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*Article*

# Changes in Gingival Crevicular Fluid Endocan (ESM-1) Levels as a Potential Biomarker After Non-Surgical Periodontal Treatment in Periodontitis Patients

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**Abstract: Background:** This study aims to investigate ESM-1 (endocan) levels in periodontitis patients before and after nonsurgical periodontal treatment by analyzing the relationship between VEGF-A and TNF- $\alpha$  in gingival crevicular fluid (GCF). **Methods:** This study included 26 periodontally healthy people in the control group (Group 1) and 27 patients with Stage III-Grade B periodontitis (Group 2). Demographic and periodontal variables were assessed. GCF samples were collected from every subject both before and six weeks following non-surgical periodontal therapy (NSPT). Using an enzyme-linked immunosorbent test, biomarker levels were determined. **Results:** Periodontitis patients showed higher ESM-1 levels than controls, though not significantly ( $p > 0.005$ ). ESM-1 levels decreased significantly after treatment ( $p = 0.001$ ). VEGF-A levels did not differ significantly between periodontitis patients and controls ( $p > 0.005$ ) and decreased following treatment without statistical significance ( $p > 0.005$ ). TNF- $\alpha$  levels were significantly higher in periodontitis patients than controls ( $p = 0.000$ ) and decreased significantly after treatment ( $p = 0.000$ ). A significant correlation found between TNF- $\alpha$  and both PPD and iCAL in the control group ( $p < 0.05$ ). In the periodontitis group, VEGF levels showed a significant correlation with GI ( $p < 0.05$ ). Significant correlations were identified between ESM-1 and VEGF-A, ESM-1 and TNF- $\alpha$ , as well as VEGF-A and TNF- $\alpha$  in both the control group and the following treatment ( $p < 0.05$ ). **Conclusions:** ESM-1 and TNF- $\alpha$  levels decreased with non-surgical periodontal treatment in GCF. Within the limits of the study, ESM-1 levels in periodontal tissues indicate that they may be indicator of periodontal disease prognosis.

**Keywords:** endocan; ESM-1; periodontitis; vascular endothelial growth factor a; tumor necrosis factor-alpha; gingival crevicular fluid

## 1. Introduction

Periodontitis has been known as an inflammatory chronic disease which destroys the tissue that supports teeth [1]. Events including vascular dilatation, papillary permeability, and extravasation of leukocytes are part of inflammation, which happens as the body reacts to pathogens. Polymorphonuclear leukocytes (PMNLs) are the initial cells to arrive at the site of inflammation. Leukocyte migration against microbial biofilm is a process that begins with endothelial cells [2]. In the gingival sulcus, PMNLs participate in the immunological response to periodontopathogens during the initial stage of host defense [3]. Inflammatory response increases with cytokine and chemokine production [4].

Instead of relying exclusively on clinical indicators, gingival crevicular fluid (GCF) may be employed to characterize a site at a molecular level because it is a site-specific exudate that reflects the inflammatory process pathologically [5].

Tumor necrosis factor (TNF)-alpha is a key proinflammatory cytokine that has a critical part in the inflammatory response within periodontal tissues [6]. Ligand for receptor activator of nuclear

factor-kappa B and matrix metalloproteinases are secreted by TNF- $\alpha$ , which is linked to bone resorption and connective tissue deterioration [7]. When it deals with periodontal disease, TNF is a crucial biomarker that may bring on the diagnosis, prognosis, and treatment options. GCF TNF levels rise with inflammatory diseases like periodontitis, which aids in the degradation of inflamed periodontal tissues [8].

Angiogenesis has a crucial role in the progression of inflammatory disorders [9]. Although many growth factors and cytokines have a role in controlling angiogenesis, the most effective element that targets the vascular endothelium is growth factor of vascular endothelial (VEGF) [10]. The active degradation of periodontal structures has been linked to increased TNF- $\alpha$  in GCF, and these mediators are major stimulators of VEGF. Thus, by enhancing vascular permeability and angiogenesis, VEGF can accelerate the course of periodontal disease [11].

Endothelial cells secrete a variety of chemicals, one of which is endothelial cell-specific molecule-1 (ESM-1) [12]. The permeable proteoglycan ESM-1(endocan) participates in numerous significant biological events, including cell transformation, cell proliferation, migration vascularization and tumor metastasis [12–14].

Overexpression of ESM-1 has been observed in inflammatory disorders, cardiovascular illnesses, sepsis, cancer, and obesity [15]. An increase in ESM-1 has been found in inflammatory disorders [12,14], and inflammation is known to play a significant role in the pathogenetic alterations of periodontitis. The regulating role of endocan in angiogenesis and inflammatory reactions is crucial [16]. The expression of VEGF may determine the angiogenic effects of endocan. According to certain research, VEGF can increase endocan activity [13,17,18]. The expression of endocan is greatly increased by inflammatory signaling pathways when proinflammatory cytokines such as TNF- $\alpha$  are activated [19]. Endocan expression is enhanced, leading to inflammatory reactions, by TNF- $\alpha$  released by monocytes or macrophages. [16,20,21].

This study aims to compare the levels of endocan in individuals having periodontitis both prior to and following nonsurgical periodontal therapy by analyzing the interaction between the proinflammatory mediator TNF- $\alpha$  and the angiogenic factor VEGF-A.

## 2. Materials and Methods

### 2.1. Population of the Study

We conducted the current investigation from February 2020 to September 2020 at the Alanya University Dentistry Faculty, Periodontology Department, Antalya, Türkiye. Participants who requested dental treatment or gingival evaluation at the periodontology outpatient clinic were assigned to the examination. This study included 26 periodontally and systemically healthy people in the group control (Group 1) and 27 people with Stage III-Grade B periodontitis who are systemically healthy (Group 2). The investigation procedure was achieved in compliance with the Declaration of Helsinki, as updated in 2013, and accepted by the ALKU Faculty of Medicine Ethics Committee (date: January 2020, Protocol No. 15-02). The investigation's objective and methodology were elucidated to each participant. Written informed permission was acquired from each participant.

### 2.2. Criteria of Inclusion and Exclusion

Smokers, pregnant and breastfeeding women, and those with systemic disorders (including obesity, rheumatoid arthritis, and diabetes mellitus) were excluded from the study. Additionally, individuals who got any periodontal therapy or used antibiotics or anti-inflammatory medication in the previous 6 months were not included. A thorough full-mouth clinical periodontal and radiographic assessments were used to determine the selection requirements. All participants were in a state of systemic health. Participants devoid of allergy, inflammatory, or autoimmune conditions were also incorporated into the study.

### 2.3. Periodontal Examination

A full-mouth clinical radiographic periodontal test was used to evaluate the following inclusion criteria: gingival index (GI) [22]; plaque index (PI) [23]; interproximal clinical attachment level (iCAL); bleeding on probing (BOP) [24]; and probing depth (PD). Individuals were required to possess 20 natural teeth at minimum, excluding 3rd molars. According to the most recent classification by AAP and EEP, all individuals were diagnosed with Stage III and Grade B periodontitis. A percentage of bone loss per age between 0.25 and 1.0, with 30% or more sites showing a PD > 5mm and CAL  $\geq$  5mm [25]. BOP < 10% and PPD  $\leq$  3mm were indicators of periodontal health (control group). We only included millimeter-level values that were more than 90% identical between the baseline and 48-hour points [26].

### 2.4. GCF Collection

GCF was collected the day after the measurements. So that GCF could not be contaminated with blood. For the periodontitis groups, specimens were taken from mesial areas the single-rooted teeth with PPD  $\geq$  5mm. Teeth with single-root were elected randomly in the control group. The sample collecting site was left in isolation, and steering clear of the marginal gingiva, supragingival plaque was excised by a curette. Paper strips (Oralflow Inc. USA) were employed to gather the samples of GCF. The strips were placed inside the crack and held there for 30 seconds when a little amount of stiffness was felt [27]. Strips with a noticeable bloodstain were not included. 200 $\mu$ l of phosphate-buffered saline (PBS) were included to the microcentrifuge tubes holding the paper strips. We hold the tubes stored till the beginning of analysis at -80°C.

### 2.5. Periodontal Therapy

The non-surgical periodontal therapy (NSPT) was started and completed by the same researcher (BK). Following the collection of baselines GCF data, patients with periodontitis were given NSPT, which included teaching them proper dental hygiene practices and performing planning of scaling and root (SRP) with the usage of curettes in addition to manual scalers. Every patient receives SRP once a week, arranged in quadrants. Antibiotics were not part of the therapy plan. Local anesthetic was administered when needed. For the control group, it was only about brushing and flossing. In the dental hygiene instructions, that have been reviewed weekly throughout the course of research, participants learned the modified Bass technique, which included both the brushing approach and dental flossing. Patients with periodontitis were re-evaluated for six weeks following the end of periodontal therapy, and further GCF samples were collected. We retrieved GCF samples from their first locations.

### 2.6. GCF ELISA Method for Endocan, TNF- $\alpha$ and VEGF

As mentioned prior to this, VEGF-A, TNF- $\alpha$ , and ESM-1 (Bioassay Technology Laboratory, China) values in GCF were measured with a sandwich enzyme-linked assay technique which is immunosorbent, following the manufacturer-provided methodology [28]. The total quantity of GCF samples was ascertained from standard curves.

The VEGF-A test had an analytical sensitivity of 10.42 ng/L and a calibration range of up to 6000 ng/L. Intra-assay coefficient of variation (CV) were < 8% and inter-assay CV <10%, respectively. The TNF- $\alpha$  test had an analytical sensitivity of 1.52ng/L and a calibration range of up to 900ng/L. Both the intra and inter-assay CVs were <10%. The ESM-1 test had an analytical sensitivity of 2.56 ng/L and a calibration range of up to 2000 ng/L. The intra-assay CV is <8%, inter-assay CV is <10%.

### 2.7. Statistical Analysis

The sample size was calculated as 52 for an effect size of 0.85, a power of 0.95, and a significance level of 0.05. Tests of Shapiro-Wilk and Kolmogorov-Smirnov were employed to assess if the data is normally distributed. Comparisons of clinical and biochemical values between groups were analyzed

by nonparametric tests. If the data was not normally distributed, Mann-Whitney U tests were addressed for post-hoc group analysis. The Wilcoxon test was used to evaluate paired biochemical and periodontal data (for baseline and for 6 weeks). The relationship between GCF endocan, TNF- $\alpha$ , and VEGF-A values and clinical periodontal markers was examined using the Spearman rank correlation test. The study's data underwent statistical analysis utilizing SPSS Ver22.0. and P scores <0.05 have been accepted to determine statistical significance.

3. Results

3.1. Clinical Parameters

Tables 1 and 2 provide a summary of the patients' clinical periodontal assessments and demographic characteristics. Age and sex did not significant differences across the groups (p >0.05). Group 2 exhibited a statistically significant increase in all clinical measures as compared to Group 1 (p<0.05). Six weeks following NSPT, patients in Group 2 showed a substantial decrease in all clinical measures in the complete mouth (P <0.05).

Table 1. Demographic characteristics of the study groups.

|               | Group 1<br>(control)<br>(n=26) | Group 2 (before<br>treatment)<br>(n=27) | Group 2 (after<br>treatment)<br>(n=27) |
|---------------|--------------------------------|-----------------------------------------|----------------------------------------|
| Age (mean±SD) | 41.5±7.5                       | 45.5±9.0                                | 45.5±9.0                               |
| Sex           | Male                           | 13 (50%)                                | 15 (55.6%)                             |
|               | Female                         | 13 (50%)                                | 12 (44.4%)                             |

Table 2. Clinical periodontal parameters of the study groups.

|           | Group 1 (C) | Group 2 (before<br>treatment) (BT) | Group 2 (after<br>treatment) (AT) | p                            |
|-----------|-------------|------------------------------------|-----------------------------------|------------------------------|
| PI        | 0.73±0.45   | 2.55 ± 0.64                        | 0.85±0.60                         | <0,05 (BT-AT,<br>BT-C)       |
| GI        | 0.69±0.47   | 2.44±0.57                          | 0.88±0.64                         | <0,05 (BT-AT,<br>BT-C)       |
| PD (mm)   | 2.26±0.66   | 5.77±1.01                          | 3.70±1.10                         | <0,05 (BT-AT,<br>BT-C, AT-C) |
| BOP (%)   | 0.00±0.00   | 73.33±8.77                         | 10.55±4.87                        | <0,05 (BT-AT,<br>BT-C, AT-C) |
| iCAL (mm) | 2.26 ± 0.66 | 6.29±1.48                          | 4.22 ± 1.50                       | <0,05 (BT-AT,<br>BT-C, AT-C) |

Values are presented as mean ± SD. P < 0.05; statistically significant difference. Abbreviations in parentheses represent groups with statistically significant differences.

3.2. Biochemical Findings

The total amounts of VEGF-A, TNF- $\alpha$ , and ESM-1 are displayed at Table 3. The total ESM-1 values were elevated in Group 2 in comparison with Group1, but this difference was not significant (p>0.05). Following therapy, there was a significant decrease in ESM-1 values (p=0.001) (Table 4). In GCF, there was no significant difference in total VEFG-A levels between two groups (p>0.05). The levels of VEFG-A were shown to decrease in periodontitis patients following therapy. However, no statistically significant difference was measured (p>0.05) (Table 3 and Table 4). The total TNF- $\alpha$  score was observed to be considerably greater in Group 2 patients than Group 1 (p=0.000). After receiving



therapy for periodontitis, it was demonstrated that TNF- $\alpha$  levels statistically reduced ( $p=0.000$ ) (Tables 3 and 4).

**Table 3.** GCF Endocan, VEGF-A and TNF- $\alpha$  levels.

| Parameter                | Group 1 (Control)    | Group 2 (before treatment) (BT) | Group 2 (after treatment) (AT) |
|--------------------------|----------------------|---------------------------------|--------------------------------|
| ESM-1 (ng/30 sn)         | 1686.22 $\pm$ 199.20 | 1706.71 $\pm$ 195.65            | 1541.27 $\pm$ 178.62           |
| TNF- $\alpha$ (ng/30 sn) | 209.09 $\pm$ 17.16   | 236.51 $\pm$ 22.00              | 188.77 $\pm$ 17.36             |
| VEGF-A (ng/30 sn)        | 1601.06 $\pm$ 169.25 | 1695.76 $\pm$ 184.00            | 1424.70 $\pm$ 172.47           |

Data are presented as the mean  $\pm$  standard deviation.

**Table 4.** Comparing biochemical levels pairwise.

|               |                            | t      | df     | p      |
|---------------|----------------------------|--------|--------|--------|
| ESM-1         | Group 2 (BT)-Group 2 (AT)  | 3,55   | 26     | 0.001* |
|               | Group 1-Group 2 (AT)       | 2.791  | 51     | 0.007* |
|               | Group 1-Group 2 (BT)       | -0.378 | 51     | 0.707  |
| TNF- $\alpha$ | Group 2 (BT)- Group 2 (AT) | 43,798 | 26     | 0.000* |
|               | Group 1-Group 2 (AT)       | 8.787  | 51     | 0.000* |
|               | Group 1-Group 2 (BT)       | 5.069  | 51     | 0.000* |
| VEGF-A        | Group 1-Group 2 (BT)       | 1.951  | 51     | 0.057  |
|               |                            | z      | p      |        |
|               | Group 1-Group 2 (AT)       | -4.715 | 0.000* |        |
| VEGF-A        | Group 2 (BT)- Group 2 (AT) | 3.027  | 0.002  |        |

\* $P < 0.05$ ; statistically significant difference.

3.3. Correlations

Table 5 shows the correlation coefficients between the GCF contents of ESM-1, VEGF-A, and TNF- $\alpha$  with the periodontal clinical parameters. The total level of TNF- $\alpha$  in the control group was

statistically significant correlated with both PPD and iCAL ( $p<0.05$ ). VEGF values and GI were shown to be significantly correlated in the periodontitis group ( $p<0.05$ ). ESM-1 values were shown to be significantly correlated with VEGF-A ( $p<0.05$ ), ESM-1 values and TNF- $\alpha$  ( $p<0.05$ ), and VEGF-A values and TNF- $\alpha$  values in both the control and received therapy for periodontitis group ( $p<0.05$ ).

**Table 5.** Correlations between GCF ESM-1, VEGF-A, TNF-  $\alpha$  levels, and periodontal clinical parameters.

| Parameter               | Group 1 (Control) |        | Group 2 (before treatment) (BT) |        | Group 2 (after treatment) (AT) |        |
|-------------------------|-------------------|--------|---------------------------------|--------|--------------------------------|--------|
|                         | r                 | p      | r                               | p      | r                              | p      |
| ESM-1 to GI             | 0.144             | 0.481  | -0.079                          | 0.696  | 0.061                          | 0.761  |
| ESM-1 to PPD            | 0.118             | 0.565  | -0.008                          | 0.968  | 0.026                          | 0.899  |
| ESM-1 to iCAL           | 0.118             | 0.565  | 0.038                           | 0.851  | -0.064                         | 0.753  |
| VEGF-A to GI            | 0.111             | 0.589  | -0.446*                         | 0.020* | 0.105                          | 0.601  |
| VEGF-A to PPD           | 0.208             | 0.307  | -0.023                          | 0.909  | 0.277                          | 0.161  |
| VEGF-A to iCAL          | 0.208             | 0.307  | 0.075                           | 0.709  | 0.133                          | 0.509  |
| TNF- $\alpha$ to GI     | -0.033            | 0.872  | 0.029                           | 0.886  | 0.159                          | 0.429  |
| TNF- $\alpha$ to PPD    | 0.414*            | 0.035* | 0.067                           | 0.740  | 0.032                          | 0.874  |
| TNF- $\alpha$ to iCAL   | 0.414*            | 0.035* | ,074                            | 0.712  | 0.096                          | 0.635  |
| ESM-1 to VEGF-A         | 0.491*            | 0.011* | 0.247                           | 0.215  | 0.599*                         | 0.001* |
| ESM-1 to TNF- $\alpha$  | 0.529*            | 0.005* | 0.262                           | 0.187  | 0.593*                         | 0.001* |
| VEGF-A to TNF- $\alpha$ | 0.399*            | 0.043* | 0.298                           | 0.131  | 0.417*                         | 0.030* |

Pearson’s correlation, r. \*Significant at the 0.05 level.

4. Discussion

This study assessed the levels of ESM-1, VEGF-A, and TNF- $\alpha$  in gingival crevicular fluid in periodontal disease prior to and following non-surgical periodontal therapy. The hypothesis of the investigation was that ESM-1, VEGF-A and TNF- $\alpha$  values would be higher in the periodontitis than control group and would decrease after NSPT. However, VEGF-A and ESM-1 levels in both groups and VEGF-A levels before and after periodontal therapy were not found to be statistically significant. This may be related to the severity and grade of periodontitis.

Serum-like GCF, which is found in gingival sulcus, contains inflammatory cells and mediators in people with periodontitis as opposed to healthy tissue, and its content is linearly connected with the degree of inflammation. Consequently, it serves as an advantageous diagnostic instrument to identify the pathological alterations associated with periodontal disease [29].

GCF and its inflammatory-related compounds have diagnostic and prognostic utility in evaluating the pathophysiology of periodontal disorders [30]. Preceding research showed that biomarkers were more accurately measured when expressed as total GCF quantities in the GCF for each sample period, as opposed to concentration [31,32]. Consequently, in our investigation, biomarkers were quantified as a total amount.

VEGF-A can enhance vascular permeability, perhaps exacerbating inflammation during the initial phases of periodontal problems. The involvement of VEGF in the pathophysiology of periodontal problems has shown inconsistent findings, with VEGF expression reported as increasing [33–36], reduced [37], or unchanged [10] during the disease process. In our study, there was no significant difference in total VEGF-A levels in the periodontitis and control groups. Pradeep et al. [33] and Jayara et al. [34] observed that VEGF-A levels in GCF of periodontitis patients were statistically significantly reduced following NSPT. However, Afacan et al. [38] determined that there was no statistically significant difference in total VEGF-A levels in GCF with advanced periodontitis following NSPT. Our study found no statistically significant difference in total VEGF-A levels after NSPT in the periodontitis group. It's likely that these differences in the study results are because

VEGF expressions may be linked to both the preservation of periodontal health and periodontal tissue destruction [39,40].

There are no contradictory results concerning TNF- $\alpha$  in the pathophysiology of periodontal diseases, in contrast to investigation involving VEGF-A. Periodontal disease is clearly related to elevated TNF- $\alpha$  levels [41]. Like other studies, the current results demonstrate that periodontitis group had a significantly higher level in TNF- $\alpha$  levels in the GCF. Also, the expected fall in TNF- $\alpha$  values in GCF after periodontal therapy.

Türer et al. and Tayman et al. found GCF ESM-1 levels to be significantly higher in the periodontitis group than in the control group [42,43]. In our study, ESM-1 levels were higher in the periodontitis group than in the control group, but this difference was not found to be statistically significant. This may be due to the stage of periodontitis.

Türer et al. and Kumar et al. found that GCF ESM-1 and TNF- $\alpha$  levels decreased statistically after NSPT in the periodontitis group. [42,44]. Similarly in this study, we found that after NSPT, levels of TNF- $\alpha$  and ESM-1 decreased. This could be because the endothelium function improves and the proinflammatory influence of ESM-1 gradually decreases. The reduction in GCF ESM-1 and TNF- $\alpha$  levels after NSPT may suggest possible prognostic relevance in assessing ESM-1 levels throughout periodontal therapy.

Furthermore, clinical periodontal markers exhibited a considerable reduction following NSPT in comparison to pre-treatment levels. This indicates that periodontal treatment to be effective, furthermore the inflammation was diminished.

The correlations between GCF VEGF-A, ESM-1, and TNF- $\alpha$  levels and periodontal disease were also assessed. TNF- $\alpha$  significantly correlated with PPD and iCAL in the control group. VEGF levels and GI were significantly correlated in periodontitis group. ESM-1 levels were significantly related to TNF- $\alpha$ , VEGF-A, and VEGF-A and TNF- $\alpha$  values in both the control and treatment periodontitis groups. Based on these results, ESM-1 may have an inflammatory effect in periodontitis.

Ultimately, these findings suggest that reduced levels of TNF- $\alpha$  and ESM-1 in periodontitis patients following NSPT may be associated with ESM-1 and periodontal inflammation.

## 5. Conclusions

The results indicate that GCF ESM-1 levels were elevated but not statistically significant in periodontitis patients; these levels, along with TNF- $\alpha$ , were considerably reduced following NSPT. The fact that ESM-1 levels are not significantly higher in periodontitis patients is due to the stage and grade classification of periodontitis. These findings indicate that ESM-1 levels in periodontal tissues indicate that they may be indicator of periodontal disease prognosis. Limited research exists in the literature about the relation between ESM-1 and periodontitis. Further research with larger populations is needed to confirm the findings and determine if ESM-1 is an inflammatory marker for periodontal disease.

**Author Contributions:** Conceptualization, methodology, formal analysis, investigation, all authors; resources, data curation, writing—original draft preparation, B.K.; writing—review and editing, visualization, project administration, funding acquisition, B.K. and K.S. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Alanya Alaaddin Keykubat University (protocol code 15-02; date of approval January 2020). All research was conducted in accordance with relevant guidelines and regulations.

**Informed Consent Statement:** Informed consent was obtained from all the subjects involved in this study.

**Data Availability Statement:** The data can be provided upon reasonable request to the corresponding author.



**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|               |                                         |
|---------------|-----------------------------------------|
| ESM-1         | Endothelial cell-specific molecule-1    |
| GCF           | Gingival crevicular fluid               |
| NSPT          | Non-surgical periodontal therapy        |
| PMNL          | Polymorphonuclear leukocytes            |
| TNF- $\alpha$ | Tumor necrosis factor-alpha             |
| VEGF          | Vascular endothelial growth factor      |
| PI            | Plaque index                            |
| GI            | Gingival index                          |
| iCAL          | Interproximal clinical attachment level |
| BOP           | Bleeding on probing                     |
| PD            | Probing depth                           |
| CV            | Coefficient of variation                |

## References

1. Nascimento, G.G.; Alves-Costa, S.; Romandini, M. Burden of severe periodontitis and edentulism in 2021, with projections up to 2050: The Global Burden of Disease 2021 study. *J. Periodontol. Res.* **2024**, *59*, 823-867.
2. Freire, M.O.; Van Dyke, T.E. Natural resolution of inflammation. *Periodontol 2000*. **2013**, *63*, 149-164.
3. Del Fabbro, M.; Francetti, L.; Pizzoni, L.; Weinstein, R.L. [Congenital neutrophil defects and periodontal diseases]. *Minerva Stomatol.* **2000**, *49*, 293-311.
4. Meyle, J.; Chapple, I. Molecular aspects of the pathogenesis of periodontitis. *Periodontol 2000*. **2015**, *69*, 7-17.
5. Barros, S.P.; Williams, R.; Offenbacher, S.; Morelli, T. Gingival crevicular fluid as a source of biomarkers for periodontitis. *Periodontol 2000*. **2016**, *70*, 53-64.
6. Boyce, B.F.; Li, P.; Yao, Z.; Zhang, Q.; Badell, I.R.; Schwarz, E.M.; O'Keefe, R.J.; Xing, L. TNF-alpha and pathologic bone resorption. *Keio. J. Med.* **2005**, *54*, 127-131.
7. Kitaura, H.; Marahleh, A.; Ohori, F.; Noguchi, T.; Nara, Y.; Pramusita, A.; Kinjo, R.; Ma, J.; Kanou, K.; Mizoguchi, I. Role of the Interaction of Tumor Necrosis Factor- $\alpha$  and Tumor Necrosis Factor Receptors 1 and 2 in Bone-Related Cells. *Int. J. Mol. Sci.* **2022**, *27*, 1481.
8. Madureira, D.F.; Lucas De Abreu Lima, I.; Costa, G.C.; Lages, E.M.B.; Martins, C.C.; Aparecida Da Silva, T. Tumor Necrosis Factor-alpha in Gingival Crevicular Fluid as a Diagnostic Marker for Periodontal Diseases: A Systematic Review. *J. Evid. Based. Dent. Pract.* **2018**, *18*, 315-331.
9. Güneri, P.; Unlü, F.; Yeşilbek, B.; Bayraktar, F.; Kokuludağ, A.; Hekimgil, M.; Boyacioğlu, H. Vascular endothelial growth factor in gingival tissues and crevicular fluids of diabetic and healthy periodontal patients. *J. Periodontol.* **2004**, *75*, 91-97.
10. Unlü, F.; Güneri, P.G.; Hekimgil, M.; Yeşilbek, B.; Boyacioğlu, H. Expression of vascular endothelial growth factor in human periodontal tissues: comparison of healthy and diabetic patients. *J. Periodontol.* **2003**, *74*, 181-187.
11. Shibuya, M. Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. *Genes Cancer.* **2011**, *2*, 1097-1105.
12. Lassalle, P.; Molet, S.; Janin, A.; Heyden, J.V.; Tavernier, J.; Fiers, W.; Devos, R.; Tonnel, A.B. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. *J. Biol. Chem.* **1996**, *271*, 20458-20464.
13. Sarrazin, S.; Adam, E.; Lyon, M.; Depontieu, F.; Motte, V.; Landolfi, C.; Lortat-Jacob, H.; Bechard, D.; Lassalle, P.; Deledre, M. 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.

14. Roudnicky, F.; Poyet, C.; Wild, P.; Krampitz, S.; Negrini, F.; Huggenberger, R.; Rogler, A.; Stöhr, R.; Hartmann, A.; Provenzano, M.; Otto, V.; Detmar, M. Endocan is upregulated on tumor vessels in invasive bladder cancer where it mediates VEGF-A-induced angiogenesis. *Cancer Res.* **2013**, *73*, 1097-1106.
15. Yilmaz, M.I.; Siriripol, D.; Saglam, M.; Kurt, Y.G.; Unal, H.U.; Eyileten, T.; Gok, M.; Cetinkaya, H.; Oguz, Y.; Sari, S.; Vural, A.; Mititiuc, I.; Covic, A.; Kanbay, M. Plasma endocan levels associate with inflammation, vascular abnormalities, cardiovascular events, and survival in chronic kidney disease. *Kidney Int.* **2014**, *86*, 1213-1220.
16. Scuruchi, M.; Aliquò, F.; Avenoso, A.; Mandraffino, G.; Vermiglio, G.; Minuti, A.; Campo, S.; Campo, G.M.; D'Ascola, A. Endocan Knockdown Down-Regulates the Expression of Angiogenesis-Associated Genes in IL-1 $\beta$  Activated Chondrocytes. *Biomolecules.* **2023**, *13*, 851.
17. Renne, E.; Mellberg, S.; Dimberg, A.; Petersson, L.; Botling, J.; Ameer, A.; Westholm, J.O.; Komorowski, J.; Lassalle, P.; Cross, M.J.; Gerwins, P. Endocan is a VEGF-A and PI3K regulated gene with increased expression in human renal cancer. *Exp. Cell. Res.* **2007**, *313*, 1285-1294.
18. Yazici, S.E.; Gedik, M.E.; Leblebici, C.B.; Kosemehmetoglu, K.; Gunaydin, G.; Dogrul, A.B. Can endocan serve as a molecular "hepatostat" in liver regeneration?. *Mol. Med.* **2023**, *29*, 1-16.
19. Lo Gullo, A.; Mandraffino, G.; Rodríguez-Carrio, J.; Scuruchi, M.; Sinicropi, D.; Postorino, M.; Morace, C.; Giuffrida, C.; Sciortino, D.; Gallizzi, R.; Loddo, S.; Zito, C.; Squadrito, G. Endocan and Circulating Progenitor Cells in Women with Systemic Sclerosis: Association with Inflammation and Pulmonary Hypertension. *Biomedicines.* **2021**, *9*, 533.
20. Scuruchi, M.; D'Ascola, A.; Avenoso, A.; Mandraffino, G.; Campo, S.; Campo, G.M. Endocan, a novel inflammatory marker, is upregulated in human chondrocytes stimulated with IL-1 beta. *Mol. Cell. Biochem.* **2021**, *476*, 1589-1597.
21. Liu, S.; Bai, T.; Feng, J. Endocan, a novel glycoprotein with multiple biological activities, may play important roles in neurological diseases. *Front. Aging. Neurosci.* **2024**, *16*, 1438367.
22. Löe, H.; Silness, J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. *Acta. Odontol. Scand.* **1963**, *21*, 533-551.
23. Silness, J.; Löe, H. Periodontal Disease in Pregnancy. II. Correlation Between Oral Hygiene and Periodontal Condition. *Acta. Odontol. Scand.* **1964**, *22*, 121-135.
24. Ainamo, J.; Bay, I. Problems and proposals for recording gingivitis and plaque. *Int. Dent. J.* **1975**, *25*, 229-235.
25. Papapanou, P.N.; Sanz, M.; Buduneli, N.; Dietrich, T.; Feres, M.; Fine, D.H.; Flemmig, T.F.; Garcia, R.; Giannobile, W.V.; Graziani, F. et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J. Clin. Periodontol.* **2018**, *45*, Suppl 20, 162-170.
26. 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.
27. Griffiths, G.S. Formation, collection and significance of gingival crevice fluid. *Periodontol 2000.* **2003**, *31*, 32-42.
28. Basim, P.; Argun, D. A Comparison of the Circulating Endocan Levels between the Inflammatory and Malignant Diseases of the Same Organ: The Breast. *J. Invest. Surg.* **2021**, *34*, 1207-1213.
29. Barros, S.P.; Williams, R.; Offenbacher, S.; Morelli, T. Gingival crevicular fluid as a source of biomarkers for periodontitis. *Periodontol 2000.* **2016**, *70*, 53-64.
30. Buduneli, N.; Kinane, D.F. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. *J. Clin. Periodontol.* **2011**, *38*, Suppl 11, 85-105.
31. Lin, S.J.; Chen, Y.L.; Kuo, M.Y.; Li, C.L.; Lu, H.K. Measurement of gp130 cytokines oncostatin M and IL-6 in gingival crevicular fluid of patients with chronic periodontitis. *Cytokine.* **2005**, *30*, 160-167.
32. Lamster, I.B.; Oshrain, R.L.; Fiorello, L.A.; Celenti, R.S.; Gordon, J.M. A comparison of 4 methods of data presentation for lysosomal enzyme activity in gingival crevicular fluid. *J. Clin. Periodontol.* **1988**, *15*, 347-352.

33. Pradeep, A.R.; Prapulla, D.V.; Sharma, A.; Sujatha, P.B. Gingival crevicular fluid and serum vascular endothelial growth factor: their relationship in periodontal health, disease and after treatment. *Cytokine*. **2011**, *54*, 200-204.
34. Pannicker, J.J.; Mehta, D.S. Effects of scaling and root planing on gingival crevicular fluid vascular endothelial growth factor level in chronic periodontitis patients with and without diabetes mellitus: A clinicobiochemical study. *J. Indian. Soc. Periodontol.* **2016**, *20*, 244-248.
35. Afacan, B.; Öztürk, V.Ö.; Paşalı, Ç.; Bozkurt, E.; Köse, T.; Emingil, G. Gingival crevicular fluid and salivary HIF-1 $\alpha$ , VEGF, and TNF- $\alpha$  levels in periodontal health and disease. *J Periodontol.* **2019**, *90*, 788-797.
36. Gündoğar, H.; Üstün, K.; Şenyurt, S.Z.; Özdemir, E.Ç.; Sezer, U.; Erciyas, K. Gingival crevicular fluid levels of cytokine, chemokine, and growth factors in patients with periodontitis or gingivitis and periodontally healthy subjects: a cross-sectional multiplex study. *Cent Eur J Immunol.* **2021**, *46*, 474-480.
37. Chapple, C.C.; Kumar, R.K.; Hunter, N. Vascular remodelling in chronic inflammatory periodontal disease. *J. Oral. Pathol. Med.* **2000**, *29*, 500-506.
38. Afacan, B.; Keleş Yücel, Z.P.; Paşalı, Ç.; Atmaca İlhan, H.; Köse, T.; Emingil, G. Effect of non-surgical periodontal treatment on gingival crevicular fluid hypoxia inducible factor-1 alpha, vascular endothelial growth factor and tumor necrosis factor-alpha levels in generalized aggressive periodontitis patients. *J. Periodontol.* **2020**, *91*, 1495-1502.
39. Keles, G.C.; Cetinkaya, B.O.; Eroglu, C.; Simsek, S.B.; 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.
40. Cetinkaya, B.O.; Keles, G.C.; Ayas, B.; Sakallioğlu, E.E.; 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.
41. Van Dyke, T.E.; Lester, M.A.; Shapira, L. The role of the host response in periodontal disease progression: implications for future treatment strategies. *J. Periodontol.* **1993**, *64*, 8 Suppl, 792-806.
42. Türer, Ç.C.; Durmuş, D.; Ballı, U.; Güven, B. Effect of Non-Surgical Periodontal Treatment on Gingival Crevicular Fluid and Serum Endocan, Vascular Endothelial Growth Factor-A, and Tumor Necrosis Factor-Alpha Levels. *J. Periodontol.* **2017**, *88*, 493-501.
43. Tayman MA, Önder C, Kurgan Ş, Serdar MA, Günhan M. Endocan (ESM-1) levels in gingival crevicular fluid correlate with ICAM-1 and LFA-1 in periodontitis. *Braz. Oral. Res.* **2020**, *35*.
44. Kumar, G.; Ponnaiyan, D.; Parthasarathy, H.; Tadepalli, A.; Veeramani, S. Evaluation of Endocan and Tumor Necrosis Factor- $\alpha$  as Inflammatory Biomarkers in Type 2 Diabetes and Periodontal Disease. *Genet. Test. Mol. Biomarkers.* **2020**, *24*, 431-435.

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