitis patients, and no study has reported its level in gingivitis patients. Therefore, the present study has been carried out to measure the level of OSM in gingival crevicular fluid of gingivitis and chronic periodontitis patients and effect of periodontal therapy on OSM concentration in GCF of chronic periodontitis patients. *Design:* A total of 60 subjects were divided into three groups ($n = 20$) based on gingival index (GI), probing pocket depth (PPD) and clinical attachment loss (CAL): healthy (group I), gingivitis (group II) and chronic periodontitis (group III). A fourth group (group IV) consisted of 20 subjects from group III, 8 weeks after treatment i.e. scaling and root planning (SRP). GCF samples were collected from all the groups to estimate the levels of OSM using Enzyme-Linked ImmunoSorbentAssay (ELISA). *Result:* The mean OSM concentrations in GCF was found to be the highest in group III i.e. 0.81 ± 0.33 pg/μl. The mean OSM concentration in group I was 0.0689 ± 0.022 pg/μl in GCF and in group IV it was 0.0943 ± 0.037 pg/μl). For group II the mean OSM concentration in GCF 0.125 ± 0.023 pg/μl, was fell in between the concentrations obtained in groups I and III. *Conclusions:* The greater the amount of periodontal tissue destruction there is substantial increase in GCF OSM concentrations. Since, OSM levels are positively correlated with PPD and CAL, it can be considered as an inflammatory biomarker in periodontal diseases.

1. Introduction

Periodontal diseases are chronic inflammatory diseases triggered in response to the periodontopathogens and its clinical outcome is highly influenced by the host immune response. Components of microbial dental plaque have the capacity to activate the local host response by inducing the initial infiltrate of inflammatory cells including lymphocytes, macrophages and polymorphonuclear leukocytes (PMNs) [1]. Sites with periodontal inflammation contain plasma cells, T-lymphocytes and macrophages; the later two cell populations producing many types of cytokines [2]. Cells that produce cytokines include the macrophages/monocytes, dendritic cells, lymphocytes, neutrophils, endothelial cells, and fibroblasts. These cytokines have important pro-inflammatory effects and are related to periodontal tissue destruction that involves the stimulation of bone resorption and induction of tissue degrading proteinases [3]. As several pro-inflammatory cytokines and chemokines, responsible for tissue destruction are secreted in GCF, it possess a great potential for serving as diagnostic or prognostic markers of the periodontal health, disease and healing after therapy. Again the collection of GCF is a relatively simple, noninvasive, and site specific procedure [4].

Oncostatin M (OSM) a gp 130 cytokine, is a member of the IL-6 family of cytokines, which includes IL-6, IL-11, leukemia inhibitory factor (LIF) and ciliary neurotrophic factors (CNTFs), has been demonstrated to fulfill Koch's postulates as an inflammatory mediator [5]. In the cascade of periodontal inflammation, human T cells and monocyte lineages can synthesize and secrete large amounts of OSM and IL-6 in response to bacterial products. The gp 130 family of cytokines appeared to play a key role in regulating periodontal bone resorption by acting on both osteoblasts and osteoclasts through autocrine IL-6 and paracrine receptor activator of nuclear factor-kappa B ligands (RANKL) regulation [6]. OSM induces prolonged expression of P-selectin [7] and E-selectin in human endothelial cells, which modulate leukocyte adhesion. OSM also induces endothelial cell expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and OSM plays an important role for recruiting leukocytes to inflammatory sites [5].

Furthermore, OSM alone may stimulate the production of IL-6, or it may act synergistically with IL-6 or TNF-α to, respectively, up-regulate the production of metalloproteinases [MMPs] or augment IL-6 production [8,9]. Recently, study by Lin et al. demonstrated that increased amounts of OSM and IL-6 in the GCF were positively correlated to the severity of periodontitis [10]. Increased

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OSM expression and its role in pathogenesis of various systemic diseases such as rheumatoid arthritis [11], multiple myeloma [12], atherosclerosis [5], wound biology [13], obesity [14] and Kaposis sarcoma [15] have been evaluated. Also, role for OSM have been investigated in periodontal diseases (other than periodontitis) like apical periodontitis [16] and cyclosporin induced gingival overgrowth [17].

Till date, no study has reported GCF OSM levels in various stages of periodontal disease like periodontal health, gingivitis and chronic periodontitis and nor correlated OSM levels before and after periodontal therapy. Thus, in view of the aforementioned findings, this clinico-biochemical study was undertaken to estimate the GCF OSM levels in subjects with clinically healthy periodontium, gingivitis and chronic periodontitis and subsequently, after initial periodontal therapy i.e. scaling and root planning (SRP) in the chronic periodontitis subjects.

2. Materials and methods

The study population consisted of 60 subjects (30 women and 30 men; age range: 25–40 years) attending the outpatient clinic of the Department of Periodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India. Written informed consent was obtained from those who agreed to participate voluntarily and ethical clearance was obtained from the Institution's Ethical Committee. *Inclusion criteria* include subjects: 1. with age group 25–40 years; 2. who have not received periodontal therapy, within preceding 6 months; and 3. should have at least 20 natural teeth. *Exclusion criteria* include subjects: 1. with systemic diseases like rheumatoid arthritis, diabetes, hypertension, tumors, gross oral pathology, etc.; 2. the post menopausal and pregnant women; 3. aggressive periodontitis patients; 4. taken any medication like antibiotics/anti-inflammatory drugs or received periodontal therapy in the preceding 6 months; and 5. smokers.

Each subject underwent a full mouth periodontal probing and charting, along with periapical radiographs using the long-cone technique. Radiographic bone loss was recorded dichotomously (presence or absence) to differentiate chronic periodontitis patients from other groups. Furthermore, no delineation was attempted within the chronic periodontitis group based on the extent of alveolar bone loss.

Based on the gingival index (GI) [18], probing pocket depth (PPD), clinical attachment loss (CAL) and radiographic evidence of bone loss, subjects were categorized into three groups. Group I (healthy) consisted of 20 subjects with clinically healthy periodontium, with a GI = 0, PPD $\leqslant$ 3 mm and CAL = 0, with no evidence of bone loss on radiograph. Group II (gingivitis) consisted of 20 subjects who showed clinical signs of gingival inflammation, GI > 1, PPD $\leqslant$ 3 mm and had no attachment loss (CAL = 0) or radiographic bone loss. Group III (chronic periodontitis) consisted of 20 subjects who had signs of clinical inflammation, GI > 1, a PPD $\geqslant$ 5 mm in 30% of sites and CAL > 2 mm in 30% of sites with radiographic evidence of bone loss. Patients with chronic periodontitis (group III) were treated with a nonsurgical approach (i.e. scaling and root planing) and GCF samples were collected from the same sites 6–8 week after the treatment to constitute group IV (the after-treatment group). Descriptive statistics of the study groups has shown in Table 1.

2.1. Site selection and fluid collection

All the clinical and radiological examinations, group allocation and sampling site selection were performed by one examiner and the samples were collected on the subsequent day by a second examiner. This was undertaken to prevent the contamination of GCF with blood associated with the probing of inflamed sites. Only one site per subject was selected as a sampling site in gingivitis and periodontitis groups (groups II and III) whereas, in the healthy group to ensure the collection of an adequate amount of GCF, multiple sites with absence of inflammation were sampled. In gingivitis patients, the site with the highest clinical signs of inflammation (i.e. redness, bleeding on probing and edema) in the absence of clinical attachment loss was selected. In chronic periodontitis patients, sites with CAL > 2 mm were identified using a University of North Carolina (UNC-15) periodontal probe (CAL was measured from the cementoenamel junction to the depth of the periodontal pocket). The site showing the highest PPD ($\geqslant$5 mm) with CAL and signs of inflammation (i.e. bleeding on probing) along with radiographic confirmation of bone loss was selected for the sampling and the same test site was selected for sampling after treatment. On the subsequent day, after gently drying the area, supragingival plaque was removed without touching the marginal gingiva and the area was isolated using cotton rolls to avoid saliva contamination. GCF was collected by placing the microcapillary pipette at the entrance of the gingival sulcus, gently touching the gingival margin. From each group, a standardized volume of 1 μl was collected using the calibration on white color-coded 1–5 μl calibrated volumetric microcapillary pipettes (Sigma–Aldrich, St. Louis, MO, USA). Each sample collection was allotted a maximum of 10 min and the sites which did not express any GCF within the allotted time were excluded. This was carried out to ensure atraumatism, and the micropipettes that were suspected to be contaminated with blood and saliva were excluded from the study. The collected GCF samples were immediately transferred to airtight plastic vials and stored at minus 70 °C until assayed.

2.2. OSM assay

The samples were then assayed for OSM levels by using Human OSM ELISA Kit obtained from KRISHJEN BioSystems, Mumbai, India (Catalog No. KB 100-H Oncostatin M). Samples were analyzed at Department of Microbiology, Kempegowda Institute of Medical Sciences, Bangalore, India.

Table 1
Descriptive statistics of the study population showing mean, standard deviation and range for the age, GI, CAL, PPD and GCF OSM concentrations. Clinical data are related to the sites selected for GCF sampling.

Groups		Age (years)	GI	CAL (mm)	PPD (mm)	GCF OSM (pg/μl)
Group I (n = 20)	Mean ± SD	28.20 ± 4.31	0	0	1.9 ± 0.73	0.068 ± 0.022
	Range (min, max)	(25, 39)	–	–	(1, 3)	(0.035, 0.100)
Group II (n = 20)	Mean ± SD	26.90 ± 2.84	1.9 ± 0.53	0	2.6 ± 0.51	0.125 ± 0.023
	Range (min, max)	(25, 34)	(1.1, 2.8)	–	(2, 3)	(0.102, 0.175)
Group III (n = 20)	Mean ± SD	34.30 ± 6.61	2.15 ± 0.43	5.8 ± 1.13	7.5 ± 1.84	0.81 ± 0.33
	Range (min, max)	(25, 42)	(1.40, 2.8)	(5, 8)	(6, 11)	(0.33, 1.52)
Group IV (n = 20)	Mean ± SD	34.30 ± 6.61	0.33 ± 0.49	2.9 ± 1.85	3.9 ± 2.51	0.094 ± 0.037
	Range (min, max)	(25, 42)	(0.0, 1.4)	(1, 6)	(2, 8)	(0.055, 0.160)