Angiogenesis Concept in Odontogenic Keratocyst: A Comparative Study

Abstract
Context: Recent reports have indicated that angiogenesis possibly affects the biologic behavior of the lesions. Aim: Given the different clinical behaviors of odontogenic keratocyst (OKC), the present study was undertaken to evaluate the concept of angiogenesis in pathogenesis and clinical behavior of OKC. Setting and Design: This experimental study was carried out on 22 and 24 samples of OKCs and dentigerous cysts (DCs), respectively. Methods: Immunohistochemical staining was approached using CD34 and vascular endothelial growth factor (VEGF) antibodies. The expression of VEGF was first reported by determining the counts of stained cells, including epithelial cells, fibroblasts, and endothelial cells, followed by the percentage of stained cells in each sample based on a 0–2 scoring system. The counts of CD34+ cells were reported in each group in the form of means ± standard deviations. In addition, the patterns of blood vessels in the samples prepared from the walls of both cysts were evaluated. Statistical Analysis Used: Mann–Whitney U-test, Chi-squared test, and t-test were used for analysis of data, and statistical significance was defined at p < 0.05. Results: The expression percentage and scores of VEGF and the mean expression rate of CD34 were significantly higher in OKCs than DCs (p = 0.045, 0.000, and <0.001). No significant difference was detected in the vascular patterns of these lesions (p = 0.58). Finally, there was a strong correlation between the expressions of the two markers in the samples (Correlation coefficient = 0.766). Conclusion: The present results indicate the angiogenesis may play an important role in the pathogenesis and the unique clinical behavior of OKC.

Keywords: Angiogenesis, CD34, dentigerous cyst, immunohistochemistry, odontogenic keratocyst, vascular endothelial growth factor

Introduction
Dentigerous cyst (DC) is one of the most common developmental odontogenic cysts, with good prognosis and a low recurrence rate, which comprises approximately 20% of all the oral cavity cysts.[1] Odontogenic keratocyst (OKC) has been classified in the odontogenic tumors group in some references due to its histopathological characteristics and specific clinical course, high recurrence rate, and invasive behavior.[2] OKC was described by Philipse for the first time in 1956. It comprises 3%–11% of all odontogenic cysts.[3]

Based on recent reports, the angiogenesis rate of lesions affects their biologic behavior,[4,5] and novel treatment modalities have been based on decreasing its rate.[6] Evaluation of angiogenesis is carried out through different markers; vascular endothelial growth factor (VEGF) is a multifunctional cytokine expressed under various conditions and has a role in increasing angiogenesis and vascular permeability; it also promotes proliferation and migration of endothelial cells. This cytokine is synthesized by different kinds of cells and functions as a principal regulator of physiologic and pathologic angiogenesis.[8,9] Previous studies have shown that solid tumors cannot develop and metastasize without a proper blood supply; indeed, the tumors with expression of VEGF can develop and metastasize.[10,11] On the other hand, CD34 is a glycosylated membrane glycoprotein of the sialomucin family. It is a pan-endothelial marker and adhesive molecule with a molecular weight of 110–120 KD, which has a major role in identifying the density of blood vessels in pathologic lesions.[12] It is expressed in the normal and neoplastic endothelial cells of blood vessels and used as a selective vascular marker for the qualitative assessment of angiogenesis in various lesions.[13,14] Considering the differences in the clinical behaviors of jaw cysts and a lack of sufficient information about the relevant biologic factors and also the importance of early diagnosis and treatment...
in prevention or at least decreasing the odds of recurrence and malignant transformations, the present study was undertaken to evaluate the concept of angiogenesis in the pathogenesis and clinical behaviors of OKC using VEGF and CD34 markers.

**Methods**

Tissue sections from paraffin block samples selected from OKC and DC lesions underwent immunohistochemical evaluation using VEGF antibody and CD34 antibody manufactured by Dako Company (Denmark), using streptavidin-biotin-peroxidase technique. During the staining procedure, placental tissue and hemangioma were used as positive controls for VEGF and CD34, respectively. Evaluation of angiogenesis for VEGF was carried out according to a technique described by Rubini et al. In this context, the expression of VEGF was determined by counting the number of stained cells, including epithelial cells, fibroblasts, and endothelial cells, in five microscopic fields. Then, the mean percentages of positive cells were determined in each sample and classified as follows:

- Score 0: ≤10% of cells exhibited VEGF staining
- Score 1: 10%–50% of cells exhibited VEGF staining
- Score 2: >50% of cells exhibited VEGF staining.

Figures 1 and 2 demonstrate VEGF expression in OKC and DC, respectively.

In the next stage, evaluation of angiogenesis was carried out by the expression of CD34 according to a technique described by Weidner as described briefly below. The lesions were visualized under a light microscope at a magnification of ×100. Three areas that exhibited the maximum number of blood vessels were selected and evaluated at ×400. The average frequency of stained endothelial cells was calculated in five microscopic fields of the three above-mentioned areas and recorded as the mean ± standard deviation (SD) for each sample. Then, the pattern of blood vessels in the cyst walls was evaluated, too. The pattern was recorded as circumferential if the stained vessels were parallel to the basement membrane of the epithelium, and the directional pattern was recorded if the blood vessels were perpendicular to or oblique to the basement membrane.

A Nikon light microscope (Japan) was used for cellular evaluations at ×400. Figures 3 and 4 represent CD34 expression in DC cyst wall as a circumferential and directional pattern, respectively. Mann–Whitney U-test, t-test, and Chi-squared test were used for statistical analyses. Statistical significance was defined at \( P < 0.05 \).

**Results**

**Expression of vascular endothelial growth factor**

As shown in Table 1, the minimum and maximum expression rates of VEGF in DC were 4% and 40.2%, respectively, with a mean of 20.2% and SD of 11.86%. In addition, the minimum and maximum expression rates of VEGF in OKC were 14% and 79%, respectively, with a mean of 52.6% and SD of 19.98%.

The T-test showed a significant difference in the expression of VEGF between OKC and DC, i.e., the expression of VEGF in OKC was significantly higher than that in DC (\( P = 0.045 \)).

**Table 1: Comparison of vascular endothelial growth factor expression in dentigerous cyst and odontogenic keratocyst**

| Lesion | \( n \) | Minimum | Maximum | Mean±SD |
|--------|-------|---------|---------|--------|
| DC - VEGF | 24 | 4 | 40.2 | 20.2±11.86 |
| OKC - VEGF | 22 | 14 | 79 | 52.6±19.98 |

SD=Standard deviation, OKC=Odontogenic keratocyst, DC=Dentigerous cyst, VEGF=Vascular endothelial growth factor growth factor expression in odontogenic keratocyst by ×400

Figure 2: Vascular endothelial growth factor expression in dentigerous cyst by ×400
of 1. In addition, 6 OKC samples (27.3%) exhibited a score of 1 and 16 samples (72.7%) had a score of 2; no samples had a score of 0.

Mann–Whitney U-test showed a significant difference in VEGF scores between DC and OKC ($p = 0.000$), i.e., OKC samples exhibited significantly higher scores in terms of VEGF expression.

In the next stage, Chi-squared test was used to evaluate the relationship between the expressions of VEGF in different cells irrespective of the lesion type.

As shown in Table 3, five cases (20.8%) of DC exhibited no epithelial cell staining and 19 (79.2%) cases exhibited epithelial cell staining. In this context, two cases (9.1%) of OKC exhibited no epithelial cell staining and twenty (90.9%) cases exhibited epithelial cell staining. Chi-squared test showed no significant differences in the expression of VEGF in epithelial cells between DC and OKC ($p = 0.268$).

As shown in Table 4, 15 (62.5%) cases of DC samples exhibited no endothelial cell staining and 9 (37.5%) exhibited endothelial cell staining. Furthermore, 15 (68.2%) OKC samples exhibited no staining of epithelial cells and 7 (31.8%) exhibited epithelial cell staining. Chi-squared test revealed significant differences in the staining of endothelial cells between OKC and DC ($p = 0.037$), with significantly higher rate of endothelial cell staining in OKC. In relation to fibroblasts, since 100% of the cells in both cysts exhibited staining, no statistical analysis was carried out.

Expression of CD34

As shown in Table 5, the minimum and maximum expression rates of CD34 in DC were 8.8 and 31.78, respectively, with a mean and SD of 19.27 ± 6.18. On the other hand, the minimum and maximum expression rates of CD34 in OKC were 13.6 and 45.1, respectively, with a mean and SD of 34.62 ± 7.31.

Man–Whitney U-test showed a significant difference in the expression of CD34 between DC and OKC, with a higher expression rate in OKC ($p < 0.001$).
In the next stage, the vascular patterns were evaluated in the cyst walls that had exhibited CD34 expression.

As shown in Table 6, in 62.5% and 37.5% of DC samples, the vascular patterns in the cyst walls were directional and circumferential, respectively. In OKC samples, 54.5% and 45.5% of the patterns were directional and circumferential, respectively. Chi-squared test revealed no significant differences in cyst wall vascular patterns between the two cysts ($p = 0.58$).

**Correlation between the expression of vascular endothelial growth factor and CD34**

There was a strong correlation between the expression of VEGF and CD34 in all the study samples (Pearson’s correlation coefficient = 0.766).

**Discussion**

The results of the present study showed a significant difference in the expression of VEGF between the two cysts evaluated, with significantly higher VEGF expression in OKC compared to CD. However, there was no significant difference in the expression of VEGF in epithelial cells between the two cysts; however, in relation to the expression of VEGF in the endothelial cells, there was a significant difference between these two cysts, with significantly higher endothelial cell staining in OKC. Consistent with the results of the present study, other studies showed significantly higher expression of VEGF in OKC compared to DC. On the other hand, Nonaka et al. showed a significant relationship between the expression of VEGF, vascular density, and angiogenesis, concluding that higher expression of VEGF was correlated with greater vascular density and higher inflammatory infiltration.

The present study showed a significantly higher mean expression rate of CD34 in OKC compared to DC ($p < 0.001$). In addition, no significant differences were detected between the vascular patterns in the cyst walls of the two lesions; however, there was a high correlation in the expression of the two studied markers in all the samples. In this context, some reports showed significant differences in the mean vascular densities using CD34 and CD105 markers between OKC, DC, and solid ameloblastoma, consistent with the results of the present study. They concluded that angiogenesis might be a possible mechanism involved in the different biologic behaviors of OKC, DC, and solid ameloblastoma. They suggested that vascular density is probably one of the mechanisms involved in the invasive biologic behaviors in ameloblastoma and OKC.

On the other hand, Gadbail et al. showed no significant differences in vascular densities, which does not coincide with the results of the present study; however, they reported that tumor angiogenesis has an important role in the invasion of injured tissues, progression of disease, and biologic behaviors of odontogenic tumors, ameloblastoma, and DC.

Furthermore, Seifi et al. showed the circumferential vascular pattern in ameloblastoma and directional pattern in OKC and follicular cyst. They also concluded that an increase in the mean microvasculature involved in the invasive behavior was consistent with those of the present study and showed no significant differences in the vascular pattern of cyst walls between DC and OKC.

Currently, angiogenesis has been introduced as one of most important factors of stroma involved in the progression of tumors. Previously, CD34 has been used for the evaluation of microvascular density in different tissues. Expression of VEGF has also been evaluated in pathologic lesions, with some researchers attributing its expression to poor prognosis in breast cancers. In addition, an increase in the expression of VEGF might be considered a first step in metastasis, which involves changes in the angiogenesis process. Although VEGF is associated with poor survival, its exact mechanism in the progression of tumors is not clear. In addition, it has been demonstrated that when VEGF is released, it might induce some responses, resulting in cell survival, mobilization, or differentiation.

Some studies have reported that angiogenesis and its relevant factors are necessary for the development of pathologic lesions. These studies have shown that when VEGF signals are inhibited, angiogenesis and the progression of pathologic lesions stop as a result. In addition, VEGF facilitates extravasation in pathologic lesions by inducing permeability of blood vessels, resulting in the creation of a matrix to support the growth of endothelial and tumoral cells, allowing the infiltration of pathologic lesions into adjacent tissues.

Very limited studies have been carried out in relation to the expression of VEGF in cysts, especially in OKC; the results...
of which have shown an increase in the expression of this marker in OKC. In the cystic lesions, it seems that VEGF has a progenitor state relative to other vascular markers and is expressed in epithelial cells and fibroblasts in addition to endothelial cells, it can increase vascular permeability, too; so, promoting extravasation of plasma proteins, finally leading to fluid accumulation. It appears the expression of VEGF in cystic lesions has a particular effect on the accumulation of fluid within OKC and an increased cyst growth.[17] On the other hand, since in the present study, the expression of VEGF in endothelial cells of OKC was higher than that in DC, it appears; in addition to fluid accumulation, VEGF can affect the ever-increasing growth of the cyst by affecting angiogenesis in the stroma of this cyst. Therefore, considering the explained mechanism of VEGF, leading to results of our study and due to strong correlation between two vascular markers studied in this report, it appears angiogenesis might have an important role in the differences observed in clinical behavior of OKC, i.e., since VEGF induces formation, proliferation, and migration of endothelial cells,[29] it can induce an increase in vascular density, playing a role in the more aggressive behavior of OKC compared to DC. Therefore, angiogenesis and evaluation of vascular density might be an important aim for developing treatment modalities based on decreasing vascular density, especially during recurrence of odontogenic tumors.[11]

Conclusion

In the present study, a higher angiogenic activity was shown in OKC compared to DC, use of VEGF and CD34 markers, which might highlight the concept of angiogenesis in the pathogenesis and unique clinical behavior of OKC. However, further studies are necessary in this field.

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Conflicts of interest

There are no conflicts of interest.

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