International Journal of Dental Sciences and Research, 2017, Vol. 5, No. 2, 35-38
Available online at http://pubs.sciepub.com/ijdsr/5/2/5

DOI:10.12691/ijdsr-5-2-5

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

Alaa Abdelhamid*

Periodontology and Oral Medicine Department, Qassim College of Dentistry, Qassim University, Saudi Arabia
*Corresponding author: dr.alaa.abdelhamid@qudent.edu.sa

Abstract Vascular endothelial growth factor (VEGF) is a homodynamic protein produced by different cells as endothelial cells, macrophages, T-cells. In epithelial and endothelial cells, VEGF has been reported more in periodontitis than in sites with gingivitis, suggesting that it could be a crucial factor for the initiation of gingivitis and its transition to periodontitis. **Objective**: To determine the effect of non-surgical periodontal treatment on VEGF expression on gingival tissues with chronic periodontitis. **Methods**: Gingival samples (2-3 mm) were collected immediately before and 4 weeks after non-surgical periodontal therapy from a 48-year-old male with chronic periodontitis. The samples were treated with immunohistochemically. **Results**: A decrease in VEGF expression was observed in gingival tissues following non-surgical periodontal treatment. **Conclusion**: VEGF is continually produced and expressed in healthy and diseased gingival tissues; non-surgical periodontal therapy with antibiotics combination significantly affects the expression pattern of VEGF as a biomarker.

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

Cite This Article: Alaa Abdelhamid, "Effect of Vascular Endothelial Growth Factor Expression in the Destruction and Healing Stages of Chronic Periodontal Disease: A Case Report." *International Journal of Dental Sciences and Research*, vol. 5, no. 2 (2017): 35-38. doi: 10.12691/ijdsr-5-2-5.

1. Introduction

VEGF is a homodynamic protein developed by many cell including endothelial cells. [1,2] It is a multifunctional angiogenic mediator that powerfully increases microvascular permeability, stimulates the proliferation of endothelial cells and induces the expression of proteolytic enzymes and the migration of endothelial cells, monocytes and osteoblasts, all of which are essential for angiogenesis; It is highly produced in different human inflammatory conditions as periodontitis, granuloma, radicular cysts and pulpitis. [3-8]

Several researchers have based their work on the angiogenic factors that lead to the development of periodontal disease over the last years. Increased VEGF expression was found in patients with periodontitis more than those with gingivitis endothelial vascular cells, neutrophils, plasma cells, pockets, and gingival epithelium. [9] Also, the increased expression of VEGF in endothelial and epithelial cells was reported less in gingivitis than in periodontitis, indicating it is possibly an important factor for the initiation of gingivitis and its transition to periodontal diseases. [10-13]

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

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. Diagnosis was confirmed by both clinical and radiographic examination as 60% of sites with periodontal disease had moderate to severe chronic periodontitis with a loss of at least 3 mm of attachment.

The patient was clearly introduced to the purpose and aims of the study and his signed authorization was obtained in advance. The Protocol was approved by the Ethical Committee at Qassim University.

The following parameters were recorded: Plaque Index (PI) [14], Gingival index (GI) [15], pocket depth (PD), Attachment Loss (CLA) in addition to periapical radiographs. Clinical periodontal parameters and radiographs were determined on the baseline and 4 weeks after periodontal treatment. The patient was not a smoker and did not receive periodontal therapy for the previous 6 months.

3. Periodontal Therapy

Patients underwent oral hygiene and professional tooth cleaning instructions. Thorough full mouth scaling and root planning were performed by curettes and ultrasonic

scalers. Debridement was performed on two baseline sessions and 4 weeks later. The patient was instructed to use chlorhexidine in the mouth wash and antibiotic regimen in combination of 500 mg Amoxicillin and 250 mg metronidazole 3 times/day for a week.

The patient was directed to follow strict periodontal maintenance program for plaque control by using extra soft toothbrush three times a day with regular tooth paste and by using interdental tooth picks with weekly regular recalls for 4 weeks.

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

Fixation of samples was performed in formalin then placed in cassettes. Samples dehydration was done by immersing them in a series of solutions of ethanol with increasing concentration up to the point of purity, water-free alcohol is then reached. Sample clearing is performed using Xylene and is then embedded by an infiltration of paraffin wax, which is then conformed in blocks and then cut to 6um thickness. VEGF immune staining was assessed in endothelial and epithelial cells of sub-epithelial connective tissue vessels by Image optical density (IOD) immune staining.

Each image of a tissue slide was captured using a 40x lens (Bar=50) with a 16-bit high resolution digital camera (2048 X1536 pixel) numerical aperture. Images were perceived and recorded using Olympus microscope – equipped with a Spot Digital Camera – using the image J software (MATLAB) program.

IOD (Image optical density) of VEGF immune staining was assessed for maximum, minimum and integrity of color intensity based on Gray-level data acquisition analysis. The assessment was done by reading 10 fixed areas in one image. The mean values of each reaction were based on the mean number of pixels. The IOD based on Gray-level transition probabilities in the images (digitalized) varied from weak to Intense.

5. Results

It was found that the expression of VEGF was brown cytoplasmic staining in the keratinocytes. Expression was found in all epithelium layers and even included the basal cells. This expression was found to range from moderate to strong according to the inflammatory cell infiltration degree. (Figure 1 and Figure 2) The expression of VEGF was cut off in some areas that showed severe involvement of periodontitis. The mean of gingival VEGF stained cells was (92.428±3.25) at the base line and (88.714±2.66) after 4 weeks.

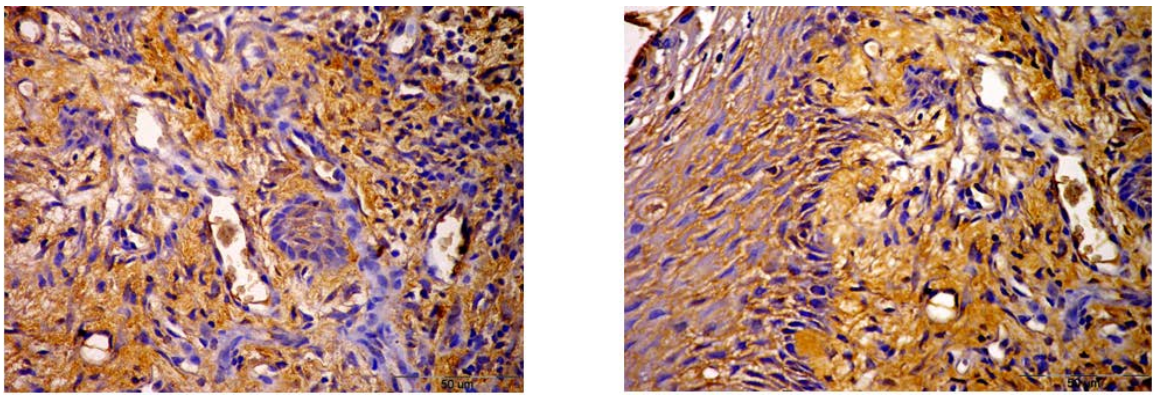

Figure 1. Photomicrographs of gingival tissue before periodontal therapy showing intense brown staining of VEGF in the both epithelial layer and lamina propria , also show the new small capillaries in both layer

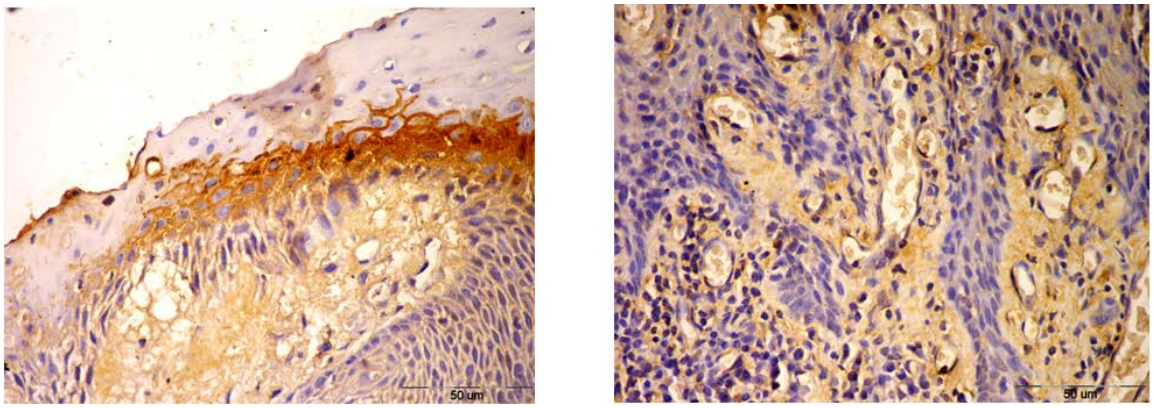

Figure 2. Photomicrographs of gingival tissue after 4 weeks periodontal therapy showing VEGF expression in the epithelial layer and moderate at the lamina propria

6. Discussion

Gingivitis and periodontitis are both processes of host mediated inflammation which is all started by oral bacterial insults, resulting in severe change in the structure and function of the supporting dentition. Although the igniting factor in this process could be directly related to the occupation of host tissues by pathogenic organisms, the rate of progression and degree of destruction is also related to the severity of the invasive bacteria and the host magnitude. Periodontal diseases treatment is performed by controlling related pathogens, similar to the treatment of other infectious diseases in the body. The aim of periodontal treatment is to eliminate or decrease the periodontal pathogens from the oral cavity and consequently the subgingival response to this infection. [16]

In this study the treatment regimen consisted of scaling and root planing together with a combination of antibiotics including metronidazole and amoxicillin. Root planing was performed to remove diseased and necrotic cementum and to leave the periodontal pocket with minimal number of pathogenic bacteria. The logic for the use of systemic antibiotics was to speed up the suppression of specific microbial species and the initiation of a compatible host microflora. The high effectiveness of Amoxicillin against most periodontal pathogens clearly justifies its use in the combination therapy. The narrow spectrum of Metronidazole makes it work specifically with anaerobic microorganisms that are related to periodontal diseases. [17] Pavicic et al (1994) found that together both antibiotics has an enhanced effect on A. Actinomycetemcomitans. [18]

Our findings reported that after periodontal therapy a significant decrease in the expression of VEGF in gingival tissue was clearly noticed. The expression of VEGF in gingival tissue was severe at deep pocket sites prior to periodontal therapy, while moderate to weak reactions were observed after 4 weeks of treatment. These results were to those of a study performed on 2004 that similarly reported increased VEGF in all periodontal tissues of patients with periodontitis. [19]

The bleeding of the Inflamed periodontal tissues after minor stimulation is attributed to the disorganized interstitial matrix and fragile vasculature associated with inflammation. Conversely, absence of bleeding after stimulation is an indication of periodontal health. However, at a histological level, inflammatory cells can be detected in clinically healthy tissues. This may indicate that the presence of VEGF in GCF of healthy sites could indicate sub-clinical levels of inflammation or healing following the microbial assault which take place with even the minimal microflora associated with gingival health. Also the presence of VEGF in healthy sites may be related to physiological angiogenesis in the gingival or periodonta1 environment. [20]

VEGF concentrations within tissues adjacent to periodontal pockets (>6 mm) could have been reduced (compared to that in tissues adjacent to 4 to 6 mm depths) due to enzymes present within that microenvironment which may have degraded the VEGF in situ. In addition, previous studies indicated that the bioavailability of VEGF may be affected when combined with heparin. [21,22] Thus, VEGF in tissues adjacent to >6 mm periodontal pockets could have been hidden 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 suppressed VEGF production by hypoxia. [23]

As a result, VEGF could be considered as a factor in the etiology of gingivitis and its progression to periodontitis, mainly by promoting the expansion of the vascular network.

7. Conclusion

VEGF is continuously produced in both healthy and diseased gingival tissues. Non-surgical periodontal antibiotic combination treatment significantly influences the expression of VEGF which serves as a biomarker.

References

[1] Brown LF, Yeo KT, Berse B, Yeo TK, Senger DR, Dvorak HF, van de Water L. Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. J Exp Med. 1992; 176: 1375-9.

[2] Robinson C J. and Stringer S E. The splice variants of vascular endothelial growth factor (VEGF) and their receptors. J Cell Sci 2001; 114: 853-65.

[3] Graziani F, Vano M, Viacava P, Itro A, Tartaro G, Gabriele M. Microvessel density and vascular endothelial growth factor (VEGF) expression in human radicular cysts. Am J Dent. 2006; 19: 11-4.

[4] Duyndam MC, Hilhorst MC, Schlüper HM, Verheul HM, van Diest PJ, Kraal G, Pinedo HM, Boven E. Vascular endothelial growth factor overexpression stimulates angiogenesis and induces cyst formation and macrophage infiltration in human ovarian cancer xenografts. Am J Pathol. 2002; 160(2): 537-48.

[5] Artese L, Rubini C, Ferrero G, Fioroni M, Santinelli A, Piattelli A. Vascular endothelial growth factor (VEGF) expression in healthy and inflamed human dental pulps. J Endod. 2002; 28: 20-3.

[6] Yao DF, Wu XH, Zhu Y, Shi GS, Dong ZZ, Yao DB, Wu W, Qiu LW, Meng XY. Quantitative analysis of vascular endothelial growth factor, microvascular density and their clinicopathologic features in human hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int .2005; 4: 220-6.

[7] Leonardi R, Caltabiano M, Pagano M, Pezzuto V, Loreto C, Palestro G. Detection of vascular endothelial growth factor/ vascular permeability factor in periapical lesions. J Endod. 2003; 29: 180-3.

[8] Metwaly H, ElDeeb A. Co-expression of matrix metalloproteinase-2 (MMP-2) and vascular endothelial growth factor in odontogenic cysts (VEGF). Egyptian Dental Journal. 2009; 55: 1401-10.

[9] Vascular endothelial growth factor in human periodontal disease. Booth, V, et al., et al. November 1998, Journal of Periodontal Research, Vols. 33, No.8, pp. 491-499.

[10] Vascular endothelial growth factor in gingival tissues and crevicular fluids of diabetic and healthy periodontal patients. Guneri, P, et al., et al. January 2004, Journal of Periodontology, Vols. 75, No.1, pp. 91-97.

[11] Involvement of vascular endothelial growth factor, CD44 and CD 133 in periodontal disease and diabetes: an immunohistochemical study. Lucarini, G, et al., et al. January 2009, Journal of Clinical Periodontology, Vols. 36, No.1, pp. 3-10.

[12] Vascular endothelial growth factor expression levels of gingiva in gingivitis and periodontitis patients with/without diabetes mellitus. Keles, G C, et al., et al. July 2010, Inflammation Research, Vols. 59, No.7, pp. 543-549.

[13] Matrix molecules and growth factors as indicators of periodontal disease activity. Giannobile, W V, Al-Shammari, K F and Sarment, D P. February 2003, Periodontology 2000, Vols. 31, No.1, pp. 125-134.

[14] Silness J and Loe N: - periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal conditions Acta Odontologica Scandinivica. 1964; 24: 747-759.

[15] Löe H and Silness J. Periodontal disease in pregnancy I. prevalence and severity. Acta Odontologica Scandinavica. 1965; 21: 533-551.

[16] Sakallioglu EE, Aliyev E, Lutfioglu M, Yavuz U, Acikgoz G. Vascular endothelial growth factor (VEGF) levels of gingiva and gingival crevicular fluid in diabetic and systemically healthy periodontitis patients. Clin Oral Investig Clin Oral Investig. 2007 Jun; 11(2): 115-20.

[17] Walker CB, Gordon JM, Mangnusson I, and William BC:- Arole for antibiotic in the treatment of refractory periodontitis.J Periodontol. 1993; 64: 772-781.

[18] Pavicic MJAMP, Van Winkelhoff AJ, Douque NN and Steuren Rwp: - Microbiological and clinical effects of metronidazole and amoxicelline on Actinobacillus actinomycetemcomitans associated periodontitis. J Periodontol.1994; 21: 107-112.

[19] Guneri P, Unlu F, Yesilbek B, Bayraktar F, Kokuludag A, Hekimgil M, et al. Vascular Endothelial Growth Factor in Gingival Tissues and Crevicular Fluids of Diabetic and Healthy Periodontal Patients. Journal of Periodontology. 2004 January; 75 No.1: p. 91-97.

[20] Egelberg J. Permeability of the dento-gingival blood vessels. Clinicafly heaIthy gingivae. J Periodont Res. 1966. 1276-286.

[21] Park JE, Keller G-A, Ferrara N. The vascular endothelial growth factor (VEGF) isoforms: Differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. Mol Biol Cell 1993; 4: 1317-1326.

[22] Houck KA, Leung DW, Rowland AM, Winer J, Ferrara N. Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms. J Biol Chem 1992; 267: 26031-26037.

[23] Liu Y, Christou H, Mouta T, et al. Carbon monoxide and nitric acid suppress the hypoxic induction of vascular endothelial growth factor gene via the 5' enhancer. J Biol Chem 1998; 273: 15257-15262.