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Gingival crevicular fluid and serum vascular endothelial growth factor: Their relationship in periodontal health, disease and after treatment

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ABSTRACT

Objectives: The levels of vascular endothelial growth factor (VEGF) in gingival crevicular fluid (GCF) correlate well with clinical parameters of periodontal disease. The present study was designed to assess the relationship between clinical parameters and VEGF levels of the GCF from inflammed gingiva, periodontitis sites and treated periodontitis sites, and to correlate them to the serum VEGF levels.
Design: Thirty, gender and age-matched subjects were divided into three groups- health, plaque-induced gingivitis and chronic periodontitis group, based on gingival index score and clinical attachment level. The fourth group consisted of 10 subjects in the periodontitis group, 6–8 weeks after nonsurgical treatment. Total of eighty samples (forty GCF and forty serum samples) were collected and quantified for VEGF using enzyme immunoassay.
Results: The highest mean GCF and serum VEGF concentrations were observed in the periodontitis group and lowest in the healthy group. Periodontitis group showed significant reduction in VEGF levels in both GCF and serum samples after treatment.
Conclusions: GCF and serum VEGF levels increased progressively with the disease severity and decreased after treatment of periodontal disease. Moreover GCF and serum VEGF levels correlated positively with clinical periodontal parameters. The study indicates the key role of VEGF in periodontal disease as a inflammatory biomarker.

1. Introduction

Periodontal disease is a chronic inflammatory disease of the highly vascularized supporting tissues of the teeth, with episodes of active destruction and periods of quiescence [1]. Angiogenesis, the formation of new blood vessels emerging from pre-existing endothelial vasculature, is an integral component of the pathogenesis of various disorders, such as tumor growth, diabetic retinopathy and chronic inflammation, including periodontitis [2]. The periodontal vasculature is affected profoundly during the periodontal disease progression [3].

VEGF, also known as vascular permeability factor or vasculotropin, is a 45-kd homodimeric glycoprotein with potent vascular permeability and angiogenic effects [4,5]. VEGF causes these effects primarily by promoting endothelial cell proliferation, secretion of proteolytic enzymes, chemotaxis, and migration [6]. Moreover it increases the permeability of fluids and proteins 50,000 times more than histamine [7]. The VEGF-A gene has been mapped to human chromosomes 6p21.3, which can be alternatively spliced to generate at least 5 distinct isoforms of 121, 145, 165, 189, and 206 amino acids (termed VEGF-A121, VEGF-A145, VEGF-A165, VEGF-A189, and VEGF-A206, respectively) [8,9]; a variant of VEGF-A165, namely, VEGF-A165b, has also been identified [10].

Studies regarding the role of VEGF in the pathogenesis of periodontal disease have showed conflicting results, suggesting that VEGF expression is increased [1,11], decreased [12], or unaffected [3] during disease. In our previous study, we have shown that the gingival crevicular fluid (GCF) VEGF levels show an increasing pattern from health to plaque-induced gingivitis. In addition, significant reduction in GCF VEGF levels were noticed after initial nonsurgical treatment (Scaling and root planing) of chronic periodontitis suggesting that VEGF levels in GCF may be considered a marker of periodontal destruction [13]. As an extension to the aforementioned study, the present study was designed to correlate the GCF VEGF levels with the serum VEGF levels, in patients with clinically healthy periodontium, in patients with plaque-induced gingivitis and chronic periodontitis, and after scaling and root planing (SRP) of periodontitis subjects.

2. Material and methods

The study population consisted of 30 subjects, whose age (30–50 year) and sex (15 women and 15 men) were matched,

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attending the outpatient clinic of the Department of Periodontics, Government Dental College and Research Institute. Ethical clearance for the study protocol was obtained from the ethical committee, Government Dental College, Bangalore. The patients were explained regarding the study procedure and written informed consent was obtained from those who agreed to participate voluntarily in this study. Patients were excluded from the study if they had aggressive periodontitis, smoking habit, alcoholism, gross oral pathology, tumors, or any other systemic disease that can alter the course of periodontal disease; were taking medication, such as phenytoin, cyclosporins, or calcium channel blockers; or had taken antibiotics, anti-inflammatory drugs, or received periodontal therapy in the preceding 6 months. The duration of the study was 3 months.

2.1. Clinical examination

Each subject underwent a full-mouth periodontal probing and charting, and periapical radiographs were taken using the long-cone technique. Radiographic bone loss was recorded dichotomously [presence or absence of crestal bone loss of 6 mm (distance measured from cementoenamel junction to alveolar bone crest)] to differentiate patients with chronic periodontitis from other groups. Furthermore, no delineation was attempted within the chronic periodontitis group based on the extent of alveolar bone loss.

Subjects were initially categorized into three groups based on the gingival index (GI), [14], clinical attachment level (CAL), and radiographic evidence of bone loss. Group 1 (healthy) consisted of 10 subjects with clinically healthy periodontium, GI = 0 (absence of clinical inflammation), and CAL = 0, with no evidence of bone loss on radiographs. Group 2 (plaque-induced gingivitis) consisted of 10 subjects who showed clinical signs of gingival inflammation, GI > 1, and had no CAL or radiographic bone loss. Group 3 (chronic periodontitis) consisted of 10 subjects who had signs of clinical inflammation, GI > 1, and CAL $\geqslant$ 2 mm, with radiographic evidence of bone loss. Patients with chronic periodontitis (group 3) were treated with SRP, and GCF and serum samples were collected 6–8 weeks after the treatment to constitute group 4 (the after-treatment group). SRP was completed in two sessions within 24 h under local anesthesia, if needed [15]; using periodontal hand instruments (Gracey curets, Hu-Friedy, Chicago, IL, USA). The patients were given instructions for self-performed plaque-control measures: twice-daily toothbrushing using the modified Bass brushing technique with a soft toothbrush and regular toothpaste and interdental cleaning once-daily.

2.2. Site selection and fluid collection

All clinical examinations, radiographs, group allocations, and sampling site selections were performed by one examiner and the samples were collected on the subsequent day by a second examiner. This was to prevent contamination of GCF with blood associated with the probing of inflammed sites. Only one site per subject was selected for GCF sample collection. In patients with plaque-induced gingivitis (group 2), the site with the greatest clinical signs of inflammation (i.e., redness, bleeding on probing, and edema), in the absence of CAL was selected. In patients with chronic periodontitis (group 3), the site showing the greatest CAL using a Williams graduated periodontal probe, and signs of inflammation, along with radiographic confirmation of bone loss, was selected for sampling; the same test site was selected for sampling after treatment (group 4). On the subsequent day, after gently drying the area, supragingival plaque was removed without touching the marginal gingiva, and the area was isolated with 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. A standardized volume of 1 μl was collected using the calibration on white color-coded 1–5 l calibrated volumetric microcapillary pipettes (Sigma–Aldrich, St. Louis, MO, USA). Each sample collection was allotted a maximum of 10 min, and the sites that 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 air-tight plastic vials and stored at −70 °C until assayed.

2.3. Serum collection

Two milliliters (ml) of blood was collected from the antecubital fossa by venipuncture using 20-gauge needle with 2 ml syringes and immediately transferred to the laboratory. Blood samples were allowed to clot at room temperature and after 1 h it was centrifuged at 3000*g* for 5 min to separate serum component. Serum was extracted from blood and stored in aliquotes at −70 °C till the time of assay procedure.

2.4. VEGF assay

The samples were assayed for VEGF levels using commercially available quantitative sandwich enzyme immunoassay kit (R&D Systems, Minneapolis, MN USA) as instructed by the manufacturer. After appropriate dilution of samples, 100 μl of samples were added to the wells which were covered with the adhesive strip and incubated at 37 °C for 2 h. Each well was aspirated and washed; the process was repeated twice for a total of three washes. About 200 μl VEGF conjugate was added to each well, covered with a new adhesive strip, and incubated at 37 °C for 2 h. Aspiration/wash was repeated. A 200 μl substrate solution was added to each well, protected from light, and incubated at room temperature for 25 min. To terminate the reaction, 50 μl stop solution was added to each well. The plate was tapped gently to ensure thorough mixing. The optical density of each well was determined within 30 min using a microplate reader set to 450 nm. The concentrations of VEGF in the tested samples were estimated using the standard curve plotted using the optical density values with the standards.

2.5. Statistical analysis

All data were analyzed using a software program (SPSS statistical package, SPSS, Chicago, IL, USA). Sample size of 10 has been taken which was found to be adequate to achieve power with confidence interval of 80% at p value <0.05. The Kruskal–Wallis test, Mann–Whitney U-test and Wilcoxon signed rank test were carried out to compare VEGF levels between groups. The Spearsman's rank correlation test was used to compare VEGF levels between groups and clinical parameters. $p < 0.05$ were considered statistically significant.

3. Results

All the samples, in each group tested positive for the presence of VEGF. VEGF concentrations obtained for all the groups for both GCF and serum are shown in Table 1. The mean VEGF concentrations in GCF and serum was observed to be the highest in group III i.e., 96.75 pg/ml and 227.20 pg/ml respectively and lowest in group I i.e., 40.67 pg/ml and 96.25 pg/ml, respectively. The mean VEGF concentration in group II (56.07 pg/ml and 190.0 pg/ml) and group IV (53.8 pg/ml and 163.7 pg/ml) fell between the highest and lowest values. The Kruskal–Wallis test showed that the difference in levels of VEGF among these groups was statistically significant at