Vascular Endothelial Growth Factor in Gingival Tissues of Diabetic and Healthy Periodontal Patients

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Abstract: Background: Vascular endothelial growth factor (VEGF) played an important role in the development of chronic inflammatory diseases. In diabetes mellitus VEGF plays a major role in diabetic microangiopathy and causes an increased angiogenic response. Recently VEGF has attracted attention as a potential inducer of periodontal disease progression. On that account, the aim of this study was to evaluate VEGF expression in healthy versus diabetic periodontally diseased patients. **Subjects and methods:** Twenty-Four subjects were participated in this study. They were divided equally into three groups: group I non diabetic, none periodontally affected subjects (controls); group II periodontally affected patients (P); and group III periodontally affected type II diabetic patients (DP). All participants were evaluated clinically and histologically by the use of monoclonal antibodies (anti-VEGF) for immunohistochemical expression of VEGF in gingival tissues. **Results:** The three studied groups demonstrated a statistically significant difference in respect to the clinical parameters of periodontal disease. Histologically, both experimental groups expressed significantly increased level of VEGF when compared to controls. Moreover, the expression of VEGF was higher in DP group than P group. However, this increase was not significant. **Conclusion:** VEGF overexpression in diabetic patients with periodontitis depicts the primary role in promoting the extravasation of inflammatory cells, suggesting a useful antiangiogenic therapy for management of chronic periodontitis.
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1- Introduction

Periodontitis represents one of the major dental pathologies that affect human populations worldwide at high prevalence rates [(1)]. It is a chronic inflammatory lesion that involves the periodontium. It is well known that periodontitis is caused by the interaction between periodontopathogens, almost gram-negative bacteria, and the host immune response to the chronic infection which results in tissue destruction [(2)]. Progression of periodontal disease is episodic in nature on a tooth site level, but more recently, it has been realized that it is principally patient-based rather than site-based; the host related risk factors could be the key to better understand disease evolution [(3)]. The available evidence shows that important risk factors for periodontal disease relate to poor oral hygiene, tobacco use, excessive alcohol consumption, stress, and diabetes mellitus (DM) [(4,5)].

DM is a chronic inflammatory disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs. Several pathogenic processes are involved in the development of diabetes [(6)].

DM and periodontitis represent common chronic diseases that may have a reciprocal influence [(7)]. DM is an important and independent risk factor for the development of gingivitis and periodontitis and periodontal disease is one of the major oral problems encountered in patients with DM [(8)]. Regarding this aspect, a two-way relationship has been hypothesized between periodontitis and DM where, on one hand, periodontitis might represent a distant source of low grade systemic inflammation and impaired metabolic control. On the other hand, microangiopathy and an increased angiogenic response, typical of DM are correlated to the progression of periodontitis [(9-11)].

In the development of chronic inflammatory disease, like DM and periodontitis, an important role is played by angiogenic factors [(12)]. Studies have demonstrated that vascular endothelial growth factor (VEGF) is a key regulator of physiological and pathological angiogenesis. It is a proinflammatory angiogenic glycoprotein that induces endothelial cell proliferation, stimulates angiogenesis and increases vascular permeability [(13)]. It is primarily mitogenic for endothelial cells and its expression was initially found to be markedly increased in rapidly growing, highly vascularized tumors [(13-15)]. But recently VEGF attracts the attention as one of the most important

angiogenic factors involved in the pathogenesis of several chronic inflammatory diseases. [12]

In DM, angiogenesis is thought to be an essential process involved in the pathogenesis and progression of this disease. Studies on the mechanism and molecular involvement in the pathogenesis of diabetic microvasculopathy have suggested that VEGF plays a major role in microangiopathy and causes an increased angiogenic response. Therefore, in patients with diabetes, important microvascular complications, such as tissue ischemia, angiogenesis, permeability in many organs, and alteration in blood glucose levels, could be related to VEGF [16, 17].

In the last decade, many groups focused their research on the angiogenic factors that contribute to progression of periodontal disease. In periodontitis patients, VEGF was detected within vascular endothelial cells, neutrophils, plasma cells, and junctional, pocket and gingival epithelium [18]. Moreover increased VEGF expression was reported in epithelial cells and endothelial cells in periodontitis more than in gingivitis -affected sites, suggesting that VEGF could be an important growth factor for the onset of gingivitis and its progression to periodontitis [19-22].

Thus; the aim of this study was to compare the expression of the VEGF in gingival tissues of diabetic and healthy periodontal patients by immunohistochemical analysis.

2.Subjects and Methods:

Study Population

Twenty- Four subjects were participated in this study under an informed protocol (# EA/30/3013) approved by the Ethical Committee of Qassim University. Research objectives were explained to the patients, and all patients provided written informed consent before being included in the study.

Participants were divided equally into three groups: group I non diabetic, none periodontally subjects (controls); group II periodontally affected patients (P); and group III periodontal affected type II diabetic patients (DP). All Participants were enrolled from the flow to dental clinic, College of Dentistry, Qassim University, and Diabetes and Endocrinology Center in Burydah city, Qassim, KSA.

Inclusion criteria for controls were: absence of important systemic diseases, both previous and ongoing and absence of any ongoing drug therapy. The diagnosis of type II diabetes mellitus based on medical history and specific laboratory analysis for each patient including at least two glycosylated hemoglobin (HbA1c) assay results, obtained in the 6 months prior to the study, and fasting plasma glucose level. The diagnosis of generalized severe, chronic periodontitis was made based on the presence of >30% of measured sites with clinical attachment loss (AL) >5 mm [23].

Determination of Periodontal Status

All participants were evaluated clinically. The following clinical and periodontal parameters were assessed by the same examiner: plaque index [PI] [24]; gingival index [GI] [24]; probing depth [PD]; and clinical attachment loss [CAL]. The PD and CAL were assessed at six sites around each tooth for the whole mouth excluding third molars.

Gingival Biopsies

A marginal gingival biopsy of the size ranging from 1-1.5 mm maintaining the scalloped contour was obtained from experimental and control sites providing tissue from histological examination. Marginal gingiva of the tooth with residual deep pocket was the preferred site for biopsy. For control group the gingival biopsy was obtained during surgical removal of wisdom tooth or during extraction for orthodontic treatment.

Immunohistochemistry

Samples were fixed in formalin, and then each was placed in suitable labeled cassettes. Dehydration of the samples was carried out by immersing them in a series of alcohol solutions of increasing concentration. Samples clearing followed using Xylene and then embedding by thorough infiltration with paraffin wax, which formed into blocks and sectioned to 6um thickness. VEGF immuno-staining was evaluated in epithelial cells and endothelial cells of subepithelial connective tissue vessels using Image optical density (IOD) of immuno-staining.

The image of each slide of tissue in both control and experimental groups were captured using a 40 X objective (Bar = 50) with numerical aperture of a high resolution of 16-bit digital camera (2048 X1536 pixel). Images were viewed and recorded using Olympus microscope – equipped with Spot digital camera, using computer program MATLAB software (image J).

Image optical density (IOD) of immuno-staining of VEGF was evaluated by the maximum, minimum and integrity of intensity color based on Gray-level acquisition, analysis of the data, were carried out by reading 10 fixed areas in one image (10 images for each case). The mean values of each reaction were based on the mean of pixel number. The IOD based on Gray-level transition probabilities in digitalized images ranged from Intense to weak between all groups showing density of about (100 up to 180) in Diabetic group, (100 up to 170) in periodontal group, and (100 up to 130) in Healthy controls.

Statistical Analyses

All collected data were analyzed using the Statistical program for Social Science (SPSS) Version 20. Descriptive statistics were obtained using frequency count and Percentage. Independent- T test was used to compare means for two groups of cases. Correlation between the results was acquired using Bivariate correlation test. Analysis of variance (ANOVA) was used to compare statistics of different groups with Significance set at P <0.05.

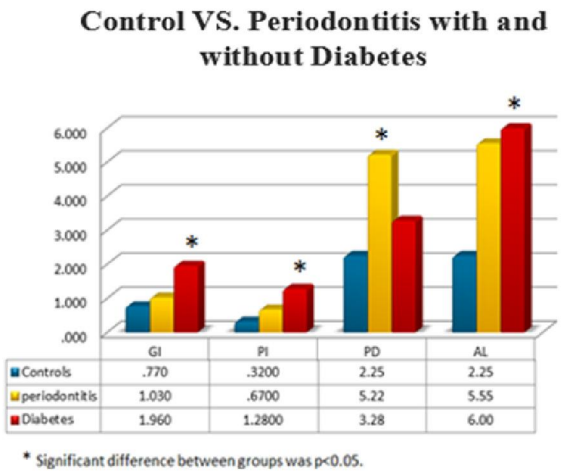
3. Results

In this study, the data obtained was subjected to appropriate statistical analysis. Regarding the clinical parameters of periodontal disease, there was a statistically significant difference between the three studied groups [p>0.05] (Graph-1).

When comparing GI and PI between DP group and controls there was a statistically significant difference. However, the difference between P and control groups regarding the same indices was not significant.

Regarding PD, there was no significant difference between DP and controls. On Contrast, a statistically significant difference was observed between P and control groups. Furthermore, a significant difference was found between each of the experimental groups versus the control group in terms of CAL (Graph-1).

When comparing DP and P groups regarding GI, PI, and PD, There was a statistically significant difference. On the Contrary, no significant difference was recorded between the two groups regarding the CAL.

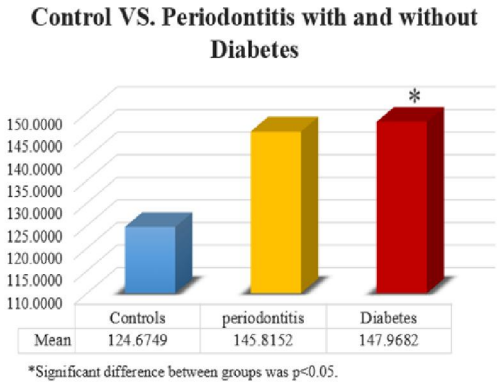


Graph- 1: Comparison between all studied groups in terms of the mean of clinical parameters

The mean of gingival VEGF stained cells was (147.96±5.93) in diabetic patient with periodontitis and was (124.67±4.96) in the control group, and the difference between the groups was statistically significant (p>0.00). VEGF-positive cells were significantly increased in the gingival tissues of patients with periodontitis (145.81±12.59) compared to controls (P >0.00), whereas, the difference in the VEGF levels was not statistically significant between the Diabetic with periodontitis and periodontitis groups (p<0.684).

A significant difference was found among the three groups regarding VEGF expression in gingival tissues (P <0.00) (Graph-2).

Immunohistochemically, the gingival expression of VEGF in endothelium and connective tissues (CT) was strong at sites of DP (Figure 1- A, B), while moderate reaction was observed in P group (Figure 2- A, B) versus weak reaction in healthy controls (Figure 3- A, B).



Graph- 2: Comparison between all studied groups in terms of the mean of VEGF expression

These finding were correlated to the clinical results that reported significant increase in the gingival inflammation at sites of periodontitis complicated by DM versus periodontitis alone (Table 1).

Table. 1: Correlation between GI and VEGF expression

	VEGF	
GI	Sig.	.027
* Correlation is significant at the 0.05 level (2-tailed).		

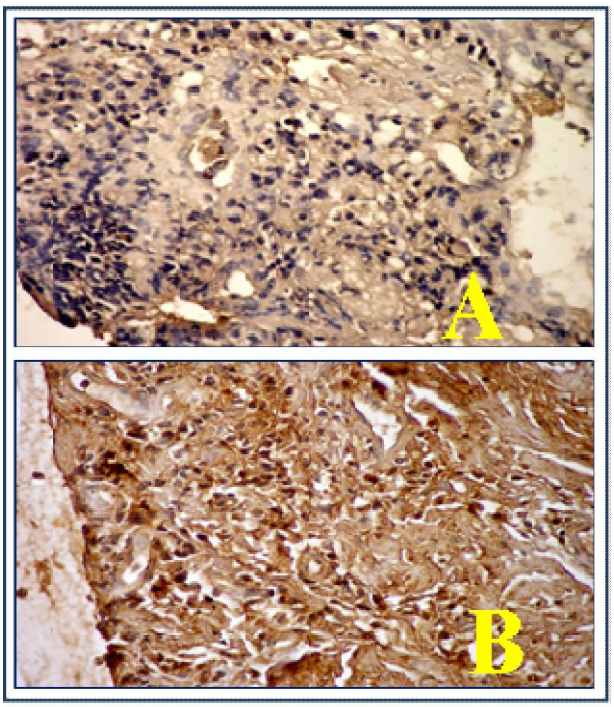


Figure 1 (A, B): Photomicrographs of gingival tissues from the (DP) group showing intense brown staining of VEGF in the endothelial cells of blood capillaries and CT.

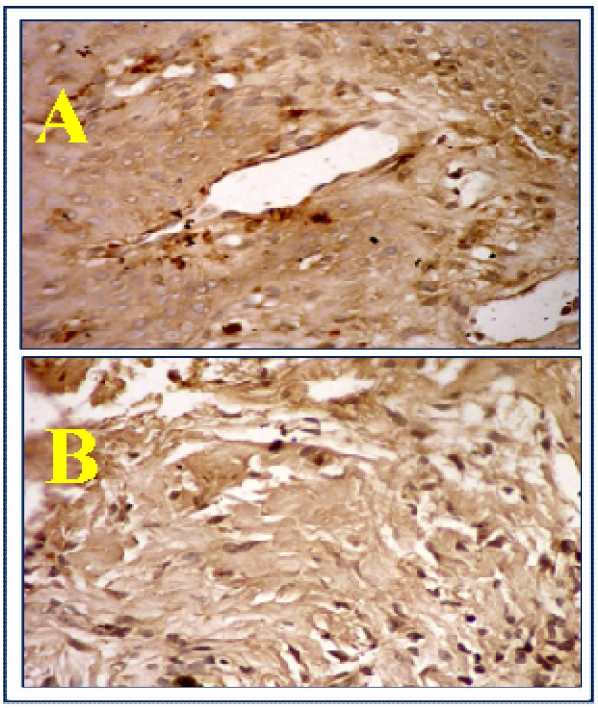


Figure 3 (A, B): Photomicrographs of gingival tissues from the control group showing weak brown staining of VEGF in the endothelial cells of blood capillaries and CT

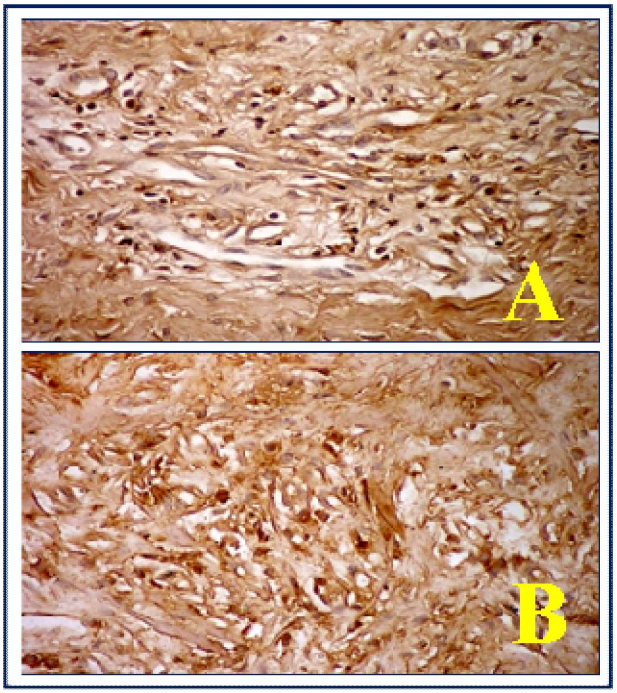


Figure 2 (A, B): Photomicrographs of gingival tissues from the (P) group showing moderate brown staining of VEGF in the endothelial cells of blood capillaries and CT.

4. Discussion:

It is widely accepted that DM aggravates both severity and progression of periodontal disease and poor metabolic control of DM is associated with the severity of periodontitis [25]. Both DM and periodontitis represent common chronic inflammatory diseases affected by angiogencety[19].

VEGF, one of the most potent angiogenic mediators, has been detected in the vascular endothelial cells, inflammatory cells, and junctional, sulcular and gingival epithelium of periodontal tissue. Evidence for the role of VEGF in inflammatory periodontal disease has been supported in some clinical studies[26-29]. It has been demonstrated that DM may have an inductive effect on the VEGF levels of the periodontium during periodontal disease [25].

This study was conducted to evaluate the level of VEGF in diabetic and non-diabetic periodontally affected patients versus healthy controls.

In the present study, several clinical Parameters were used. Gingival Index [24] was used for the assessment of the gingival condition and records qualitative changes in the gingiva. Our findings showed a significant increase in the GI between DP and control group. In contrast, there is no significant increase in GI when comparing between controls and P patients with the P group having the highest mean. When the three groups compared together, a

significant difference was found in favor to the DP group.

Periodontal pockets are pathognomonic signs of periodontal disease and therefore attain critical status in diagnosis of periodontitis. When compared between controls and each of the experimental groups, there are significant differences in probing depth. In contrast to that, there is no significant difference among P and DP patients, because of recessions were more pronounced in DP group than in P group.

Clinical attachment loss is a sign of destructive periodontal disease. The current research reported a significant difference among control, P and DP groups, whereas there is no significant differences between P and DP.

Recently VEGF has attracted attention as a potential inducer of periodontal disease progression and correlates with inflammatory resolution and periodontal tissue healing [30].

Our findings reported a significant difference among the three groups for VEGF expression in gingival tissues. The expression of VEGF in gingival tissues was intense at sites of DP, while moderate reaction was observed in P group versus weak reaction in Healthy control. These findings were correlated to the GI (Table. 1).

Accordingly, the present results revealed that VEGF level in the tissue samples of the DP and P groups were found to be significantly higher than those of control group. These findings were in agreement with Pelin Güneri study in 2004, that reported VEGF is increased in all periodontal tissues of periodontitis and type 2 diabetic patients. He concluded that DM might have an additive effect on the VEGF levels of both healthy and diseased gingival tissues [31]. Moreover, our findings were also in consistent with Aspriello, *et al.,* 2009 who concluded that VEGF acts as a potent and pleiotropic inflammatory agent in periodontitis, especially when further aggravated by diabetes [32].

Furthermore, when comparing DP and P groups, VEGF expression was higher in DP group, whereas, this difference was not statistically significant (p<0.684). These findings were in accordance with a study conducted by Sakallıoğlu, *et al.,* 2007, who stated that VEGF level was significantly higher in the gingiva of the diabetic more than that of the non-diabetic patients and this increase in VEGF production has been shown to be induced in gingival fibroblasts by the periodontal pathogens *P. gingivalis* and *A. actinomycetemcomitans* [25].

Conclusion:

VEGF acts as a potent and pleiotropic inflammatory agent in periodontitis, especially when further aggravated by type II diabetes mellitus, suggesting a useful antiangiogenic strategy for periodontitis treatment. However further researches have to be conducted to evaluate the effect of periodontal therapy on the level of VEGF in gingival tissues of diabetic and non diabetic periodontal affected patients.

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