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Effect of Vascular Endothelial Growth Factor Expression in the Destruction and Healing Stages of Chronic Periodontal Disease: A Case Report

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Abstract Vascular endothelial growth factor (VEGF) is a homodynamic proinflmmatory protein produced by many types of cells including endothelial cells, macrophages, activated T-cells and epidermal keratinocytes. VEGF was reported in epithelial cells and endothelial cells in periodontitis more than in gingivitis affected sites, suggesting that it could be an important growth factor for the onset of gingivitis and its progression to periodontitis. **Objective**: To evaluate the effect of non-surgical periodontal therapy on the expression of vascular endothelial growth factor (VEGF) in gingival tissues affected by chronic periodontitis. **Methods**: Gingival samples (2-3mm) were collected from a 48 year old male with chronic periodontitis immediately before and 4 weeks after non-surgical periodontal therapy. The tissue samples were processed using immunohistochemical technique. **Results**: A decrease in VEGF expression in gingival tissues was recorded after non-surgical periodontal therapy. **Conclusion**: VEGF is continually produced and expressed in healthy and diseased gingival tissues; non-surgical periodontal therapy with antibiotics combination greatly affects the expression the patterns of VEGF as biomarker.

Keywords: *chronic periodontitis, non-surgical periodontal therapy, vascular endothelial growth factor*

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1. Introduction

Vascular endothelial growth factor (VEGF) is a homodynamic proinflmmatory protein produced by many types of cells including endothelial cells, macrophages, activated T-cells and epidermal keratinocytes. [1,2] VEGF is a multifunctional angiogenic mediator that potently increases microvascular permeability, stimulates endothelial cell proliferation and induces proteolytic enzyme expression and the migration of endothelial cells, monocytes and osteoblasts, all of which are essential for angiogenesis. It is over expressed in various human tumors and inflammatory conditions such as periapical granuloma, pulpitis, periodontitis and radicular cysts. [3-8].

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, junctional pocket and gingival epithelium. [9] The increased expression of VEGF was reported in epithelial cells and endothelial cells in periodontitis more than in gingivitis affected sites, suggesting that it could be an important growth factor for the onset of gingivitis and its progression to periodontitis. [10,11,12,13]

The case presented is a short term non-surgical treatment of a male with chronic periodontitis with the evaluation of vascular endothelial growth factor expression.

2. Case Report

A 48 year old Egyptian male presented to the outpatient clinic, College Of Dentistry, Qassim University, Saudi Arabia suffering from periodontal diseases. The diagnosis was confirmed by both clinical and radiographic examination as 60% of periodontal sites were moderate to severe chronic periodontitis with a minimum of 3 mm attachment loss.

The patient was informed about the nature and objectives of the study and his full signed consent was obtained prior to the study. The study complied with the rules set by the International Conference on Harmonization of Good Clinical Practice Guidelines and the Declaration of Helsinki The protocol was reviewed and approved by the Ethical Committee at the College of Dentistry, Qassim University.

The following parameters were recorded: Plaque Index (Pl-I) [14], Gingival index (GI) [15], Probing pocket depth (PD), Clinical Attachment Loss (CLA) in addition to radiographic examination (periapical radiographs) using long cone parallel technique. The clinical periodontal parameters and radiographs were determined at base line and 4 weeks after periodontal therapy. The patient was not a smoker and has not received any periodontal therapy during the last 6 months.

3. Periodontal Therapy

Patient was subjected to oral hygiene instructions and professional tooth cleaning. Full mouth supra and subgingival scaling and root planning was performed using ultrasonic scalers and periodontal curettes. Debridement was completed on two sessions at base line and 4 weeks afterwards. The patient was instructed to use chlorhexidine mouth wash and antibiotic regimen in the form of a combination of 500 mg Amoxicillin and 250 mg metronidazole 3 times/day for 7 days.

The patient was instructed to follow a maintenance program for mechanical plaque control by using a soft tooth brush three times daily with regular tooth paste and the use of interdental tooth picks with regular recall visits every 7 days for 4 weeks interval.

4. Methodology

4.1. Gingival Tissue Specimens

After topical anesthesia using 20% Benzocaine, an intra-sulcular incision was made in the periodontal pocket with a #15 blade on the palatal/lingual part of the tooth with periodontal disease (PD >5 mm). This was performed at base line and 4 weeks after periodontal treatment.

4.2. 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 ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached. Samples clearing are performed by using Xylene and then they are embedded by thorough infiltration with paraffin wax which are then formed into blocks and sectioned to 6um thickness. VEGF immune staining was evaluated in epithelial cells and endothelial cells of sub-epithelial connective tissue vessels using Image optical density (IOD) of immune staining

The image of each slide of tissue was captured using a 40x 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 immune staining of VEGF was evaluated by the maximum, minimum and integrity of intensity color based on Gray-level acquisition analysis of the data. The analysis was 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.

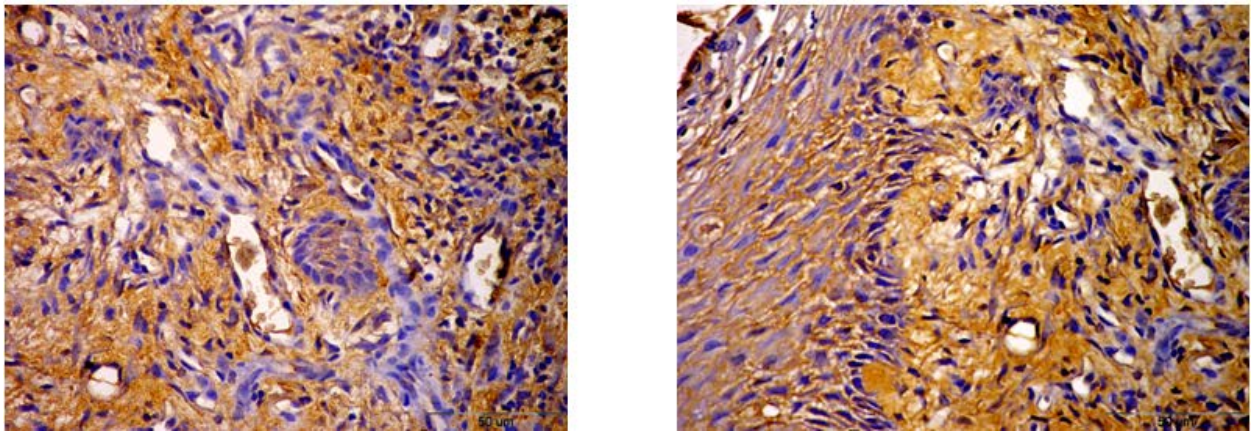

Figure 1. Photomicrographs of gingival tissue before periodontal therapy showing intense brown staining of VEGF in the both epithelial layer and lamina propria , also show the new small capillaries in both layer

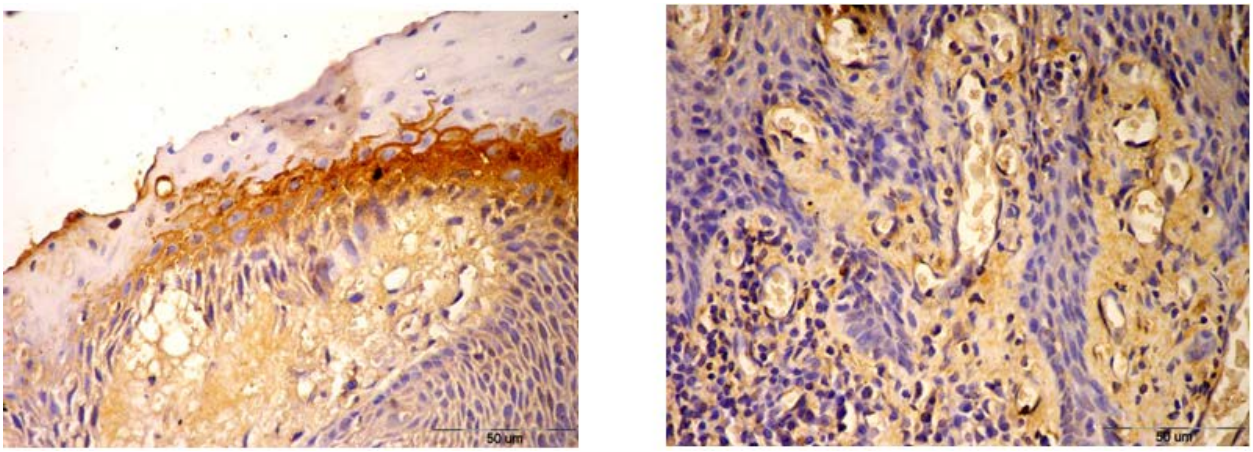

Figure 2. Photomicrographs of gingival tissue after 4 weeks periodontal therapy showing VEGF expression in the epithelial layer and moderate at the lamina propria

5. Results

The expression of VEGF was found as brown cytoplasmic staining in the keratinocytes. The expression was observed in all layers of epithelium including the basal cells but it varied from moderate to strong according to the degree of inflammatory cell infiltrates. (Figure 1 & Figure 2) The expression of VEGF was interrupted in some areas with sever periodontal involvement. The mean of gingival VEGF stained cells was (92.428±3.25) at the base line and (88.714±2.66) after 4 weeks.

6. Discussion

Periodontal disease encompassing both gingivitis and periodontitis is a host mediated inflammatory process initiated by oral bacterial insult which may result in significant alterations in the normal structure and/or function of the supporting tissues of the dentition. Although colonization of host tissues by pathogenic organisms is the initiating factor in this disease process, the associated rate of progression and degree of destruction are dependent upon both the virulence of the invading organisms and the magnitude/persistence of the host. Treatment of periodontal diseases has a great deal in common with the treatment of infectious diseases elsewhere in the body by controlling the putative pathogens. The goal of periodontal therapy is the elimination or reduction of periodontal pathogens from the oral cavity and the subgingival area response to this infection. [16]

In the present study the treatment protocol consisted of scaling and root planing in conjunction with antibiotic combination of amoxicillin and metronidazole. The goal of root debridement was to remove microbiologically contaminated cementum and to eliminate or reduce the number of pathogenic bacteria in the periodontal pocket below their disease inducing levels. The rational for the use of systemic antibiotics was to rapidly suppress target microbial species and faster the establishment of a host compatible microflora. Metronidazole has a narrow spectrum and works specifically on anaerobic microorganisms associated with periodontal diseases. Amoxicillin is very effective against most periodontal pathogens. [17] Pavicic et al (1994) found that both antibiotics act synergistically on A. Actinomycetemcomitans. [18]

Our findings reported a significant decrease in VEGF expression in gingival tissues after periodontal treatment. The expression of VEGF in gingival tissues was intense at sites of deep pocket at before periodontal therapy, while moderate to weak reaction was observed after 4 weeks of treatment. These findings were in agreement with Pelin Güneri study that was done on 2004 that reported VEGF is increased in all periodontal tissues of periodontitis patients. [19]

Inflamed periodontal tissues bleed after gentle stimulation and this is attributed to the disorganized interstitial matrix and fragile neovasculature associated with inflammation. Conversely, a lack of bleeding after stimulation is taken to indicate periodontal health. However, at a histological level, inflammatory cells can be detected in clinically healthy tissues. Therefore the presence of VEGF in GCF from healthy sites may reflect sub-clinical levels of inflammation or healing following the microbial assault which occurs with even the sparse microflora in gingival health. Also the presence of VEGF in healthy sites may be relevant to physiological angiogenesis in the gingival/periodontal environment. [20]

VEGF concentrations within tissues adjacent to>6 mm periodontal pockets could have been reduced (as compared to that in tissues adjacent to 4 to 6 mm depths) due to enzymes present within that microenvironment which could have degraded the VEGF in situ. In addition, previous studies indicate that the bioavailability of VEGF may be affected when bound to heparin. [21,22] Thus, VEGF in tissues adjacent to >6 mm periodontal pockets could have been masked by the extracellular matrix and unavailable for assay, thereby reducing its concentration in the tissue assay. Reduced concentrations of VEGF in tissues adjacent to >6 mm periodontal pockets could also be the result of sup- pressed VEGF synthesis by hypoxia. [23]

Thus, VEGF is likely a factor in the etiology of gingivitis and its progression to periodontitis, possibly by initiating expansion of the vascular network.

7. Conclusion

VEGF is continually produced and expressed in healthy and diseased gingival tissues; non-surgical periodontal therapy with antibiotics combination greatly affects the expression the patterns of VEGF as biomarker.

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