ORIGINAL RESEARCH PAPER

Volume-8 | Issue-4 | April-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

VASCULAR ENDOTHELIAL GROWTH FACTOR IN DENTIGEROUS CYST AND RADICULAR CYST : A COMPARATIVE STUDY

Oral Pathology

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ABSTRACT

Background VEGF is a potent proangiogenic cytokine which acts on the vasculature by inducing the proliferation, differentiation and migration of vascular endothelial cells. Angiogenesis has been evaluated in various human lesions including breast cancer, head and neck squamous cell carcinoma, lichen planus and ameloblastoma. Very little data were found on angiogenesis, as assessed by immunohistochemically in odontogenic cysts. The objective of the present study was to evaluate vascular endothelial growth factor (VEGF) expression in different types of odontogenic cysts

Methodology: A retrospective study was carried out comprising a total of 18 cases , 9 cases of dentigerous cyst and 9 cases of radicular cyst. The sections were stained immunohistochemically with VEGF antibody and were evaluated for the presence, and intensity of the immunoreactive cells. Statistical analysis was carried out using Chi-square test to intercompare the VEGF expression between Dentigerous Cyst And Radicular Cyst

Results: VEGF expression in the epithelium and connective tissue was significantly higher in compared to dentigerous and radicular cyst.

Conclusion: The present study data supports the literature finding that angiogenesis can be important in the progression and enlargement of odontogenic cysts

KEYWORDS

Odontogenic cyst, Dentigerous Cyst, Radicular Cyst, VEGF.

INTRODUCTION

Cysts of the jaws are probably the most common destructive bone lesions in the human maxillofacial skeleton. The head and neck region and the jaw in particular, collectively comprise one of the most common sites for the occurrence of cysts.[1]

Odontogenic cysts are derived from the epithelium which is associated with the development of the dental apparatus. They are either of developmental origin or may result from inflammation.[2]

The most common odontogenic cysts are radicular cysts, odontogenic keratocysts and dentigerous cysts.[2] Odontogenesis is controlled by interactions between the epithelial and mesenchymal components of developing dental tissues. The connective tissue stroma has an essential role in preservation of epithelial tissues and minor alterations in the epithelium are followed by corresponding changes in the stroma, such as angiogenesis.[3]

Angiogenesis is the process of formation of new microvessels from the preexisting vasculature. It is thought to be initiated by an increase in the level of angiogenic stimuli and a concomitant decrease in the level of angiogenic inhibitors..[4] The angiogenic factors play a role as important mediators of tumor angiogenesis in epithelial odontogenic tumors and their alterations might be associated with tumoriogenesis or malignant transformation of odontogenic tumors.

VEGF has been implicated as an important factor in granulation tissue development and cyst enlargement. Also, it is now assumed that the critical event in the regulation of angiogenesis is the signaling cascade involving vascular endothelium growth factor (VEGF).[5]

Vascular endothelial growth factors (VEGF) comprise a family of multifunctional proteins mainly involved in normal and pathologic angiogenesis, defined as the formation of new vessels by sprouting of the pre-existing endothelium.[6]

Therefore, the present study attempted to assess the VEGF expression, in , dentigerous cyst and radicular cyst and correlate the role of VEGF and angiogenesis with cyst proliferation.

MATERIALS AND METHODS

The present study was carried out on 18 archival tissues which included nine cases of DC and nine cases of RC. Formalin fixed-paraffin embedded tissues were sectioned at 3-4 micron thick sections. Routine staining protocols were carried out for hematoxylin and eosin (H and E) and immunohistochemical (IHC) technique for VEGF.

Determination of VEGF expression

VEGF expression was determined by immunohistochemistry using a rabbit polyclonal anti-VEGF antibody (BioGenex, USA), to assess intensity.

The immunoexpression of VEGF in the epithelial was qualitatively evaluated at 400x magnification. performing an adaptation of the method proposed by Leonardi, et al. (2003)[9]

the immunoexpression of the vegf was classified according to the following scores: 0 – no staining; 1 – weak, staining in 11-25% of cells; score 2 – moderate, staining in 26-75% of cells; 3 – strong, staining in more than 76% of cells.

VEGF expression in connective tissue was semi-quantitatively evaluated, calculating the percentage of VEGF-positive vessels, fibroblasts, and inflammatory cells in ten representative fields by using a microscope at 400× magnification. The cases were divided in the following categories: score 0 (≤ 10% positive cells); score 1 (> 10% and <50% positive cells); score 2 (≥ 50% positive cells)[10].

The Chi-square test was applied to intercompare the VEGF expression between dentigerous cyst and radicular cyst. *P* values <0.05 were considered to be statistically significant.

RESULTS

The present study was carried out on 18 formalin-fixed paraffin-embedded tissues of 9 cases of Dentigerous cyst And 9 cases of Radicular cyst. Immunohistochemical staining was carried out with VEGF antibody to evaluate the VEGF expression in Dentigerous Cyst and Radicular Cyst.

On inter-comparison of VEGF expression in the epithelium between Dentigerous Cyst and Radicular Cyst

Positive immunoreactivity was observed in all cases of Dentigerous cyst where as in 66.66% cases of Radicular cyst.

In Dentigerous Cyst Strong expression was observed in 4cases (44.44%), moderate expression in 2 cases (22.22%) ,weak expression in 3 cases (33.33%) . In Radicular Cyst Strong expression was observed in 1case (11.11%), moderate expression in 1case (11.11%) ,weak expression in 4 cases (44.44%) and 3 cases (33.33%) revealed negative staining .A significant difference was observed with the p value of (0.018) on Inter- comparison of VEGF expression in the epithelium between Dentigerous Cyst And Radicular Cyst.

On inter-comparison of VEGF expression in the connective tissue between Dentigerous Cyst and Radicular Cyst. In Dentigerous Cyst Strong expression was observed in 3cases (33.33%), moderate expression in 5 cases (55.55%) ,weak expression in 1 case (11.11%). In Radicular Cyst Strong expression was observed in 4cases (44.44%), moderate expression in 4 cases (44.44%),weak expression in 1 case (11.11%). A significant difference was observed with the p value of (0.0155) on Inter- comparison of VEGF expression in the connective tissue between, Dentigerous cyst and Radicular cyst.

Inter-comparison of VEGF expression in the epithelium between Dentigerous Cyst and Radicular Cyst revealed statistically significant results with the p value of (0.0321).

Inter-comparison of VEGF expression in the connective tissue between Dentigerous Cyst and Radicular Cyst revealed a non-significant difference with the p value of (0.145).

Table 1: Intercomparison Of Vegf Presence In Epithelium Between Dentigerous Cyst And Kcot

Presence	Dentigerous cyst (n=10)		Radicular CYST(n=10)	
	Frequency	Percent	Frequency	Percent
Negative	1	10	2	20
Mild	3	30	3	30
Moderate	3	30	2	20
Strong	3	30	3	30
p* value	0.0321 (S)			

Table 2 : Intercomparison Of Vegf Presence In Connective Tissue Between Dentigerous Cyst And Radicular Cyst

Presence	DENTIGEROUS cyst (n=10)		Radicular cyst (n=10)	
	Frequency	Percent	Frequency	Percent
Score 1	1	10	1	10
Score 2	5	50	3	30
Score 3	4	40	6	60
p* value	0.145 (NS)			

DISCUSSION

Odontogenic cysts comprise a group of osseo-destructive lesions that frequently affect the jaws; in this group, periapical cysts, dentigerous cysts, and keratocystic odontogenic tumors (previously termed odontogenic keratocysts) can collectively represent up to 95% of all diagnoses.[11-13] These cysts can arise from epithelial components of the tooth germ, the reduced epithelium of the enamel organ, epithelial rests of Malassez, remnants of dental lamina, or the basal layer of the oral epithelium.[14,15]

Odontogenesis is controlled by interactions between the epithelial and mesenchymal components of developing dental tissues. These interactions have been considered to play an important role in the tumorigenesis of odontogenic lesions.[16] The connective tissue stroma has an essential role in preservation of epithelial tissues and minor alterations in the epithelium are followed by corresponding changes in the stroma such as angiogenesis.[17] Vascular Endothelial Growth Factor (VEGF) is considered the first factor which maintains its position as the most critical driver of vascular formation and is required to initiate the formation of immature vessels.[18]

In the present study majority of the cases manifested in males and in the mandible as compared to maxilla. These finding were in accordance with the results of Ledesma-Montes et al 2000[19], Avelar et al 2009[13], Khosravi N et al 2013[20] & Ramachandra S et al 2014[21] who conducted a study on the prevalence of odontogenic cysts and observed that males were more commonly affected than females and mandible was more common site. The results of present study were in disagreement with studies conducted by Ochsenius et al 2007[22] and de Souza et al 2010[17] who found maxilla as the common site for the odontogenic cysts.

In the present study statistically significant differences in VEGF expression in the epithelium (0.018) and connective tissue (0.0155) were observed between Dentigerous Cyst and Radicular Cyst. These results suggest that the critical event in the regulation of angiogenesis is the signaling cascade involving vascular endothelium growth factor (VEGF). This conclusion is based first of all on the biological properties of this growth factor.

On literature review only one study was found comparing VEGF expression in KCOT, DC and RC by G. K. Mitrou et al. (2009)[23] who observed immunoreactivity for VEGF in 35 cases out of 37 OKs and all DCs and RCs, as well as adjacent endothelial cells, fibroblasts, and inflammatory cells. Our results were consistent with the findings of G. K. Mitrou et al. (2009) as we also observed positive expression of VEGF in the epithelium of all the cases of DC 9(out of 9) and RC 9(out of 9) with all cases of DC, while only 66.66% cases of RC 6(out of 9) revealing positive VEGF expression in the connective tissue.

Mitrou et al[23] concluded that VEGF expression in the lining epithelium of DC, and RC was not associated with the presence of inflammation. Although it can be assumed that pre-existing inflammation may up regulate VEGF expression, particularly in RCs, their findings are suggestive of a role for VEGF regardless of inflammation.

A significant difference in the epithelium (0.0321) and non significant difference in the connective tissue (0.145) was observed on inter-comparision of the VEGF expression between Dentigerous Cyst and Radicular Cyst. These results were in accordance with that of G. K. Mitrou et al. (2009)[23] who also observed no statistical significant difference between DC and RC (P 0.26).

Maiara de Moraes et al in 2013[24] compared the VEGF and angiogenic index in RC and DC. They observed higher expression of VEGF in the epithelium and capsule of Radicular Cyst as compared to Dentigerous Cyst and suggested that the RANK and RANKL play an important role in bone resorption in DC and the hemorrhagic areas in the capsule of DC could be explained by increased vessel's number and the higher VEGF expression in RC might be related to nature of these lesions, where the inflammatory process contributes significantly to these findings.

The epithelium status of the RCs has been suggested as a reliable histological parameter of biological activity and/or inactivity of cystic growth. The epithelial expression of VEGF in DCs might constitute an additional mechanism for the enlargement of these lesions, maintaining the stimulus for angiogenesis and vascular permeability. Results from studies on brain tumor cysts suggest that VEGF might enhance accumulation of cyst fluid altogether with an increase in oxygen supply boosting the development of the cyst.

Vascular endothelial growth factor has been studied in inflammatory periapical lesions. Leonardi et al.in 2003[9] found that VEGF was expressed in the epithelial component in six (out of 6 cases) radicular cysts, 17 periapical granulomas with epithelial proliferation, and Malassez's rests, while reaction of fibroblasts and inflammatory cells was heterogeneous. Graziani et al. in 2006[25] found heterogeneous, weak-to-moderate expression of VEGF in the lining epithelium in 24(out of 24 cases) radicular cysts, and strong in the connective tissue. Both VEGF expression and CD34 microvascular density (MVD) were higher in cysts showing more intense inflammation.

Both studies (Leonardi et al.in 2003[9] & Graziani et al. in 2006)[25] emphasized the association of VEGF expression with inflammation and pointed to the up-regulation of cytokines that induced VEGF expression, such as IL-1a, IL-6, TGF-b, IGF-1, in periapical lesions as a possible mechanism.

Accumulation in the cystic fluid of KCOT, DC and Radicular Cysts of serum proteins from the vasculature have been thought to elevate the hydrostatic pressure and maintain their expansion. Also, mechanical forces have been shown to up-regulate VEGF and VEGF inducing growth factors (TGF, PDGF, and FGF) in endothelial cells.[26]

Various factors such as cytokines interleukin-1, growth factors like transforming growth factors (TGFs), fibroblast growth factor (FGF), regulate the expression of VEGF. Piatelli A et al in 2004[27] carried out a study to evaluate the positivity of TGF-β1 in odontogenic cysts. They observed strong positivity in both the epithelium and stromal cells in 9 out of 10 cases of KCOT, DC revealed epithelial positivity in 13 cases out of 27 and stromal cell positivity in 7 cases out of 27, where as all the RCs present an epithelium that was negative for the TGF-β1 with slight positivity was present in stromal cells.(11of 30 cases).They concluded from their study that TGF-β1 is important in angiogenesis and may play a pivotal role in many cellular activities like, regulation of cellular proliferation, differentiation.

Thus, it would be reasonable to suggest that VEGF expression in epithelial cells and capsular fibroblasts and vessels, might form a cellular network sharing regulation by the stimulatory signals promoting angiogenesis and also VEGF induces an increase in cystic pressure and is induced by the pressure exerted on the lining epithelium from the cystic fluid and may further play a role in cystic proliferation.

CONCLUSION

It is the well known fact that the VEGF expression is regulated by various stromal factors, such as transforming growth factors (TGFs), insulin-like growth factor (IGF) 1, fibroblast growth factor (FGF) and

various studies have concluded the increased expression of these growth factors in the KCOT than in the other odontogenic cysts like dentigerous cyst and radicular cyst.

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