

Research Paper: Angiogenesis: A Critical Role in Locally Aggressive Behavior of Keratocystic Odontogenic Tumor



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Citation: Bashardoust N, Bagheri S, Attarbashi S, Alizadeh N, Modabbernia Sh. Angiogenesis: A Critical Role in Locally Aggressive Behavior of Keratocystic Odontogenic Tumor. Journal of Dentomaxillofacial Radiology, Pathology and Surgery. 2017; 6(4):97-102.

doi: <http://dx.doi.org/10.32598/3dj.6.4.97>

Funding: See Page 100

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Article info:

Received: 10 Aug 2017

Accepted: 05 Nov 2017

Available Online: 01 Dec 2017

Keywords:

Odontogenic cysts,
Odontogenic tumors,
Angiogenesis, Vascular
endothelial growth factor

ABSTRACT

Introduction: Odontogenic cysts are osteodestructive lesions affecting the jaws. Odontogenic keratocyst is an odontogenic cyst with highly aggressive features. Multicystic ameloblastoma is a benign epithelial odontogenic tumor with more aggressive behavior than dentigerous cyst and odontogenic keratocyst. Invasion and metastasis of tumors require increased blood vessels during tumorigenesis. This study aimed to perform an immunohistochemical evaluation of blood vessels density by vascular endothelial growth factor expression.

Materials and Methods: This study was conducted on 16 odontogenic keratocysts, 18 dentigerous cysts, and 10 ameloblastomas. Immunohistochemical staining was performed using antibody against vascular endothelial growth factor. Kruskal-Wallis test and Chi-square test were used for data analysis.

Results: The highest percentage of cases with score 1 was observed in dentigerous cyst and lowest percentage was seen in ameloblastoma. The highest percentage of score 2 belonged to odontogenic keratocysts. No statistically significant differences were observed between dentigerous cysts, odontogenic keratocysts, and ameloblastomas regarding the vascular endothelial growth factor expression ($P=0.96$).

Conclusion: According to the result of this study, no significant differences were seen between vascular endothelial growth factor expression in ameloblastomas and odontogenic keratocysts, although vascular endothelial growth factor expression was higher in odontogenic keratocysts compared to dentigerous cysts. Perhaps the high level of vascular endothelial growth factor expression is related to odontogenic keratocyst invasive behavior.

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1. Introduction

Odontogenic cysts and tumors play an important role in oral and maxillofacial pathology [1-3]. Odontogenic Keratocyst (OKC), recently introduced as Keratocystic Odontogenic Tumor (KOT), presents with a different clinical behavior from other developmental odontogenic cysts such as Dentigerous Cysts (DC) and is more similar to some odontogenic tumors like ameloblastoma [4, 5]. The reactions between the odontogenic epithelium and its ectomesenchymal components play a role in the formation of odontogenic lesions, including odontogenic cysts [6]. Angiogenesis has a proven critical role in tumor behavior. Vascular Endothelial Growth Factors (VEGFs) include a family of multifunctional proteins which have an optional mitogenic effect on the vascular endothelial cells and are also the angiogenesis initiator in blood vessels [7, 8].

Although, angiogenesis has been assessed in many other pathologic lesions such as ameloblastoma, breast cancer, and melanoma [9], the number of studies based on angiogenesis in odontogenic lesions are insufficient. Hence, regarding different clinical behavior of odontogenic lesions, lack of information, and also the critical role of early diagnosis and treatment to reduce the risk of recurrence and malignant changes, further studies in this area are recommended. In the present study, we evaluate the VEGF expression in the epithelium of OKC, DC, and ameloblastoma to check its mitogenic activity on epithelium of odontogenic cysts and tumors.

2. Materials and Methods

The samples of this retrospective study were collected from 44 patients, including 16 samples of OKC, 18 samples of DC, and 10 samples of ameloblastoma from the Department of Oral and Maxillofacial pathology, Guilan University of Medical Sciences, Rasht, Iran. Patients without complete data and insufficient paraffin-embedded tumor material were excluded from the study. The samples with necrotic tissue, bleeding, excessive swelling, insufficient tissue and those related to recurrence of odontogenic lesions were also excluded from the study.

Immunohistochemical staining was performed on the 3- μ paraffin sections over the poly-L-lysine-coated glass slides. After heat drying, the slides were deparaffinized in xylene and rehydrated in ethanol with TUF in 90°C for 10 minutes to remove antigen's cover. After 3 times rinsing with 4°C water, they were incubated by 1:200 diluted monoclonal antibody VEGF-VG1 made by

(DAKO, Denmark). Finally, those slides were incubated by strept (avidin)-biotin-peroxidase method with diaminobenzidine 3.3 and were stained by hematoxylin. In this staining procedure, placental tissues were considered as a positive control.

The evaluation of VEGF expression was performed through Rubini et al. method [10]. The VEGF incidence was evaluated by counting the stained cells, including epithelial cells, fibroblasts, and endothelial cells in 5 microscopic fields. The average positive cell's percentage in each sample was categorized as follows: Score 0: Negative staining; Score 1 (weak): 10% of cells or less have showed the VEGF staining; Score 2 (moderate): 10% to 50% of cells have showed the VEGF staining; and Score 3 (strong): more than 50% of cells have showed the VEGF staining. Finally, the Kruskal-Wallis and Chi-square test were used for statistical analysis while the significance level was considered less than 0.05.

3. Results

A total of 44 patients (26 female, 18 male) were included in this study. VEGF score 0 immunoreexpression was observed in 5.6% of DC samples, 6.3% of OKC samples, and 10% of ameloblastoma samples. Score 1 was reported in 50% of OKC samples, 61.1% of DC samples, and 50% of ameloblastoma samples. Score 2 was reported in 37.5% of OKC samples, 27.8% of DC samples, and 40% of ameloblastoma samples. In 6.3% samples of OKC, score 3 was observed, while only 5.6% of DC samples were categorized in the same score. Although OKC was on the top of the score 2 list, there were no significant differences. Table 1 shows the details of the results. Figures 1, 2, and 3 show the VEGF immunoreexpression in DC, OKC, and ameloblastoma, respectively.

4. Discussion

Various evaluations support that different clinical behavior of OKC is due to the nature of its epithelium. The epithelium of the OKC has shown a higher rate of proliferation than other odontogenic cysts because of the strong expression of p53, proliferating cell nuclear antigen, and Ki-67 [11]. Angiogenesis has also an important role in the growth and proliferation of the tumor cells [12]. Despite the critical role of angiogenesis, there are few studies in the case of VEGF expression in odontogenic cysts. Thus higher expression of VEGF in pathologic lesions such as breast cancer is proved to result in poor prognosis and lower survival rate due to the great induction permeability of blood vessels which facilitates the growth of tumor cells [13-15].

Table 1. Analysis of VEGF score in each group using Chi-Square test

Lesion VEGF Score	No. (%)			P
	OKC	DC	Ameloblastoma	
Score 0 0%	1(6.3)	1(5.6)	1(10)	0.96
Score 1 <10%	8(50)	11(61.1)	5(50)	
Score 2 10%-50%	6(37.5)	5(27.8)	4(40)	
Score 3 50%>	1(6.3)	1(5.6)	0(0)	
Total	16(100)	18(100)	10(100)	

Abbreviations: VEGF: Vascular Endothelial Growth Factor; OKC: Odontogenic Keratocyst; DC: Dentigerous Cysts

The present study evaluated the expression of VEGF in 16 cases of OKC, 18 cases of DC, and 10 cases of ameloblastoma and showed that Microvessel Density (MVD) in OKC was higher than that in DC which is another expression for higher recurrence rate and aggressive behavior of OKC in comparison to DC. Despite no significant difference, VEGF is expressed strongly in OKC compared to ameloblastoma. This finding supports the fact that although OKC is a cystic lesion of odontogenic origin, it has an aggressive behavior like an odontogenic tumor.

Based on literature review, Mitrou et al. observed immune reactivity for VEGF in 35 cases out of 37 OKCs and all DCs and radicular cysts, as well as adjacent endothelial cells, fibroblasts, and inflammatory cells [14].

They reported that pre-existing inflammation could surely increase the expression especially in radicular cyst; however, current inflammation lacked a critical role in upregulating VEGF expression. In contrast, in the present study we observed more VEGF immune reactivity- although insignificant - in OKC than in DC [14]. Furthermore, in the present study moderate expression was observed in the majority of cases of OKC. On the other hand, most cases of DC revealed weak to moderate expression. These findings suggest that OKC has increased the level of angiogenesis which may be related to the increase in tissue metabolism and nutrition requirement of the odontogenic epithelium. These findings also could help explain the biological behavior of OKC which should be considered as a benign odontogenic tumor rather than a simple cyst.

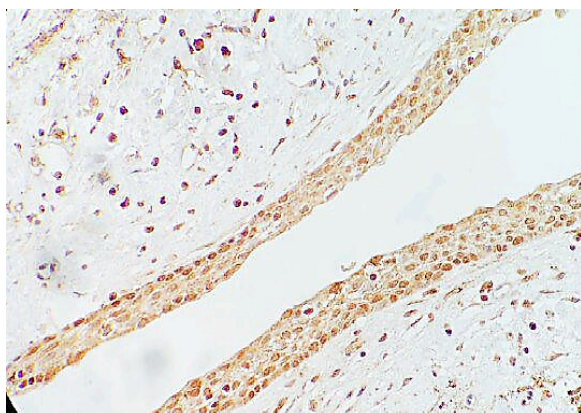


Figure 1. Immunohistochemical staining of VEGF in dentigerous cyst (x400)

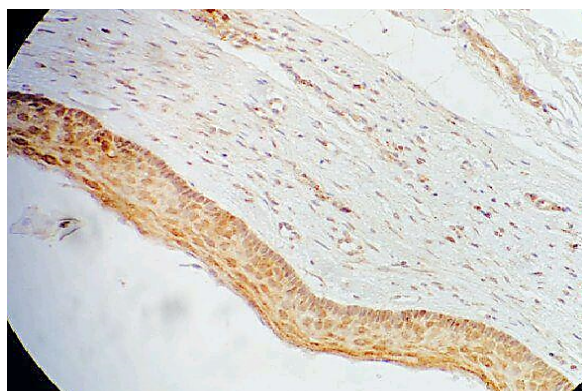


Figure 2. Immunohistochemical staining of VEGF in OKC (x400)

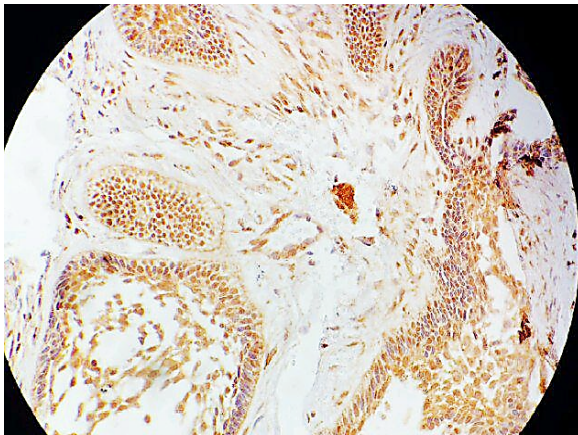


Figure 3. Immunohistochemical staining of VEGF in ameloblastoma (×400)

Sadri et al. declared that expression of VEGF and CD-34 are significantly higher in OKC compared to those in DC with insignificant differences in the pattern of expression. There was also a strong correlation between these two markers [7]. Rubini et al. found a significant and different expression of VEGF in all cell components in OKCs compared to DCs. The majority of OKCs (80%) and orthokeratinized odontogenic cysts (68%) expressed more than 50% VEGF-positive cells, whereas the majority of DCs (71%) were either negative in the epithelium or showed <10% positive cells which is somehow in consistent with our results. This higher positivity for VEGF of OKC could help describe the infiltrative characteristic of this lesion. They also indicated a correlation between VEGF expression in epithelial cells and capsular fibroblasts and vessels, which suggests that these cells might form a cellular network sharing adjustment by the stimulatory signals promoting angiogenesis [10].

Khajuria et al. revealed a significant difference between the expression of VEGF in OKC and DC which could also help clarifying the dissimilar growth pattern of these two cysts and resulted in labeling OKC as an odontogenic tumor that somehow supported the present results [15]. The result of the study of Danish Kumar et al. showed that VEGF was strongly expressed in ameloblastomas compared to KCOT. However this result is insignificant because of the same expression of VEGF in OKC which is in accordance with the present results. The finding of Danish Kumar et al. study supports that OKC has an aggressive behavior similar to odontogenic tumors. It also could suggest that CD105 is a crucial mediator of tumor angiogenesis and higher expression of CD105 might be related to tumorigenesis [12].

Seifi et al. also in a study based on the evaluation of the MVD in odontogenic lesions, declared that intratumoral MVD had a vital role in growth pattern and clinical behavior of odontogenic lesions and assumed that both ameloblastoma and OKC shared resemblances regarding angiogenesis accounting for its aggressive behavior. Their results agree with our results. Furthermore, OKC showed increase in the total vascular area and mean vascular area in comparison with other development cysts which may be related to the augmentation in tissue metabolism and nutrition requirement [7]. The present data indicate that angiogenesis plays a crucial role in the development and enlargement of odontogenic cysts similar to neoplastic conditions. The stroma of OKC could not only act as a structural support of the cyst wall but also as an important part in the neoplastic behavior of cyst.

5. Conclusion

In conclusion, although, VEGF expression may be responsible for different clinical behavior in OKC and ameloblastoma, it may also act as a mitogenic agent for epithelium of odontogenic cysts and tumors. Furthermore, the similarity between OKC and ameloblastoma, as seen in the results of the present study, suggests that OKC not only has clinical criteria of a neoplasm but has molecular similarity with a tumor, too. The role of VEGF in the pathogenesis of OKC and odontogenic cysts should be further assessed using advanced techniques with larger samples.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article. The participants were informed about the purpose of the research and its implementation stages; They were also assured about the confidentiality of their information; Moreover, They were allowed to leave the study whenever they wish, and if desired, the results of the research would be available to them.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no conflict of interest.

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