Effect of Non-Surgical Periodontal Treatment on Gingival Crevicular Fluid and Serum Endocan, Vascular Endothelial Growth Factor-A, and Tumor Necrosis Factor-Alpha Levels

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Background: Periodontitis is a chronic inflammatory disease that occurs due to the interaction between pathogenic microorganisms and host defenses. Endocan is a proteoglycan secreted by endothelial cells under the control of inflammatory cytokines. Aims of the study are to determine serum and gingival crevicular fluid (GCF) endocan levels in the pathogenesis of periodontal diseases, supported with vascular endothelial growth factor (VEGF-A) and tumor necrosis factor (TNF)-alpha levels. This study additionally aims to evaluate correlation between GCF endocan levels, VEGF-A, and TNF-α levels with periodontal probing depth (PD).

Methods: The study consists of two groups: group 1 (n = 20), healthy individuals; group 2 (n = 20), individuals with generalized chronic periodontitis (CP). Clinical measurements were recorded; GCF and serum samples were obtained from each participant before and 6 weeks after therapy. Levels of biomarkers were measured by enzyme-linked immunosorbent assay. Intergroup comparisons of biochemical and clinical parameters were analyzed by Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney *U* test using statistical software.

Results: Serum and GCF endocan, VEGF-A, and TNF-α levels were significantly higher in patients with CP than in healthy individuals ($P < 0.001$) and decreased after treatment ($P < 0.03$). A significant correlation was observed between GCF TNF-α and PD (4 mm ≤ PD ≤5 mm and PD ≥6 mm). A significant relationship was found among GCF endocan and TNF-α, VEGF-A, CAL, and GI for all groups ($P < 0.05$).

Conclusions: Endocan and TNF-α levels, both in GCF and serum, increased from health to periodontitis and decreased with non-surgical periodontal treatment. Within the limits of the study, endocan may be considered as a potential inflammatory marker for periodontal disease. *J Periodontol 2017;88:493-501.*

KEY WORDS

Aftercare; endocan proteins; gingival crevicular fluid; periodontal diseases; tumor necrosis factor-alpha; vascular endothelial growth factor a.

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Periodontal disease, characterized by periodontal tissue destruction, is a complex immune-inflammatory condition that can arise when a host organism encounters a periodontal pathogenic microorganism.[1,2] Inflammation is a response in host tissue exposed to such pathogens. The inflammatory process includes vascular dilation as well as increased capillary permeability, blood flow, and leukocyte influx. The first cells recruited to the site of inflammation are polymorphonuclear leukocytes (PMNLs), monocytes/macrophages, epithelial cells, and endothelial cells.[3] Endothelial cells are responsible for attracting leukocytes, both of which are recruited in an immediate response to a microbial biofilm.[4] With production of cytokines and chemokines, inflammatory response is amplified, and

doi: 10.1902/jop.2016.160279

so is the number of cells supplied to address microbial challenge.[5]

A significant event within periodontal pathogenicity is the host response. Inflammatory mediator levels reveal a lot about receptivity to the disease.[6] In both healthy and disease conditions, the gingival sulcus contains serum-like gingival crevicular fluid (GCF), into which cells and mediators, stimulated by the presence of oral microbes, are released in the inflammatory phase.[6,7] GCF in the gingival sulcus or periodontal pocket can indicate severity of inflammation, and its flow increases with increasing permeability of the microvasculature. Thus, GCF is used as a diagnostic and prognostic tool in determining pathogenicity of periodontal disease.[6]

Tumor necrosis factor (TNF)-alpha is an important proinflammatory cytokine that causes damage to periodontal tissue and is produced in response to bacteria and bacterial products.[8,9] It is involved in recruiting leukocytes to inflamed tissue, vascular permeability and leakage, and chemokine production.[10,11] Chemotaxis of PMNLs to the vascular epithelium and its phagocytosis is another local effect.[8] TNF-α is associated with both destruction of connective tissue and bone resorption by managing secretion of matrix metalloproteinases and receptor activator of nuclear factor-kappa B ligand (RANKL).[5] Angiogenesis is the development of new blood vasculature from existing capillaries, a process regulated by cytokines and growth factors.[12] Vascular endothelial growth factor (VEGF) is derived from platelets and plays an important role in endothelial cell proliferation, proteolytic enzyme secretion, and migration through chemotaxis; it also stimulates angiogenesis.[13,14] TNF-α induces VEGF-A, which plays important roles in periodontal health and destructive conditions.[11,14,15] Endocan, a soluble proteoglycan, was once known as endothelial cell-specific molecule-1; it is associated with systemic diseases, such as cardiovascular and inflammatory bowel diseases.[16,17] Endocan is maintained by inflammatory cytokines and is found in the vascular endothelium.[18,19] Endocan has been shown to be regulated by inflammatory mediators and angiogenic growth factors;[4,20-22] VEGF-A as well as various cytokines, such as TNF-α and interleukin (IL)-1, are also involved in the action of endocan.[19-22]

Many studies concerning periodontal disease progression have shown important roles of TNF-α and VEGF-A in the pathologic and healing periods.[15,23-27] It is clear that TNF-α levels are increased in periodontal inflammation,[23,24] whereas the role of VEGF-A in such inflammation remains unclear.[15,25-27] VEGF-A levels have been assessed in both the healing and inflammation periods of periodontal disease in different studies[15,25]. Given findings that TNF-α and VEGF-A upregulate secretion of endocan[28] the authors of this study considered that endocan levels might be increased in periodontal disease and decreased after periodontal treatment. To the best knowledge of the authors, no reported study has established the role of endocan in periodontal inflammation. Here, the primary aim was to evaluate the interplay of the angiogenic factor VEGF-A and the proinflammatory mediator TNF-α with respect to the action of endocan in chronic periodontitis (CP) patients before and after periodontal treatment. A second goal was to assess any correlation(s) between periodontal clinical parameters and biomarker values.

MATERIALS AND METHODS

Study Design

In total, 40 individuals (19 males and 21 females, aged 25 to 49 years; mean age: 37.45 ± 5.40 years), admitted to the Periodontology Department of Bulent Ecevit University, Zonguldak, Turkey, and matched for age and sex, were enrolled. The study involved two groups: healthy periodontal individuals (group 1; n = 20) and individuals suffering from generalized CP (group 2; n = 20). The research period was from April 2015 to September 2015.

The study was approved by the Ethics Committee on Human Research of Bulent Ecevit University and was conducted according to the Declaration of Helsinki, as revised in 2013 (protocol number: 2016 to 02-13/01). All individuals provided written informed consent. This study was registered at ClinicalTrials.gov (NCT02750956).

Inclusion and Exclusion Criteria and Periodontal Examination

Inclusion criteria were assessed after a radiographic and full-mouth clinical periodontal examination, including: 1) gingival index (GI);[29] 2) plaque index (PI);[30] 3) clinical attachment level (CAL); 4) bleeding on probing (BOP);[31] and 5) probing depth (PD). Participants had to possess a minimum of 20 natural teeth, not including third molars. The healthy group showed no sign of inflammation or bone or attachment loss; i.e., GI = 0 and PD ≤3 mm. For the CP group, the inclusion criteria, apart from clinically distinct signs of inflammation, were set as: 1) GI >1; 2) PD ≥5 mm; and 3) CAL ≥5 mm, with alveolar bone loss affecting >30% of the teeth, as detected on clinical and radiographic examinations. All measurements were recorded using a periodontal probe‡ by the same investigator (DD). All disease classifications were made based on the classification system published in 1999.[32]

Patients with systemic diseases, such as diabetes mellitus, rheumatoid arthritis, obesity, and cancer were excluded. Pregnant and lactating mothers, tobacco

‡ Williams probe, Hu-Friedy, Chicago, IL.

users, patients with aggressive periodontitis, and those who had ever used antibiotics or drugs or had received periodontal treatment in the last 6 months that could affect periodontal status, were excluded.

Sample Collection

Study parameters were evaluated clinically by one calibrated examiner (DD) who was masked to the purpose of the study. Calibration was performed using 10 randomly selected individuals before study measurements were taken. The examiner evaluated participants twice at an interval of 48 hours. Only measurements that were >90% similar at the millimeter level at baseline and 48 hours were accepted.[33]

GCF collection. Two areas per patient were selected as sampling sites on the day after the measurements, so contamination of GCF with blood was avoided. The mesio- and disto-buccal areas of single-rooted teeth were used for the sampling of GCF. In the healthy controls, GCF was collected from healthy sites without signs of inflammation. In patients with CP, the most inflamed areas with increased CAL were chosen for GCF sampling. Supragingival plaque was removed without making contact with the marginal gingiva on the measurement days (baseline and 6 weeks after periodontal treatment). The area was isolated using cotton rolls before beginning GCF sampling to avoid saliva contamination. The area was dried and filter paper[§] was used for collecting samples of GCF, using the intracrevicular method, which is beneficial in the sense that it is fast and easy to use, is applicable on individual sites, and causes potentially no harm when used properly.[34] Paper strips were placed in the crevice until mild resistance was felt and left there for ≈30 seconds. The strips were used to measure volume of GCF by weighing[‖] the liquid immediately after collection. Strips (blood- and saliva-contaminated strips were discarded) were placed in a microcentrifuge tube, pooled to make one sample, and stored immediately at –40°C until assayed.

Serum collection. Samples (2 mL) of peripheral blood were taken from the antecubital fossa by venipuncture and collected in gel-containing tubes.[¶] To separate the serum component, samples were centrifuged (3,000 × g, 5 minutes) and the serum was stored immediately at –40°C until assayed.

Periodontal Treatment

The same researcher (DD) carried out non-surgical periodontal treatment (NSPT). After baseline GCF and serum data were obtained, patients with CP received NSPT, consisting of oral hygiene instruction and scaling and root planing (SRP) using manual scalers and curets.[#] SRP was provided to all patients on a quadrant-by-quadrant basis, once per week, and each visit lasted 45 to 60 minutes. No antibiotic was prescribed during treatment. If required, local anesthesia was used. The control group received only oral hygiene instructions. The modified Bass technique, consisting of the brushing method and dental flossing, was described in the oral hygiene instructions, which were repeated at 1-week intervals during the study period. At 6 weeks after completion of periodontal therapy, patients with CP were re-evaluated, and further serum and GCF samples were obtained. GCF samples were collected from the sites where they were first obtained. Individuals in each group participated in follow-up evaluations, including treatment and maintenance phases, as well as sample collection, over a 3-month period. There were no dropouts.

Measurement of Endocan, VEGF-A, and TNF-α Levels

Two strips were added to a microcentrifuge tube to produce a single sample. Three hundred and fifty microliters of phosphate-buffered saline (pH 7.4) was added to tubes with the sample strips. Concentrations of VEGF-A,[**] TNF-α,[††] and endocan[‡‡] in GCF and serum were determined using a sandwich enzyme-linked immunosorbent assay method according to the protocol provided by the manufacturer, as described previously.[35] Concentrations in GCF and serum samples were determined from standard curves.

The calibration range of the VEGF-A assay was up to 1,000 pg/mL, and the analytical sensitivity was 7.9 pg/mL. The within- and total-run coefficients of variation (CV) were <6.2% and <4.3%, respectively. The calibration range of the TNF-α assay was up to 1,000 pg/mL, and analytical sensitivity was 1 pg/mL. The within- and total-run CVs were <6% and <7.4%, respectively. The calibration range of the endocan assay was up to 1,000 pg/mL, and analytical sensitivity was 1 pg/mL. The within- and total-run CVs were <10%.

Statistical Analyses

Sample size was determined using the primary outcome variable (variation in serum and GCF endocan levels after treatment). Estimates were determined on the basis of a pilot study that included 10 individuals in each group. It was estimated that a sample size of 16 participants in each group would enable a Type II error level of $\beta = 0.20$ (80% power) and a Type I error level of $\alpha = 0.05$ (5% probability). It was decided to have 20 individuals in each group to allow for potential dropouts.

§ PerioPaper, ProFlow, Amityville, NY.
‖ Precisa ES 125SM, Dietikon, Switzerland.
¶ BD Vacutainer; Becton, Dickinson, and Company; Plymouth, U.K.
# Hu-Friedy.
** eBioscience, San Diego, CA.
†† eBioscience.
‡‡ Elabscience Biotechnology, Wuhan, China.

To assess whether data were normally distributed, the Shapiro–Wilk test was used. Intergroup comparisons of biochemical and clinical parameters were assessed using the Kruskal–Wallis non-parametric test, followed by post hoc group comparisons with a Bonferroni-adjusted Mann–Whitney *U* test if the data were not normally distributed. With the Bonferroni correction, $\alpha = 0.05/2 = 0.025$ was taken to indicate statistical significance. Paired biochemical and periodontal parameters (at baseline and 6 weeks) were compared, with $\alpha = 0.05$ taken to indicate statistical significance. A χ^2 analysis was used to compare the sex ratio between groups. To determine how GCF endocan, VEGF-A, and TNF-α levels were related to clinical periodontal parameters, Spearman rank correlation test was used. Statistical software[§§] was used to carry out all the tests, and *P* values <0.05 were considered to indicate statistical significance.

RESULTS

Clinical Findings

Table 1 presents clinical findings. PD, CAL, PI, GI, and BOP were found to be significantly higher in the CP group than the healthy group ($P < 0.05$). There was a significant reduction in each of the clinical parameters in patients with CP at 6 weeks after NSPT, in the full mouth as well as at the sample sites ($P < 0.05$; Table 1). Age and sex distributions showed no significant difference between the groups ($P > 0.05$). Because of the multifactorial etiology and chronic nature of periodontal disease, participants were selected from among an otherwise healthy, non-smoking population with a narrow age range.

Biochemical Findings

GCF and serum endocan levels. The presence of endocan was seen in all samples of GCF and serum. Table 2 presents total amounts, concentrations, and serum endocan levels. In patients with CP, total endocan levels were statistically significantly higher than in the healthy controls ($P < 0.001$). Patients with CP had significantly elevated endocan concentrations and a significant reduction was found after treatment ($P < 0.001$). Serum endocan levels were also significantly higher in patients with CP than in the healthy controls ($P < 0.001$).

GCF and serum VEGF-A and TNF-α levels. VEGF-A and TNF-α were seen in all GCF and serum samples. Table 2 presents total amounts, concentrations, and serum levels of VEGF-A and TNF-α. In GCF, total VEGF-A levels were statistically significantly higher in the CP group than in the healthy controls ($P < 0.001$). There was no significant difference in total VEGF-A concentration between patients with CP and healthy controls ($P > 0.05$). A significant reduction was found in VEGF-A levels after treatment in patients with CP ($P < 0.001$). Serum VEGF-A levels were also significantly higher in the CP group than in the healthy control group ($P < 0.01$).

Furthermore, it was found that in the CP group, total TNF-α levels were statistically significantly higher than in the healthy controls ($P < 0.001$). Serum TNF-α levels were significantly higher in the CP group than in the healthy controls ($P < 0.001$). Concerning total TNF-α concentrations, no significant difference was observed between the groups ($P > 0.05$).

Effects of Periodontal Treatment on Endocan, VEGF-A, and TNF-α Levels

Findings from the current study showed that NSPT resulted in a significant decrease in total amounts, concentrations, and serum levels of endocan ($P < 0.025$). After NSPT, statistically significant differences in the total amount and concentration of endocan were observed (Table 2; $P < 0.025$).

There were also statistically significant differences in total amounts, concentrations, and serum levels of VEGF-A before and after NSPT in patients with CP. Furthermore, total amounts and serum TNF-α levels showed statistically significant differences after periodontal treatment (Table 2; $P < 0.025$). Results showed that GCF and serum VEGF-A and TNF-α levels decreased after NSPT.

Correlations

Table 3 shows correlation coefficients. There was a statistically significant positive relationship between the total amount of TNF-α compared with endocan, sample site CAL, and sample site GI in the CP group ($P < 0.05$). There was a statistically significant positive correlation between total amount of endocan and sample site GI in the CP group ($P < 0.05$). No significant correlation was found between total amount of endocan compared with CAL or VEGF-A in the CP group ($P > 0.05$). There was no significant correlation between total amounts of VEGF-A compared with endocan, TNF-α, CAL, or GI. An examination of all clinical groups showed that all evaluated markers were positively related to each other, as well as to CAL and GI ($P < 0.001$).

The Spearman rank correlations (*r*) in the CP group with regard to total amounts of endocan, VEGF-A, TNF-α, and the number of deep sites, are reported in Table 4. The total amount of TNF-α was found to have a positive and significant relationship with the number of sites with 4 mm ≤ PD ≤5 mm and PD ≥6 mm ($P < 0.05$). No significant relationship was found between the total amount of VEGF-A and endocan level between the number of sites with 4 mm ≤ PD ≤5 mm or PD ≥6 mm ($P > 0.05$).

§§ SPSS Statistics for Windows, v19.0, IBM, Armonk, NY.

Table 1.

Full-Mouth and Sample Site Clinical Parameters of the Study Groups

	Parameter	Group 1 (Control)	Group 2 (CP: before treatment)	Group 2 (CP: after treatment)
Full-mouth periodontal examination	PD* (mm)	1.58 ± 0.15 (1.62)	4.51 ± 0.44 (4.37)	2.46 ± 0.22 (2.48)
	CAL* (mm)	1.58 ± 0.15 (1.62)	5.30 ± 0.35 (5.27)	3.39 ± 0.57 (3.43)
	GI*	0.00 ± 0.00 (0.00)	2.52 ± 0.18 (2.61)	0.24 ± 0.14 (0.24)
	PI*	0.42 ± 0.14 (0.43)	2.29 ± 0.20 (2.26)	0.63 ± 0.20 (0.66)
	BOP* (%)	0.00 ± 0.00 (0.00)	88.63 ± 3.52 (88.61)	11.86 ± 2.67 (11.84)
Sampled sites periodontal examination	PD* (mm)	1.60 ± 0.50 (2.00)	6.10 ± 0.78 (6.00)	2.60 ± 0.50 (3.00)
	CAL* (mm)	1.75 ± 0.44 (2.00)	7.05 ± 0.82 (7.00)	3.90 ± 0.50 (3.00)
	GI*	0.00 ± 0.00 (0.00)	2.55 ± 0.51 (3.00)	0.50 ± 0.50 (0.51)
	PI*	0.00 ± 0.00 (0.00)	2.15 ± 0.37 (2.00)	0.50 ± 0.52 (0.50)
	BOP*	0.00 ± 0.00 (0.00)	100.00 ± 0.00 (100.00)	20.00 ± 0.00 (0.00)

Data are expressed as the mean ± SD (median). Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney *U* test (unpaired observations). Wilcoxon signed-rank test (paired observations). Bonferroni correction $\alpha = 0.05/2 = 0.025$.
* Statistically significant difference among groups ($P < 0.05$).

Table 2.

GCF and Serum Endocan, VEGF-A and TNF-α Levels in Study Groups

Parameter	Group 1 (Control)	Group 2 (CP: before treatment)	Group 2 (CP: after treatment)
Total GCF endocan (pg)	1.55 ± 0.86* (1.53)	9.82 ± 0.83 (9.87)	2.02 ± 1.71* (1.29)
Serum endocan (pg/mL)†	64.34 ± 28.08 (56.97)	196.44 ± 75.55 (193.68)	104.76 ± 32.51 (113.91)
GCF endocan concentration (pg/mL)	5.12 ± 2.98* (4.84)	12.78 ± 1.41 (13.10)	4.04 ± 3.40* (2.60)
Total GCF VEGF-A (pg)	4.66 ± 3.20* (4.12)	12.05 ± 3.65 (10.99)	3.15 ± 2.87* (2.21)
Serum VEGF-A (pg/mL)	149.76 ± 79.94* (158.88)	735.65 ± 532.67 (599.00)	253.12 ± 306.18* (76.12)
GCF VEGF-A concentration (pg/mL)	15.20 ± 10.52 (12.96)	15.60 ± 4.62 (13.88)	6.35 ± 5.89*‡ (4.18)
Total GCF TNF-α (pg)	0.15 ± 0.06* (0.12)	0.27 ± 0.04 (0.28)	0.20 ± 0.08* (0.19)
Serum TNF-α (pg/mL)†	1.80 ± 1.56 (1.25)	18.79 ± 19.09 (14.38)	5.68 ± 3.90 (6.15)
GCF TNF-α concentration (pg/mL)	0.48 ± 0.21 (0.38)	0.35 ± 0.06 (0.36)	0.40 ± 0.16 (0.39)
GCF volume (mL)†	0.31 ± 0.03 (0.31)	0.77 ± 0.05 (0.76)	0.50 ± 0.02 (0.49)

Data are expressed as the mean ± SD (median). Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney *U* test (unpaired observations). Wilcoxon signed-rank test (paired observations). Bonferroni correction $\alpha = 0.05/2 = 0.025$.
* Statistically significant difference from group 2 ($P < 0.05$).
† Statistically significant difference among groups ($P < 0.05$).
‡ Statistically significant difference from group 1 ($P < 0.05$).

Table 3.

Spearman Rank Correlation (*r*) Among Groups With Respect to GCF-Endocan, GCF-VEGF-A, GCF-TNF-α, and Sampled Site CAL and GI

	Group 1 (Control)		Group 2 (CP: before treatment)		Group 2 (CP: after treatment)		All Groups	
Parameter	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value
GCF-endocan to CAL	0.31	0.18	0.41	0.07	0.30	0.20	0.74*	<0.001*
GCF-endocan to GI	NA	NA	0.46*	0.04*	0.38	0.10	0.78*	<0.001*
GCF-VEGF-A to CAL	0.27	0.25	0.28	0.24	0.30	0.21	0.55*	<0.001*
GCF-VEGF-A to GI	NA	NA	0.25	0.28	0.38	0.10	0.67*	<0.001*
GCF-TNF-α to CAL	0.39	0.09	0.56*	0.01*	0.41	0.07	0.71*	<0.001*
GCF-TNF-α to GI	NA	NA	0.61*	0.01*	0.47*	0.04*	0.72*	<0.001*
GCF-endocan to GCF-VEGF-A	0.29	0.22	0.39	0.09	0.39	0.09	0.68*	<0.001*
GCF-endocan to GCF-TNF-α	0.34	0.15	0.50*	0.02*	0.45	0.05	0.72*	<0.001*
GCF-VEGF-A to GCF-TNF-α	0.37	0.11	0.38	0.10	0.38	0.10	0.57*	<0.001*

GCF-endocan = total amount of endocan in GCF; GCF-TNF-α = total amount of TNF-α in GCF; GCF-VEGF-A = total amount of VEGF-A in GCF; NA = not applicable.
* Statistically significant ($P < 0.05$).

Table 4.

Spearman Rank Correlation (*r*) Among CP Group With Respect to GCF-Endocan, VEGF, TNF-α Levels, and Number of Deep Sites

	Group 2 (CP: before treatment)	
Parameter	*r*	*P* value
GCF-endocan to 4 mm ≤ PD ≤5 mm	0.36	0.12
GCF-endocan to PD ≥6 mm	0.35	0.13
GCF-VEGF-A to 4 mm ≤ PD ≤5 mm	0.33	0.16
GCF-VEGF-A to PD ≥6 mm	0.32	0.17
GCF-TNF-α to 4 mm ≤ PD ≤5 mm	0.47*	0.04*
GCF-TNF-α to PD ≥6 mm	0.48*	0.03*

* Statistically significant ($P < 0.05$).

DISCUSSION

In the present study, GCF and serum levels of endocan, VEGF-A, and TNF-α in periodontal disease before and after NSPT were evaluated. To the best knowledge of the authors, no previously published study has investigated endocan levels in periodontal disease. This is, the authors believe, also the first reported study to evaluate the role of NSPT in endocan levels. Consistent with the study hypothesis, endocan, TNF-α, and VEGF-A levels were higher in the CP group than in healthy individuals and decreased after NSPT.

Serum provides information about periodontal pathogen-induced inflammation and responses.[36] A literature review showed biomarkers in serum may be linked to periodontal disease.[37-39] In GCF, inflammation-related molecules may provide reliable information about the status of periodontal disease.[40] Measurements of inflammatory mediators in GCF have been used to determine the risk of an individual developing periodontal disease.[41]

In this study, statistically significant differences between groups were observed in terms of GCF volume. This is consistent with the GCF volume differing

by inflammatory status. GCF volume was greater in patients with CP than in healthy controls, and it decreased in patients after NSPT. This supports the finding that GCF volume increases with inflammation and decreases after resolution of inflammation with NSPT.[6] The increase in GCF volume in inflamed regions would be expected to, in turn, decrease concentration of GCF elements that are produced locally in the GCF.[34,42] GCF volume, which is a dependent variable, directly influences GCF component concentrations, which are not independent variables.[42] In the present study, there was no statistically significant difference in concentrations of VEGF-A and TNF-α between patients with CP and healthy individuals, whereas concentration of endocan was significantly higher in patients with CP than in healthy individuals. This can be explained simply by the lower GCF volume in healthy periodontium. Earlier studies showed that considering biomarkers in terms of total amounts of GCF per sampling time in the GCF, rather than in terms of concentration, was more reliable.[42,43] Thus, the current study is essentially based on total amounts, although concentrations were also determined.

It has been found that VEGF-A plays a role in lesion destruction, as well as in the healing period, in periodontal disease.[15,25-27] Cetinkaya et al.[27] reported that VEGF-A expression may have a greater relationship with the healing period of periodontal disease than with the lesion destruction phase. It was also demonstrated by Keles et al.[15] that VEGF-A expression is associated with maintenance of periodontal disease and with periodontal tissue damage. However, Pradeep et al.[25] reported that GCF and serum VEGF-A concentrations showed progressive increases commensurate with the intensity of the disease and decreased after treatment of the periodontal disease. Similarly, another study reported that VEGF-A levels increased in periodontitis and decreased after periodontal treatment.[26] In the current study, total amounts and serum VEGF-A showed increases in the CP group, whereas there were significant decreases in the total and serum VEGF-A levels after periodontal treatment. According to these findings, in addition to the healing period, VEGF-A is likely also involved in the inflammation period of periodontal disease.

Unlike studies about VEGF-A, there are no conflicting findings regarding TNF-α in periodontal disease pathogenesis. It is clear that there are higher levels of TNF-α in periodontal disease.[23,24] Current findings are similar to previous reports in that TNF-α levels in serum and GCF showed a statistically significant increase in the CP group. Additionally, as predicted, TNF-α levels in GCF and serum decreased after periodontal treatment. Because endocan was examined for the first time in periodontal disease pathogenesis, another well-known inflammatory mediator, TNF-α, was also examined to confirm the inflammatory status and impact of NSPT. Thus, increased GCF and serum levels of TNF-α in the CP group indicate that patients were indeed experiencing an inflammatory phase. Furthermore, there was a decrease in TNF-α and VEGF-A levels when examined with respect to clinical periodontal parameters; this confirmed that periodontal treatment was successful and the inflammatory course had been reduced or ended. This information supports the conclusion that endocan may be a possible biomarker for periodontal disease.

According to the current study hypothesis, there were increases in serum and GCF endocan levels in the CP group, which decreased significantly after NSPT. According to these findings, GCF and serum endocan levels may be linked to the inflammatory stage of periodontal disease. To the best knowledge of the authors, no previously published study has examined endocan levels in periodontal disease and after periodontal therapy, so it is not possible to directly compare findings. These results, along with those pertaining to the TNF-α and VEGF-A levels, make it possible to predict that increased endocan levels may have diagnostic value in periodontitis. Additionally, the decrease in GCF and serum endocan levels after NSPT suggests there may be prognostic value in assessing endocan levels during periodontal treatment.

Correlations among GCF endocan, VEGF-A, and TNF-α levels and PD were also evaluated. There was no significant relationship between total amounts of endocan or VEGF-A and PD. The only correlation found was between TNF-α and PD. Theoretically, this can be explained by TNF-α being one of the cytokines in the acute-phase reaction, which encourages the inflammatory response, whereas endocan and VEGF-A primarily have effects in angiogenesis during healing from disease.

The current findings, considering all groups together, showed a positive and significant correlation between total amounts of TNF-α and VEGF-A, endocan, CAL, and GI. Similarly, there was a significant relationship between total amounts of VEGF-A and TNF-α, endocan, CAL, and GI. Pradeep et al.[25] also found a positive significant correlation between GCF VEGF-A levels and CAL in a periodontitis group. More importantly, in terms of the study hypothesis, there was a positive and significant relationship between total amounts of endocan and TNF-α, VEGF-A, CAL, and GI in all groups. This finding indicates that there may be a proinflammatory impact of endocan in CP. Finally, these results suggest that increased TNF-α and VEGF-A levels may be related to production of endocan in patients with CP.

CONCLUSIONS

From the findings reported here, GCF and serum endocan, VEGF-A and TNF-α levels were greater in

patients with periodontal tissue destruction; after NSPT, these levels decreased. The increase in levels of endocan and TNF-α in GCF may indicate that endocan can be produced by inflammatory cells in periodontium. Thus, levels of endocan in periodontal tissues suggest that it has potential as an inflammatory marker and also may be useful as a diagnostic and prognostic marker for periodontal disease. Further studies with larger populations are needed to confirm these results and to establish whether endocan is an inflammatory marker for periodontal disease.

ACKNOWLEDGMENT

The authors report no conflicts of interest related to this study.

REFERENCES

1. Paes Batista da Silva A, Barros SP, Moss K, et al. Microbial profiling in experimentally induced biofilm overgrowth among patients with various periodontal states. *J Periodontol* 2016;87:27-35.
2. Offenbacher S, Barros SP, Singer RE, Moss K, Williams RC, Beck JD. Periodontal disease at the biofilm-gingival interface. *J Periodontol* 2007;78:1911-1925.
3. Freire MO, Van Dyke TE. Natural resolution of inflammation. *Periodontol 2000* 2013;63:149-164.
4. Lassalle P, Molet S, Janin A, et al. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. *J Biol Chem* 1996; 271:20458-20464.
5. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol 2000* 2014;64:57-80.
6. Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. *Periodontol 2000* 2016;70:53-64.
7. Kinney JS, Morelli T, Oh M, et al. Crevicular fluid biomarkers and periodontal disease progression. *J Clin Periodontol* 2014;41:113-120.
8. Ebersole JL, Cappelli D. Acute-phase reactants in infections and inflammatory diseases. *Periodontol 2000* 2000;23:19-49.
9. Varghese SS, Thomas H, Jayakumar ND, Sankari M, Lakshmanan R. Estimation of salivary tumor necrosis factor-alpha in chronic and aggressive periodontitis patients. *Contemp Clin Dent* 2015;6(Suppl. 1): S152-S156.
10. Hofmann S, Grasberger H, Jung P, et al. The tumour necrosis factor-alpha induced vascular permeability is associated with a reduction of VE-cadherin expression. *Eur J Med Res* 2002;7:171-176.
11. Frank S, Hübner G, Breier G, Longaker MT, Greenhalgh DG, Werner S. Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. *J Biol Chem* 1995;270:12607-12613.
12. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol* 1995;11:73-91.
13. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 1995;146:1029-1039.
14. Shibuya M. Vascular endothelial growth factor and its receptor system: Physiological functions in angiogenesis and pathological roles in various diseases. *J Biochem* 2013;153:13-19.
15. Keles GC, Cetinkaya BO, Eroglu C, Simsek SB, Kahraman H. Vascular endothelial growth factor expression levels of gingiva in gingivitis and periodontitis patients with/without diabetes mellitus. *Inflamm Res* 2010;59:543-549.
16. Çimen T, Efe TH, Akyel A, et al. Human endothelial cell-specific molecule-1 (endocan) and coronary artery disease and microvascular angina. *Angiology* 2016;67:846-853.
17. Voiosu T, Bălănescu P, Benguş A, et al. Serum endocan levels are increased in patients with inflammatory bowel disease. *Clin Lab* 2014;60:505-510.
18. Sarrazin S, Adam E, Lyon M, et al. Endocan or endothelial cell specific molecule-1 (ESM-1): A potential novel endothelial cell marker and a new target for cancer therapy. *Biochim Biophys Acta* 2006;1765:25-37.
19. Lee W, Ku SK, Kim SW, Bae JS. Endocan elicits severe vascular inflammatory responses in vitro and in vivo. *J Cell Physiol* 2014;229:620-630.
20. Bechard D, Meignin V, Scherpereel A, et al. Characterization of the secreted form of endothelial-cell-specific molecule 1 by specific monoclonal antibodies. *J Vasc Res* 2000;37:417-425.
21. Gerritsen ME, Tomlinson JE, Zlot C, Ziman M, Hwang S. Using gene expression profiling to identify the molecular basis of the synergistic actions of hepatocyte growth factor and vascular endothelial growth factor in human endothelial cells. *Br J Pharmacol* 2003;140:595-610.
22. Aitkenhead M, Wang SJ, Nakatsu MN, Mestas J, Heard C, Hughes CC. Identification of endothelial cell genes expressed in an in vitro model of angiogenesis: Induction of ESM-1, (beta)ig-h3, and NrCAM. *Microvasc Res* 2002;63:159-171.
23. Van Dyke TE, Lester MA, Shapira L. The role of the host response in periodontal disease progression: Implications for future treatment strategies. *J Periodontol* 1993; 64(Suppl.8):792-806.
24. Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. *J Periodontal Res* 1991;26:230-242.
25. Pradeep AR, Prapulla DV, Sharma A, Sujatha PB. Gingival crevicular fluid and serum vascular endothelial growth factor: Their relationship in periodontal health, disease and after treatment. *Cytokine* 2011;54:200-204.
26. Prapulla DV, Sujatha PB, Pradeep AR. Gingival crevicular fluid VEGF levels in periodontal health and disease. *J Periodontol* 2007;78:1783-1787.
27. Cetinkaya BO, Keles GC, Ayas B, Sakallioglu EE, Acikgoz G. The expression of vascular endothelial growth factor in a rat model at destruction and healing stages of periodontal disease. *J Periodontol* 2007;78:1129-1135.
28. Rennel E, Mellberg S, Dimberg A, et al. Endocan is a VEGF-A and PI3K regulated gene with increased expression in human renal cancer. *Exp Cell Res* 2007; 313:1285-1294.
29. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963;21: 533-551.
30. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condtion. *Acta Odontol Scand* 1964;22:121-135.
31. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J* 1975;25:229-235.

32. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;4:1-6.
33. Schwarz F, Bieling K, Latz T, Nuesry E, Becker J. Healing of intrabony peri-implantitis defects following application of a nanocrystalline hydroxyapatite (Ostim) or a bovine-derived xenograft (Bio-Oss) in combination with a collagen membrane (Bio-Gide). A case series. *J Clin Periodontol* 2006;33:491-499.
34. Griffiths GS. Formation, collection and significance of gingival crevice fluid. *Periodontol 2000* 2003;31:32-42.
35. Balli U, Aydogdu A, Dede FO, Turer CC, Guven B. Gingival crevicular fluid levels of sclerostin, osteoprotegerin, and receptor activator of nuclear factor-κB ligand in periodontitis. *J Periodontol* 2015;86:1396-1404.
36. Buduneli N, Özçaka Ö, Nalbantsoy A. Salivary and plasma levels of Toll-like receptor 2 and Toll-like receptor 4 in chronic periodontitis. *J Periodontol* 2011;82:878-884.
37. Rodrigues VP, Libério SA, Lopes FF, et al. Periodontal status and serum biomarkers levels in haemodialysis patients. *J Clin Periodontol* 2014;41:862-868.
38. Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Sugita N, Yoshie H. The effect of periodontal treatment on serum leptin, interleukin-6, and C-reactive protein. *J Periodontol* 2010;81:1118-1123.
39. Caúla AL, Lira-Junior R, Tinoco EM, Fischer RG. The effect of periodontal therapy on cardiovascular risk markers: A 6-month randomized clinical trial. *J Clin Periodontol* 2014;41:875-882.
40. Buduneli N, Kinane DF. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. *J Clin Periodontol* 2011;38(Suppl. 11):85-105.
41. Champagne CM, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S. Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. *Periodontol 2000* 2003;31:167-180.
42. Lin SJ, Chen YL, Kuo MY, Li CL, Lu HK. Measurement of gp130 cytokines oncostatin M and IL-6 in gingival crevicular fluid of patients with chronic periodontitis. *Cytokine* 2005;30:160-167.
43. Lamster IB, Oshrain RL, Fiorello LA, Celenti RS, Gordon JM. A comparison of 4 methods of data presentation for lysosomal enzyme activity in gingival crevicular fluid. *J Clin Periodontol* 1988;15:347-352.

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Submitted May 3, 2016; accepted for publication November 14, 2016.