DOI: 10.7860/JCDR/2014/8450.5163

Original Article

Dentistry Section

Vascular Endothelial Growth Factor Levels in Gingival Crevicular Fluid Before and after Periodontal Therapy

PADMA R.[1], ANNAJI SREEDHARA[2], INDEEVAR P.[3], INDRANIL SARKAR[4], CHETAN S KUMAR[5]

ABSTRACT

Introduction: Of the various cytokines and growth factors regulating angiogenesis, the most potent agent acting on vascular endothelium is vascular endothelial growth factor(VEGF). The present study aims to access VEGF concentration in periodontal diseases and the effect of periodontal therapy on its concentration in gingival crevicular fluid (GCF).

Materials and Methods: Ninety five subjects (47 females and 48 males) 25- 47 y of age were divided into healthy (group-1), gingivitis (group-2), and periodontitis (group-3). GCF samples were collected using micro-capillary pipettes & were transferred immediately to plastic vials and stored at -70°C until the time of the assay. The concentration of VEGF was determined using commercially available ELISA kit.

Results: The mean VEGF concentration was highest in periodontitis patients (Group 3) (88.08±8.04pg/ml), with lowest in healthy patients (Group 1). VEGF levels reduced significantly after therapy in Groups 2 and 3.

Conclusion: VEGF levels in GCF had a significant correlation with both periodontal disease progression and healing after therapy.

Keywords: Angiogenesis, Gingival crevicular fluid, Periodontitis, Vascular endothelial growth factor

INTRODUCTION

Angiogenesis is the formation of new capillaries by the budding of endothelial cells residing in the surrounding pre-existing vessels. There is considerable evidence to indicate that vascular endothelial growth factor (VEGF) also known as vascular permeability factor or vasculotropin plays a critical role in neo-vascularization [1]. Increase in vessel profiles along the periodontal pocket wall could be a result of angiogenesis [2]. VEGF induces the permeability of fluids and proteins 50,000 times more than histamine [3]. VEGF potently increases micro-vascular permeability, stimulates endothelial cell (EC) proliferation, induces proteolytic enzymes and migration of endothelial cell and monocytes, all of which are essential for angiogenesis [4-11].

Several members of VEGF family have been described during the past few years including VEGF-A, PlGF, VEGF-B, VEGF-C, VEGF-D and VEGF-E [12]. VEGF A is a dimeric glycoprotein which has atleast nine subtypes due to the alternative splicing of the single gene; $VEGF_{121}$, $VEGF_{145}$, $VEGF_{148}$, $VEGF_{165}b$, $VEGF_{183}$, $VEGF_{189}$, $VEGF_{206}$ [13,14]. $VEGF_{121}$ does not bind heparin and is freely released from the cell. $VEGF_{165}$ readily binds to heparin and it remains bound to the cell surface or to the extracellular matrix following secretion [15]. $VEGF_{189}$ and $VEGF_{206}$ bind to heparin with greater affinity than $VEGF_{165}$ and are completely sequestrated within the extracellular matrix [15,16].

VEGF has a role in physiological (menstrual cycle, pregnancy and wound healing) and pathological conditions (diabetes, rheumatoid arthritis, psoriasis, cancer and chronic inflammation) [17-21]. VEGF is also shown to be important for memory and learning [22]. Exaggerated levels of VEGF A have been detected in tissues and biologic samples from subjects of asthma where these levels correlate directly with the disease [23] and inversely with airway function [24]. Increased levels of circulating soluble fms -like tyrosine kinase 1 (sFIT-1) and reduced levels of free placental growth factor (PlGF) and VEGF predict the subsequent development of pre-eclampsia [25].

In gingival crevicular fluid (GCF) the levels of bacterial antigens, inflammatory mediators and tissue breakdown products have been assessed as risk markers of active periodontal destruction [26]. PGE_2 in GCF is associated with destruction of periodontal attachment [27]. PGE_2 is a potent stimulant of VEGF synthesis. In addition to PGE_2, interleukin-1 (IL1) and tumour necrosis factor α (TNF α) are also implicated in the induction of VEGF [28]. Both these cytokines are released at higher levels by monocytes and macrophages from patients with periodontitis than from healthy controls particularly in response to bacterial risk factor [29,30]. VEGF expression is also reported to be regulated by the oxygen concentration of the tissues with hypoxia inducing its expression [31].

In view of the above mentioned findings this clinico-biochemical study was undertaken to investigate the VEGF levels detectable in GCF in subjects with clinically healthy gingiva, gingivitis and chronic periodontitis and after initial therapy i.e. scaling in the gingivitis subjects and scaling root planning (SRP) in periodontitis subjects.

MATERIALS AND METHODS

The study population consisted of 95 subjects (47 females and 48 males) 25-47 y of age that were selected from the out-patient section of the Dept. of Periodontics, St. Josephs Dental College, Eluru, Andhra Pradesh, India.

Exclusion criteria were: Pregnancy, lactation, Smoking, alcoholism, diabetes, hypertension, asthma, tumours, rheumatoid arthritis, cardiac diseases, long term administration of anti-inflammatory and antibiotic medication, aggressive periodontitis and previous periodontal therapy within a period of twelve months. Each subject underwent a full mouth periodontal probing and charting along with intra oral peri-apical radiographs for crestal bone loss.

The subjects were then categorised into three groups based on the clinical examination and radiographic evidence of bone loss. Forty five subjects with clinically healthy periodontium, Gingival Index (GI<1), Probing pocket depth less than or equal to 3mm (PPD ≤ 3), no clinical attachment loss (CAL=0) and no radiographic evidence of crestal bone loss were included in group 1.

Group 2 (Gingivitis group) consisted of 25 subjects with GI≥2, PPD≤3mm and CAL=0.

Group3 (Chronic periodontitis) consisted of 25 subjects with PPD ≥5mm, CAL ≥2mm and/or radiographic evidence of bone loss.

Subjects satisfying the above criteria were enrolled in the study after obtaining the ethical clearance from the institutional review board. All the recordings were performed by a single examiner. Single site was selected from each subject for GCF sampling. In the healthy subjects sampling was predetermined to be from mesio-buccal region of maxillary right first molar in the absence of which the left molar was considered. In the gingivitis patients, sites with most severe gingival inflammation were considered and in periodontitis group sites showing maximum probing pocket depth or sites with CAL ≥2mm along with radiographic confirmation of crestal bone loss was sampled. GCF samples were collected using calibrated volumetric micro capillary pipettes (TT3 Top Tech, Biomedicals, Mumbai, India). Sites selected for sampling were isolated with cotton wool rolls, supragingival plaque gently removed with a curette and the site dried with a pellet of cotton roll. GCF was collected in micropipette with 5µl capacity and colour coded markings at each µl. The pipette was kept at the entrance of gingival sulcus until the pipette is filled with 3µl of gingival fluid. Pipettes being contaminated with blood or saliva were discarded and fresh samples were again collected. The GCF samples were transferred immediately to plastic vials containing 5ml of phosphate buffered solution (PBS) and stored at -70° until the time of the assay. The concentration of VEGF was determined by using commercially available enzyme linked immunosorbent assay (ELISA) kit {R &D system Minneapolis, USA}, as instructed by the manufacturer.

Group 2 patients were treated with single sitting ultrasonic scaling.

Group 3 patients were treated with a non-surgical approach which included single sitting ultrasonic scaling and root planing (SRP) and GCF samples were collected after 12 wk.

The study was performed in accordance with the Declaration of Helsinki. All study participants gave their signed, informed consent before inclusion in the project.

STATISTICAL ANALYSIS

The mean and standard deviation was calculated for all the parameters, then these values were subjected for analysis of variance (ANOVA) and paired t-test for further analysis using SPSS software for Microsoft excel.

RESULTS

All of the samples in each group tested positive for the presence of VEGF. [Table/Fig-1] shows the mean and the standard deviation of VEGF in the three groups. The mean VEGF levels in GCF shows modest increase from 31.91± 3.06 (control/group-1) to 48.71 ± 3.61(gingivitis/group-2) while, a sharp increase is noticed in the periodontitis group tantamounting to a level of 88.08±8.04 (periodontitis/group-3). When subjected for analysis of variance test (ANOVA) the results showed a statistically significant difference in VEGF values in each group (p-value 0.00) [Table/Fig-2]. When compared between control (Group-1) and gingivitis (Group-2) group the mean difference was 16.79 and between control (group-1) and periodontitis (Group-3) the mean difference was 56.16. Similarly, when compared between gingivitis (Group-2) and periodontitis (Group-3) the mean difference was 39.36. These differences were found to be statistically significant with p-value 0.00 [Table/Fig-3]. This indicated that as the disease progressed from health to gingivitis and then to periodontitis the VEGF levels increased significantly in the GCF.

The GCF levels of VEGF were re-evaluated after 3 months of intervention. In group-2 the mean VEGF levels reduced from 48.71±3.61 to 36.72±4.27 with a mean difference of 11.98±3.74. This difference was statistically significant with p-value 0.0002 [Table/Fig-4]. Similarly in Goup-3 the mean VEGF values reduced from 88.08±8.04 to 66.39±9.07 after three months with a difference of 21.68±4.66 with a statistically significant p-value 0.00038 [Table/Fig-4].

Upon subjecting the group-2 patients for scaling and root planning, it was seen that the mean gingival index (GI) decreased from 2.24±0.18 to 0.82±0.27. When subjected to paired sample test the mean difference of GI before & after therapy was 1.41± 0.32 which is statistically significant (p-value 0.00) [Table/Fig-5] The group-3 patients upon completion of therapy showed a reduction in GI from 2.59±0.17 to 1.36±0.20. Similarly, the probing pocket depth reduced from 6.04±0.67 to 4.28±0.89. The results of the paired sample test showed the mean difference of 1.23±0.25, 1.76±0.77 and 0.36±0.48 for GI, PPD and CAL respectively. These differences were found to be statistically significant with p-value 0.00 [Table/Fig-6].This decrease in the clinical parameters suggests the efficacy of the scaling and root planing on periodontitis.

DISCUSSION

Aberrant angiogenesis is associated with lesion formation in chronic periodontitis but the mediators that contribute to angiogenesis or therapeutic agents that control the action of mediators have not been well described [32].

Periodontitis is a chronic inflammatory disease with episodes of active destruction and periods of quiescence [33]. However, individuals vary in their response to chronic gingival inflammation. VEGF one of the most potent angiogenic mediators was detectable in periodontal tissues within the vascular endothelial cells, plasma cells, macrophages and in junctional and sulcular gingival epithelium. This extensive cellular distribution shows that VEGF may play a role in the maintenance of periodontal physiology and the progression of periodontal diseases [34]. In recent years the possible role of VEGF in the pathogenesis of periodontal diseases has been investigated in clinical studies [32,35-38]. To the readers knowledge the present study is one of the few studies designed to investigate the concentration of VEGF in GCF of periodontal healthy subjects and to compare with disease subjects (gingivitis and periodontitis) also, before and after therapy in disease subjects. The patients were carefully selected to eliminate the possible alterations of hormonal conditions seen women such as in menstruation cycle and menopause, because of the possible effects of smoking on gingival microcirculation and increased inflammatory cytokine production may aggravate periodontal disease therefore, smokers were excluded from the study. The patients with psoriasis and hypersensitivity have also shown high levels of VEGF [39] levels so, they were also excluded from this study.

The mean VEGF concentration in GCF was highest in group 3 and lowest in group 1. And intermediate in group 2. The results of this study suggested that VEGF levels in GCF increased progressively from healthy to gingivitis and periodontitis subjects. The results of the present study are in accordance with those of Guneri et al., who also reported increased VEGF levels in GCF in periodontitis sites compared to healthy sites [37]. This suggests that in periodontitis patients the production of VEGF is generally upregulated. The reported increase in the release of IL-1β [40] & TNF-α from peripheral blood mono-nuclear cells of periodontitis patients compared with healthy subjects. This might explain the higher concentration of VEGF found in patients since these cytokines can induce the expression of VEGF. Johnson et al., [35] from their study suggested that VEGF is likely to be a factor in the etiology of gingivitis and its progression to periodontitis, possibly by initiating expansion of the vascular network which in turn could be initiated by Interleukin 1β concentration at sites of active periodontal inflammation. Further, Fusen et al., investigated the association between VEGF, Diabetes

	N	Mean	Std. Deviation	Std. Error	95% confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
1	45	31.9144	3.06842	0.45741	30.9926	32.8363	27.15	38.75
2	25	48.7120	3.61021	0.72204	47.2218	50.2022	42.15	55.85
3	25	88.0888	8.04723	1.60945	84.7583	91.4017	76.20	105.35
Total	95	51.1153	23.78442	2.44023	46.2701	55.9604	27.15	105.35

[Table/Fig-1]: GCF VEGF levels (pg/ml) of the three groups

ANOVA					
	Sum of squares	df	Mean square	F	Sig.
Between groups	50894.401	9	25447.200	1026.248	.000
Within groups	2281.265	92	24.796		

[Table/Fig-2]: Analysis of variance for VEGF levels in the three groups

(I)VAR00006 (J)VAR00006	Mean differences (I-J)	Std. error	Sig.	95% confidence interval	
				Lower bound	Upper bound
Tukey HSD 1.00 2.00 3.00	-16.79756 -56.16556	1.24213 1.24213	0.00 0.00	-19.7566 -59.1246	-13.8385 -56.2065
2.00 1.00 3.00	16.79756 -39.36800	1.24213 1.40844	0.00 0.00	13.8385 -42.7232	19.7566 -36.0128

[Table/Fig-3]: Intergroup comparison of VEGF levels (pg/ml) before treatment

Paired samples test								
	Mean	SD	Std Error	95% confidence intervel of the		t	Diff.	Sig.
Pair 1 gr2 before - gr2 after	11.98800	3.74417	.74883	10.44248	13.53352	16.009	24	0.0002
Pair 2 gr3 before - gr3 after	21.68920	4.66712	.93342	19.76271	23.61569	23.2356	24	0.00038

[Table/Fig-4]: Intra group comparison of VEGF levels (pg/ml) before and after therapy

	Mean	N	Std. deviation	Std. error of mean	Differences		p-value
					Mean	S.D.	
Pair 1 Glb Gla	2.2440 0.8280	25 25	0.18502 0.27917	0.3700 0.5583	1.41600	0.32104	0.00

[Table/Fig-5]: Paired sample statistics of Group 2

	Mean	N	Std. Deviation	Std. error Mean	Differences		p-value
					Mean	Std. Deviation	
Pair 1 Glb Gla	 2.5960 1.3600	 25 25	 0.17673 0.20817	 0.03535 0.04163	1.23600	0.25311	0.00
Pair 2 PDb PDa	 6.0400 4.2800	 25 25	 0.67577 0.89069	 0.13515 0.17814	1.76000	0.77889	0.00
Pair 3 CALb CALa	 2.3200 1.9600	 25 25	 0.47610 0.35119	 0.9522 0.7024	0.3600	0.48990	0.001

[Table/Fig-6]: Paired sample statistics of Group 3

mellitus and periodontitis and concluded that VEGF is increased in gingival tissues of diabetic patients especially those with periodontal diseases [40].

Contrary to the above findings Booth et al., have reported that VEGF is up-regulated in relatively healthy sites. They explained this finding to be due to the following possible mechanisms; the presence of sub-clinical levels of inflammation, healing after the microbial assault, revealing the presence of VEGF as a component of physiological angiogenesis in the gingival or periodontal environment. Similarly, Cetinkaya BO et al., investigated the association between VEGF expression and vascularisation with regard to the number and diameter of blood vessels and concluded that VEGF may be related more to the healing stage of periodontal disease than to the destruction stage [41]. In another study by Keles GC et al., on VEGF expression levels of gingiva in gingivitis and periodontitis patients with/without diabetes mellitus it was stated that VEGF expression is probably related to both maintenance of periodontal health and periodontal tissue destruction [42]. Therefore, it can be stated that studies regarding the role of VEGF in the pathogenesis of periodontal diseases have had conflicting results.

The gingivitis and periodontitis subjects were treated by non-surgical periodontal therapy (SRP) and strict oral hygiene measures were instituted. Post-treatment both the gingivitis and periodontitis group showed significant reduction in VEGF concentration of GCF [Table/Fig-3]. These results are in accordance with those of Devi V Prapulla [43]. The variability of the VEGF concentration in each group can be attributed to the different stages of the disease process at the time of collection of GCF samples. The results of our study indicate a direct correlation of VEGF values in GCF and the inflammatory condition of the periodontal tissues.

In the present study the GI scores of gingivitis group reduced significantly after therapy. The mean GI and PPD scores of periodontitis subjects also reduced significantly post therapy. According

to Becker et al., [44], the decreases in probing pocket depths have however proved to be smaller following non-surgical treatment although these differences have not always been sustained over longer periods of time. At deeper sites gain in attachment have been reported by some but not other investigators following non-surgical therapy [45]. However, according to Goodson et al., and Page and De Rouen any treatment would appear successful when the experimental sites are not actively loosing attachment [46,47]. The mean decrease in the clinical parameters (GI & PD) in the periodontitis subjects post therapy in our study suggests the efficacy of treatment procedures, however our study did not show any CAL gain.

Further, the work into the role of VEGF in the pathogenesis and healing of periodontal disease should be carried out. Longitudinal clinical research would provide further information and should monitor the levels of VEGF both during development and resolution of experimental gingivitis and periodontitis. Further emphasis should also be given to quantify the VEGF levels during treatment intervention.

CONCLUSION

In this study VEGF concentration in GCF increased proportionally with the progression of periodontal disease. Further treatment aimed at arresting periodontal disease progression resulted in improvement in the clinical parameters (GI & PD) and also, a statistically significant reduction in GCF VEGF levels proportionally confirming to its active pathological role. However, to reach a more precise conclusion longitudinal clinical studies with a larger study population and longer duration on those patients who are on maintenance program is suggested.

REFERENCES

[1] Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular Permeability factor/vascular endothelial growth factor, microvascular hyperpermeability and angiogenesis. *Am J Pathol.* 1995;146(5):1029-39.

[2] Cheryl C Chapple, Rakesh K Kumar, Neil Hunter. Vascular remodelling in chronic inflammatory periodontal disease. *J Oral Pathol Med.* 2000;29:500-06.

[3] Connolly DT, Olander JV, Heuvelman D, Nelson R, Monsell R, Siegel N, Haymore BL, Leingruber R, Feder J. Human vascular permeability factor. Isolation from U937 cells. *J Biol Chem.* 1989;264:20,017–24.

[4] Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev.* 1992;13: 18-32.

[5] Lantieri LA, Martin-Garcia N, Wechsler J, Mitrofanoff M, Raulo Y, Baruch JP. Vascular endothelial growth factor expression in expanded tissue: A possible mechanism of angiogenesis in tissue expansion. *Plast Reconstr Surg*. 1998;101:392-98.

[6] Connolly DT. Vascular permeability factor: A unique regulator of blood vessel function. *J Cell Biochem*. 1991;47(3):219-23.

[7] Sakuta T, Matsushita K, Yamaguchi N, Oyama T, Motani R, Koga T, et al. Enhanced production of vascular endothelial growth factor by human monocytic cells stimulated with endotoxin through transcription factor SP-1. *J Med Microbiol.* 2001;50(3):233-37.

[8] Leung DW, Cachianes G, Kuang WJ, Goeddel, DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science.* 1989;246(4935):1306-09.

[9] Keck PJ, Hauser SD, Krivi G, Sanjo K, Warren T, Feder J, Connolly DT. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science*. 1989; 246(4935):1309-12.

[10] Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun*. 1989;161(2):851-58.

[11] Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumour cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science.* 1983;219(4587):983-85.

[12] Takahashi H and Shibuya M. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. *Clin Sci*. 2005;109(3):227-41.

[13] Lange T, Guttmann-Raviv N, Baruch L, Machluf M, Neufeld G. VEGF 162, a new heparin binding vascular endothelial growth factor splice form that is expressed in transformed human cells. *J. Biol Chem*. 2003;278(19):17164-69.

[14] Bates DO, Harper SJ. Regulation of vascular permeability by vascular endothelial growth factors. *Vascul pharmacol.* 2002;39(4-5):229-37.

[15] Park JE, Keller GA, Ferrara N. The vascular endothelial growth factor (VEGF) isoforms: Differential deposition into the subepithelial extracellular matrix and bio activity of extracellular matrix-bound VEGF. *Mol Biol Cell*. 1993;4(12):1317-26.

[16] 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(36):26031-137.

[17] Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev.* 1997;18(1):4-25.

[18] Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature*. 1993;362(6423):841-84.

[19] Folkman J. Angiogenesis in cancer vascular, rheumatoid and other disease. *Nature Medicine.* 1995;(1):27-31.

[20] Hernandez GL, Volpert OV, Iniguez MA, Lorenzo E, Martínez-Martínez S, Grau R, et al. Selective inhibition of vascular endothelial growth factor mediated angiogenesis by cyclosporine 2: Roles of the nuclear factor of activated T cells and cyclooxygenase 2. *J Exp Med*. 2001;193(5):607-20.

[21] Cetinkaya BO, Acikgoz G, Ayas B, Aliyev E, Sakallioglu EE. Increased expression of vascular endothelial growth factor in cyclosporine A- induced gingival overgrowth in rats. *J Periodontol*. 2006;77(1):54-60.

[22] Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, et al.,VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat Genet*. 2004; 36(8):827-35.

[23] Lee YC, Lee HK. Vascular endothelial growth factor in patients with acute asthma. *J Allergy Clin Immunol.* 2001;107(6):1106-07.

[24] Hoshino M, Nakamura Y, Hamid QA. Gene expression of vascular endothelial growth factor and its receptors and angiogenesis in bronchial asthma. *J Allergy Clin Immunol.* 2001;107(6):1034-38.

[25] Richard JL, Sharon EM, Cong Qian, Kee-Hak Lim, Lucida J, Kaif Y, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med.* 2004; 350(7):672-83.

[26] Curtis MA, Gillet IR, Griffiths GS, Maiden MF, Sterne JA, Wilson DT, et al. Detection of high risk groups and individuals for periodontal diseases: laboratory markers from analysis of gingival crevicular fluid. *J Clin periodontal.* 1989; 16(1):1-11.

[27] Offenbacher S, Collins JG, Heasman PA. Diagnostic potential of host response mediators. *Adv Dent Res*. 1993;7(2):175-81.

[28] Frank S, Hubner G, Breier G, Longaker MT, Greenhalgh DG, Werner S. Regulation of vascular endothelial growth factor in cultured keratinocytes. Implications for normal and impaired wound healing. *J Biol Chem.* 1995;270(21):12607-13.

[29] Bramanti TE, Wong GG, Weintraub ST, Holt SC. Chemical characterisation and biologic properties of lipopolysaccharide from Bacteroides gingivalis strains W50, W83 and ATCC33277. *Oral Microbiol immunol.* 1989;4(4):183-92.

[30] McFarlane CG, Reynolds JJ, Meikle MC. The release of interleukin-1beta, tumour necrosis factor-alpha and interferon-gamma by cultured peripheral blood mononuclear cells from patients with periodontitis. *J Periodontal Res.* 1990;25(4):207-14.

[31] Shweiki D, Neeman M, Itin A, Keshet E. Induction of vascular endothelial growth factor expression by hypoxia and by glucose deficiency in multicell spheroids: implications for tumor angiogenesis. *Proc Natl Acad Sci (USA).* 1995;92(3): 768-72.

[32] Oyama T, Sakuta T, Matsushita K, Mauyama I, Nagaoka S, Torii M. Effects of roxythromycine on tumor necrosis factor-alpha-induced vascular endothelial growth factor expression in human periodontal ligament cells in culture. *J Periodontol*. 2000; 71(10):1546-53.

[33] Goodson JM, Tanner AC, Haffajee AD, Sornberger GC, Socransky SS. Patterns of progression and regression of advanced destructive periodontal disease. *J Clin Periodontol.* 1982;9(6):472-81.

[34] Booth V, Young S, Cruchley A, Taichman NS, Paleolog E. Vascular endothelial growth factor in human periodontal disease. *J Periodontal Res.* 1998;33(8):491-99.

[35] Johnson RB, Serio FG, Dai X. Vascular endothelial growth factors and progression of periodontal diseases. *J periodontal.* 1999;70(8):848-52.

[36] Suthin K, Matsushita K, Machigashira M, Tatsuyama S, Imamura T, Torii M, et al. Enhanced expression of vascular endothelial growth factor by periodontal pathogens in gingival fibroblasts. *J. Periodontal Res.* 2003;38(1):90-96.

[37] Guneri P, Unlu F, Yesilbek B, Bayraktar F, Kokuludag A, Hekimgil M, & Boyacioglu H. Vascular endothelial growth factor in gingival tissues and crevicular fluids of diabetic and healthy periodontal patients. *Jol of periodontal.* 2004; 75: 91-97.

[38] Booth V, Young S, Cruchley A, Taichman NS, Paleolog E. Vascular endothelial growth factor in human periodontal disease. *J Periodontal Res.* 1998;33(8):491-99.

[39] Brown LF, Olbricht SM, Berse B, Jackman RW, Matsueda G, Tograzzi KA, et al. Over expression of vascular permeability factor (VPF/VEGF) and its endothelial cell receptors in delayed hypersensitivity skin reactions. *J Immunol.* 1995;154:2801-07.

[40] Fusen U, Pelin GG, Mine H, Banu Y, Hayal B. Expression of vascular endothelial growth factor in human periodontal tissues: comparison of healthy and diabetic patients. *J Periodontol.* 2003;74(2):181-87.

[41] 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;78:1129-35.

[42] 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(7):543-49.

[43] Devi VP, Pai BS, Pradeep AR. Gingival crevicular fluid VEGF levels in periodontal health and disease. *J Periodontol.* 2007;78(9):1783-87.

[44] Becker B, Caffesse R, Ochsenbein C, Morrison E. Three modalities of periodontal therapy: II, 5-year final results. *J Dent Res.* 1990;69:219-51.

[45] Nicklaus PL, Thorkild K. Proceedings of the 1st European workshop on Periodontology. London: Quintessence publishing1994.
[46] Goodson JM. Discussion: Design issues specific to studies of periodontitis. *J Periodontal Res.* 1992;27:412-16.
[47] Page RC, De Rouen TA. Design issues specific to studies of periodontitis. *J Periodontal Res*. 1992;27(4):395-404.

PARTICULARS OF CONTRIBUTORS:
1. Head of the Department, Department of Periodontics, Coorg Institute of Dental Sciences, Virajpet, Karnataka, India.
2. Reader, Department of Periodontics, Coorg Institute of Dental Sciences, Virajpet, Karnataka, India.
3. Senior Lecturer, Department of Periodontics, St. Joseph Dental College, Eluru, Andhra Pradesh, India.
4. Post Graduate Student, Department of Periodontics, Coorg Institute of Dental Sciences, Virajpet, Karnataka, India.
5. Reader, Department of Orthodontics, St. Joseph Dental College, Eluru, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Padma R,
Head of Department, Department of Periodontics, Coorg Institute of Dental Sciences,
Virajpet, Maggula Village Post, South Kodagu, Karnataka - 571218, India.
Phone: 08971484333/08762893293, E-mail: padmasanskrithi@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jan 11, 2014**
Date of Peer Review: **Aug 15, 2014**
Date of Acceptance: **Sep 03, 2014**
Date of Publishing: **Nov 20, 2014**