

IMPACT OF INFLAMMATION ON THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN THE DENTAL PULP

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ABSTRACT

Objective: The present study was aimed to investigate immunohistochemical expression of VEGF in healthy and inflamed human dental pulps as well as the importance of VEGF in dental pulp angiogenesis and lymphangiogenesis.

Methods: Twenty-eight pulps obtained from the teeth having a clinical diagnosis of irreversible pulpitis and thirty-one samples of healthy dental pulps obtained from young patients who required extractions for orthodontic reasons were involved in the study. All cases were examined by immunohistochemistry using monoclonal mouse anti-human vascular endothelial growth factor antibody, CD34 antibody and D2-40 antibody.

Results: The comparison between the groups showed statistically significant difference between VEGF expression in healthy and inflamed human dental pulps ($p=0,004$). There was, however, a weak positive correlation between the number of CD34 positive blood vessels and VEGF expression ($r=0,228$; $p=0,253$), as well as, between the number of D2-40 positive lymphatic vessels and VEGF expression ($r=0,223$; $p=0,250$) in inflamed human dental pulp.

Conclusion: VEGF expression is upregulated during dental pulp inflammation, but does not correlate with dental pulp angiogenesis and lymphangiogenesis.

Key words: VEGF, dental pulp, inflammation, angiogenesis, lymphangiogenesis

Introduction

The intensity and duration of the tooth injury, either from caries or trauma, has important implications on the subsequent pulpal response. The acids, which are released from bacterial biofilms on the tooth surface, diffuse through dental tissues and dissolve the enamel and dentin matrix. During caries induced demineralization, biologically active molecules are released from dentin matrix. These molecules have the potential to influence cellular events in the pulp-dentin complex. Thus, for example, released angiogenic growth factors can contribute to the healing process of the dental pulp [1,2]. Pulp cells also express several pro-angiogenic growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF-2) angiogenin, angiopoietin, epidermal growth factor, heparin-binding epidermal growth factor, hepatocyte growth factor, leptin and placental growth factor [3,4].

Caries-induced pulpitis is typically accompanied by an increased number of blood and lymphatic vessels. These processes imply the formation of new blood and lymphatic vessels by "sprouting" from pre-existing ones. While increased vasodilation, which is typical for acute phase of inflammatory reaction, causes increased blood flow in the inflamed tissue, sprouting of capillaries leads to an increase in their number, improving perfusion of hypoxic tissue. However, the angiogenesis can facilitate inflammatory processes with increased delivery of nutrients, inflammatory cells and oxygen to the inflamed tissue. Thus, angiogenesis and lymphangiogenesis appear as basic processes during inflammation-induced hypoxia.

Vascular endothelial growth factor (VEGF) is a dominant growth factor controlling angiogenesis and lymphangiogenesis. It induces endothelial cell proliferation, migration and survival. It is also known as vascular permeability factor because it induces the permeability of blood vessels with unusually rapid kinetics and 50.000 times more potency compared to histamine. VEGF performs its biological effect through the interaction with transmembrane tyrosine kinase receptors (VEGFR) [5,6].

The present study aimed to investigate immunohistochemical expression of VEGF in healthy and

inflamed human dental pulps as well as the impact of VEGF on angiogenesis and lymphangiogenesis in inflamed human dental pulp.

Materials and methods

Sample selection and preparation

Twenty-eight (28) pulps were obtained from teeth having clinical diagnosis of irreversible pulpitis. Thirty-one (31) samples of healthy dental pulps were obtained from young patients who required extractions for orthodontic reasons. The pulp samples were fixed in neutral 10% buffered formalin, embedded in paraffin and cut in 4 serial sections at 4 μ m. One section was stained with hematoxylin and eosin in order to confirm clinical diagnosis. The other 3 sections were stained immunohistochemically using monoclonal mouse anti-human vascular endothelial growth factor, monoclonal mouse anti-human CD34 and monoclonal mouse anti-human D2-40.

Sections were first dewaxed for 15 min and rehydrated via graded ethanol solutions. Antigen retrieval was performed with microwave treatment in phosphate-buffered saline (PBS) (pH 9.0) for 10 min at 120W. Sections were immersed into 3% hydrogen peroxide (H₂O₂) for 10 min to block the endogenous peroxidase activity. After that, sections were incubated for 30 minutes with primary monoclonal antibodies agents: VEGF (Clone VG1; dilution 1:50; DAKO, Denmark), CD34 (Clone QBEnd-10, dilution 1:50, DAKO, Denmark) and D2-40 (Clone D2-40; dilution 1:200; DAKO, Denmark) followed by incubation with biotin-labelled secondary antibodies. A two-step technique (EnVision; Dako, Glostrup, Denmark) was used for visualization, with diaminobenzidine (DAB) as a chromogen (DAB Chromogen, Dako, Glostrup, Denmark). Finally, after being three times washed in distilled water (5 minutes each), sections were counterstained with haematoxylin, mounted and cover slipped.

Assessment of immunostaining

The assessment of immunostaining was made by experienced pathologist using light microscope (Olympus BX40 - Artisan Scientific, Champaign, Illinois, USA). Immunoreactivity for VEGF was observed to assess localization, intensity and percentage of

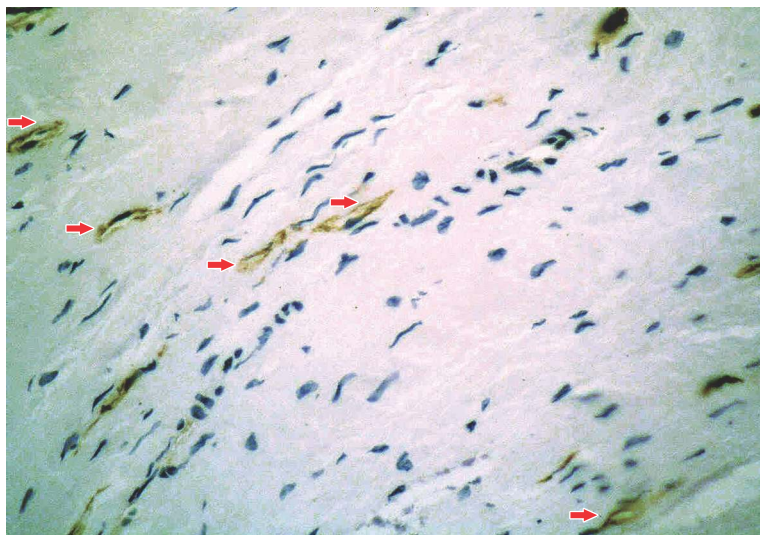


Figure 1. VEGF-positive endothelial cells (arrows) in inflamed dental pulp (IH, X250)

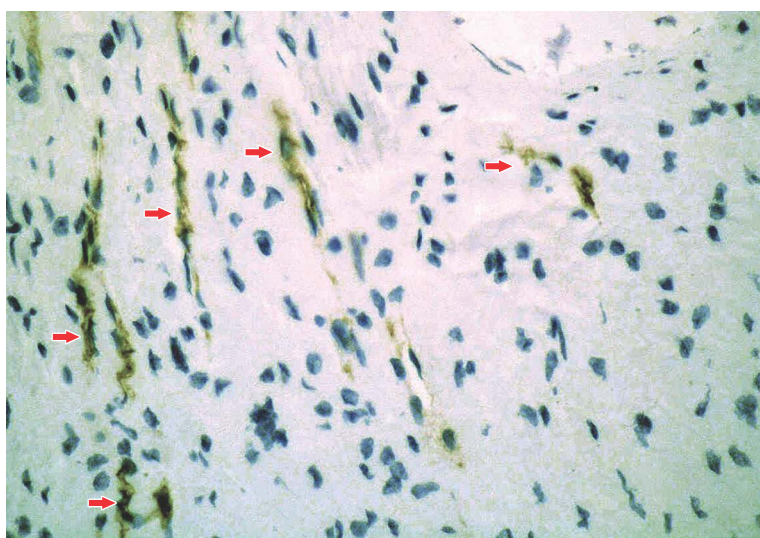


Figure 2. VEGF-positive fibroblasts (arrows) in inflamed dental pulp (IH, X400)

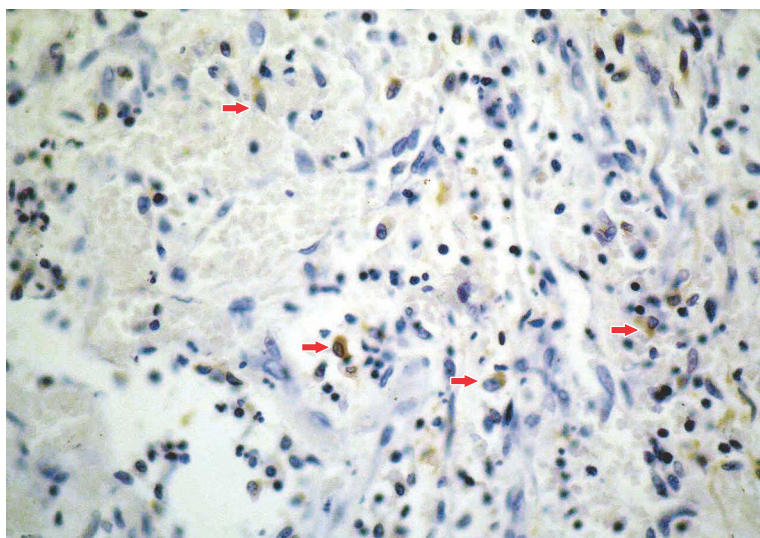


Figure 3. Cytoplasmic expression of VEGF in plasma cells (arrows) of dental pulp (IH, X400)

positive cells. Five fields of view were counted at magnification $\times 200$ in each slide. The final product of the immunohistochemical reaction for VEGF was cytoplasmic staining with granular pattern. Intensity of the staining was scored as follows: 0 - no staining, 1 - weak staining, 2 - mild staining and 3 - high intensity staining. The percentage of the VEGF positive cells were scored as follows: 0 - no positive cells; 1 - $<25\%$ positive cells and 2 - $>25\%$ positive cells.

To assess the immunoreactivity for CD34 and D2-40, sections were first scanned at low magnification ($\times 40$) to identify the areas with the highest number of blood and lymphatic vessels. Microvessel counting was done under $\times 200$ magnification from five areas for each pulp sample. To determine the number of CD34 positive blood vessels and D2-40 positive lymphatic vessels, brown-staining endothelial cells which formed slit-like or rounded luminal spaces were defined as a single countable microvessel.

Statistical analysis

The results were subjected to statistical analysis using Student's t-test for independent samples to compare differences in VEGF expression between the groups. Correlations between VEGF expression and number of CD34 positive blood vessels and between VEGF expression and number of D2-40 positive lymphatic vessels were estimated using the Pearson's correlation coefficient.

Results

The immunopositive, cytoplasmic reaction for VEGF, with varying intensity of immunolabeling, was observed in 16 (57,1%) samples of inflamed and 11 samples (35,5%) of healthy dental pulps. As shown in **Figure 1**., VEGF immunoreactivity in inflamed pulps was detected most frequently in endothelial cells ($n=11$), followed by fibroblasts ($n=9$) (**Figure 2**). VEGF expression in plasma cells was observed in only one case (**Figure 3**).

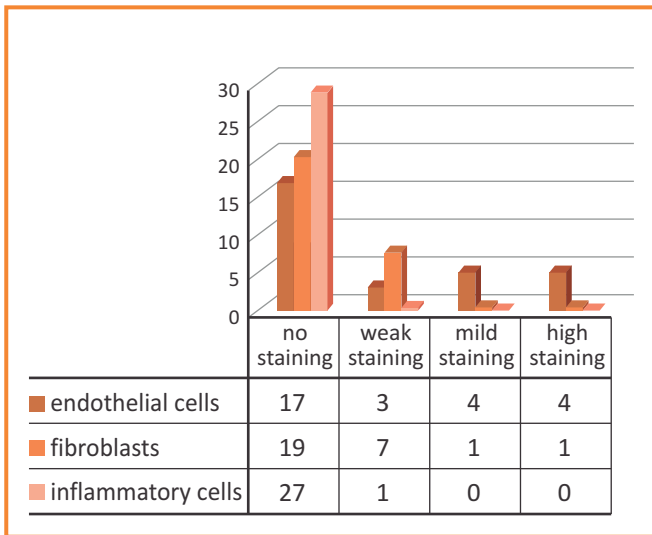


Figure 4. Staining intensity for VEGF in the different cells of inflamed pulp

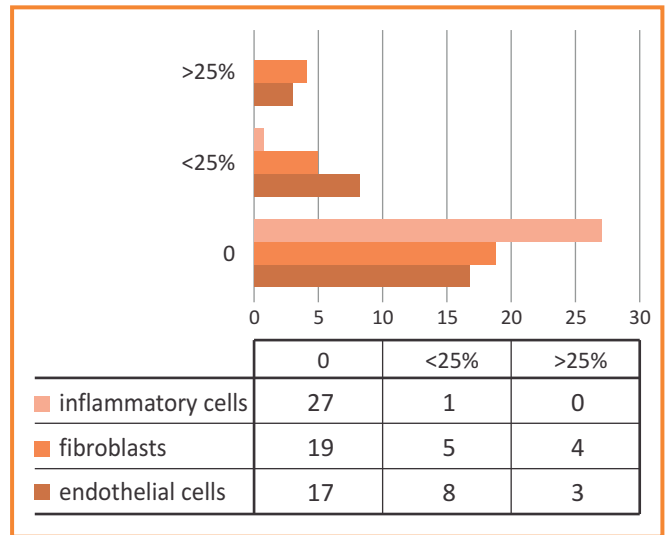


Figure 5. The percentage of VEGF positive cells in inflamed dental pulp

In most cases, weak staining intensity was recorded (Figure 4). In 11 of these cases, less than 25% represented by 7 cases, had more than 25% of the cells positive for VEGF (Figure 5). In healthy dental pulps, VEGF immunoreactivity was detected 8 times in fibroblasts and 4 times in endothelial cells with weak staining intensity in most cases (n=10). The VEGF expression in inflamed dental pulps was statistically higher (t-test, $p=0,004$) than in healthy dental pulps.

The mean number of blood vessels positive for CD34 in the inflamed dental pulp (78.93 ± 25.8) (Figure 6.) was significantly higher ($P < 0.0001$) than the mean number in the healthy dental pulp (50.4 ± 9.34). Pearson's coefficient of correlation showed no significant match between the total scores of CD34 positive endothelial cells and VEGF expression ($r=0,228$; $p=0,253$).

The mean number of lymphatic vessels positive for D2-40 in the inflamed dental pulp (8.04 ± 2.8) (Figure 7.) was significantly higher ($P < 0.0001$) than the mean number in the healthy dental pulp (3.93 ± 1.11). Pearson's coefficient of correlation between the number of D2-40 positive lymphatic vessels and VEGF expression was low, reflecting lack of significant correlation.

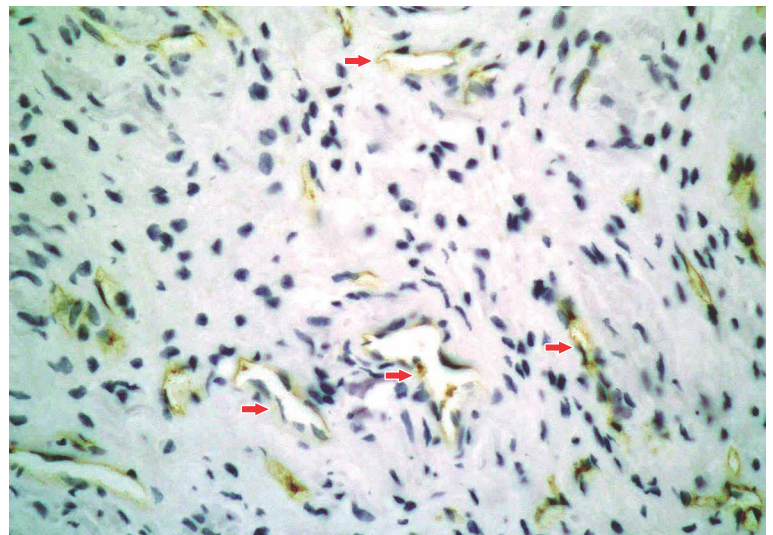


Figure 6. CD34 positive endothelial cells (arrows) in inflamed dental pulp (IH, X250)

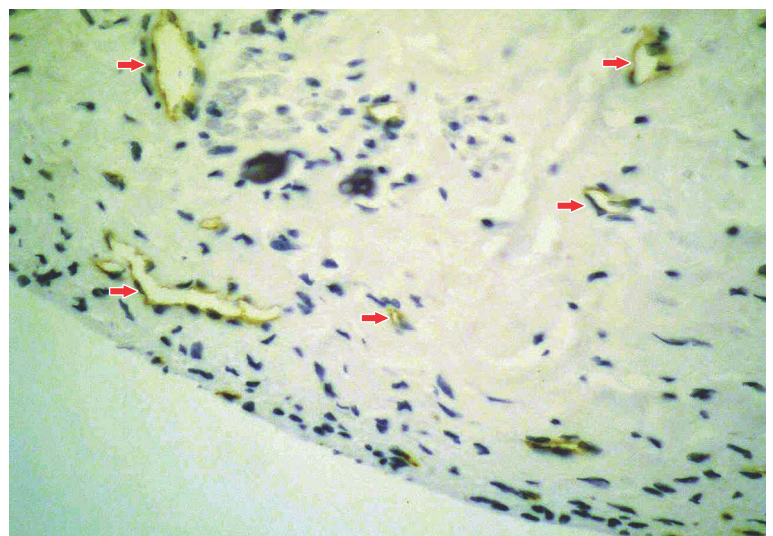


Figure 7. D2-40-positive endothelial cells (arrows) in inflamed dental pulp (IH, X250)

Discussion

There are different clinical situations, such as carious or traumatic pulp injuries, orthodontic movements or replanted avulsed teeth, requiring an increase in vascular density in the dental pulp. The VEGF, produced by various cellular types, is considered as the most essential for differentiation of the vascular system. In this study, we found that VEGF is overexpressed in inflamed pulp tissue. The VEGF expression is most often observed in endothelial cells and almost three times more frequent in inflamed compared to healthy pulp. Although higher expression of VEGF in fibroblasts after injury was detected previously [7], we noticed that the number of VEGF positive fibroblast was almost equal in both groups. Unlike previous study in which cytoplasmatic positivity to VEGF in inflammatory cells was found in very high percentage [8], we found VEGF expression in plasma cells in only one case. The intensity of expression was generally weak in both tested groups.

Chronic inflammatory diseases are often accompanied by intense angiogenesis and lymphangiogenesis. An increased number of blood and lymphatic vessels was found in the inflamed dental pulp, suggesting that inflammation contributes angiogenesis and lymphangiogenesis [9,10]. This study has once again confirmed these facts. There was, however, a weak positive correlation between the number of CD34 positive blood vessels and VEGF expression, as well as, between the number of D2-40 positive lymphatic vessels and VEGF expression in inflamed dental pulp. The lack of correlation between angiogenesis, lymphangiogenesis and VEGF expression in inflamed dental pulp supports the hypothesis that multiple angiogenic factors may play a role in the angiogenic process.

Conclusion

VEGF expression is upregulated during dental pulp inflammation, but does not correlate with dental pulp angiogenesis and lymphangiogenesis. Our findings imply that the VEGF expression is not a single specific indicator of angiogenesis and lymphangiogenesis in inflamed dental pulp.

References

1. Roberts-Clark DJ, Smith AJ. Angiogenic growth factors in human dentine matrix. *Arch Oral Biol.* 2000;45(11):1013-6.
2. Smith AJ. Vitality of the dentin-pulp complex in health and disease: growth factors as key mediators. *J Dent Educ.* 2003;67(6):678-89.
3. El Karim IA, Linden GJ, Irwin CR, Lundy FT. Neuropeptides regulate expression of angiogenic growth factors in human dental pulp fibroblasts. *J Endod.* 2009;35(6):829-33.
4. Tran-Hung L, Laurent P, Camps J, About I. Quantification of angiogenic growth factors released by human dental cells after injury. *Arch Oral Biol.* 2008;53(1):9-13.
5. Grando Mattuella L, Westphalen Bento L, de Figueiredo JA, Nör JE, de Araujo FB, Fossati AC. Vascular endothelial growth factor and its relationship with the dental pulp. *J Endod.* 2007;33(5):524-30.
6. Virtej A, Løes S, Iden O, Bletsa A, Berggreen E. Vascular endothelial growth factors signalling in normal human dental pulp: a study of gene and protein expression. *Eur J Oral Sci.* 2013 Apr;121(2):92-100.
7. Tran-Hung L, Mathieu S, About I. Role of human pulp fibroblasts in angiogenesis. *J Dent Res.* 2006;85(9):819-23.
8. 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(1):20-3.
9. Tahmišćija I, Radović S, Jukić-Krmek S, Konjhodžić-Prčić A, Šečić S, Đapo N. Assessment of angiogenesis by endoglin (CD105) in inflamed dental pulp. *Stomatološki vjesnik.* 2015;4(1):41-6.
10. Tahmišćija I, Radović S, Berisalić A, Jakupović S, Dozić A. Immunohistochemical assessment of lymphangiogenesis in inflamed human dental pulp. *Arch Orofac Sci.* 2012;7(2):63-7.