



Angiogenesis and mast cells as predictive factors in behavior of Odontogenic cysts



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Abstract:

Purpose: The aim of this study was to verify the density of mast cells (MCs) and microvessels in odontogenic cysts. Furthermore, the correlation between MCs and microvessels was evaluated to assess the contribution of MCs to angiogenesis and growth of odontogenic cysts. This approach may be a basis for the development of future pharmaceuticals addressed to MCs performance to manage odontogenic cysts.

Methods: 45 cases of odontogenic cysts consisting of 15 radicular cysts (RCs), 15 dentigerous cysts (DCs) and 15 odontogenickeratocysts (OKCs) were included in this study. Five high power fields in connective tissue wall were counted for each sample. Moreover, a total mean of five fields was calculated to calculate MVD and MCD of the samples.

Results: RC showed the highest mean numbers of microvessels ($p < 0.05$) and OKC showed the highest mean number of mast cells. A positive correlation between the numbers of MCs and microvessels was observed.

Conclusion: Increase in angiogenesis of OKC confirms that aggressive lesions require a larger number of blood vessels and highlights the concept of unique clinical behavior of this cyst. In OKCs and DCs cysts, the MVD is closely related to of the inflammatory infiltrate. Mast cells are imperative in the pathogenesis of odontogenic cysts. Mast cells have a role in promoting the growth of odontogenic cysts, particularly OKC. Further studies on the *in vivo* functions of MCs will make the concept more clear.

Introduction

Head and neck region and the jaw in particular, collectively comprise one of the most common sites for the cysts occurrence⁽¹⁾, as mandible and maxilla are the only bones in the skeleton with epithelial components⁽²⁾. The World Health Organization (WHO) originally proposed three major subdivisions of intraosseous cysts⁽³⁾. The original categories were odontogenic cysts (radicular cysts, dentigerous cysts and odontogenickeratocysts), non-odontogenic cysts (incisive canal, nasopalatine duct cysts) and non-epithelial cysts (traumatic (solitary) bone cysts)⁽⁴⁾. Odontogenic cysts are the most common type of cysts occurring within the jaws⁽⁵⁾. They arise as a result of proliferation and cystic degeneration of odontogenic epithelial rests, either result from developmental origin or may inflammation⁽⁶⁾. The most common odontogenic cysts are radicular, dentigerous and odontogenickeratocysts⁽⁷⁾.

Radicular cyst (RC) is the most common inflammatory cystic lesions of the jaw, which originates from the epithelium existing in the periodontal space or forming as a result of pulp necrosis^(8,9). This inflammatory cyst usually has a low growth rate and is asymptomatic unless there is acute inflammation in the area⁽¹⁰⁾. Dentigerous cyst (DC) and odontogenickeratocyst (OKC) are the most common developmental odontogenic cysts⁽¹¹⁾. Dentigerous cyst exhibits slow growth and minimal invasiveness⁽¹²⁾. It demonstrates an indolent behavior and seldom recurs following removal^(13,14). Odontogenickeratocyst (OKC) is a developmental odontogenic cyst with specific histopathological features and clinical behaviors, high recurrence rate, and aggressive behavior compared to other developmental odontogenic cysts⁽⁶⁾. The OKCs, similar to DCs, appear as cystic lesions, but their invasiveness and destructive growth are comparable to odontogenic tumors. It has been suggested that unknown factors integrated in the epithelium or fibrous capsule of OKCs that may be responsible for their specific biological behavior⁽¹³⁾.

Due to its aggressive behavior and tendency to high recurrence, the 2005 classification placed it as 'keratocysticodontogenictumor' in the category of the benign odontogenic tumor⁽³⁾. Recently, the status OKC was addressed and classified into the category of cyst. Based on WHO (2017) histological classification of odontogenic tumors and odontogenic cysts⁽¹⁵⁾. Many efforts have been made to understand the pathogenesis of odontogenic cysts, but many of them have been unsuccessful⁽¹⁶⁾.

Blood supply is an essential factor for the growth of odontogenic epithelium. Because there is no vascular system in the epithelium, apoptosis will happen if the connective tissue does not provide the necessary blood supply⁽¹⁷⁾. Angiogenesis occurs in physiologic and pathologic processes including embryogenesis, wound healing, and inflammation⁽¹⁸⁾. In odontogenic cysts, the connective tissue stroma has an essential role in the preservation of epithelial tissues and minor alterations in the epithelium are followed by corresponding changes in the stroma, such as angiogenesis^(17,19,13).

Mean vascular density (MVD) is a quantitative analysis of angiogenesis, which has been evaluated by using various molecules including: CD31, CD34 and CD105 (endoglin)^(20,21). CD105 (endoglin) is a homodimeric cell membrane glycoprotein and is a component of TGF- β receptor complex. This marker is an indicator of endothelial cell proliferation and is up-regulated during angiogenesis^(22,23). Moreover, the expression of CD105 is one of the most conspicuous characteristics of newly formed blood vessels; Hence, it is more appropriate to determine MVD⁽²⁴⁾. Several types of cells are associated with the development of cysts and tumors⁽¹⁶⁾. Among inflammatory cells, mast cells have been considered in growth and expansion of cysts. Mast cells are one of the defense cells of immune system with metachromatic cytoplasmic granules^(25,26).

Recently, mast cells were recognized in the pathogenesis of more aggressive pathologic lesions⁽²⁷⁾. Mast cells have an inhibitory role on the development of pathological lesions. However, stimulatory role of mast cells in the growth of pathological lesions is more prevalent and obvious than their inhibitory effect⁽²⁸⁾. With respect to several roles of mast cells such as participation in inflammation, degradation of extracellular matrix and bone resorption⁽²⁹⁾, previous studies have identified mast cells in odontogenic cysts, but there were limited studies about the role of mast cells in the pathogenesis of odontogenic cysts⁽³⁰⁾. There is a

Material and method :

In this work, the study samples included 45 paraffin blocks of odontogenic cysts (15RC, 15 DC, 15 OKC) that were collected from archival files of Oral Pathology Departments, Faculties of Dentistry, Mansoura University. Four micrometer thick tissue sections were stained through standard immunohistochemical (CD 105) and histochemical (Toluidine blue) staining procedures according to the instructions of the manufacturer. The slides were deparaffinized in xylene and then hydrated in graded alcohol series. For histochemical evaluation, the sections were stained with freshly prepared 1% toluidine blue (adjusted at pH 2.0 – 2.5) for 2-3 minutes, using Toluidine blue staining protocol for mast cells⁽³²⁾. Following dehydration and clearing. For CD105 marker, the endogenous peroxidase activity was blocked by incubating the slides with 3% hydrogen peroxide in methanol for 30 minutes. The antigen retrieval for CD105 was carried out by treating sections with proteins-K for 5 minutes. To prevent nonspecific reactions, sections were incubated with 10% serum for 10 minutes. Mouse Monoclonal Anti-Human CD105 antibody (Catalog No. AM441-5M: Ready-to-Use Antibody for Use with BioGenex Super Sensitive Detection Systems, Fremont, CA 94538 USA) were used as primary antibodies in this study. CD105 antibody was incubated at room temperature for 90 minutes in a

Results:

Vascular endothelial cells, which were stained by CD105, was observed cytoplasmic brown deposits in endothelial cells of capillary sprouts and in the newly formed tortuous small blood vessels with or without narrow lumina. On the other hand, mast cells were stained purple in their cytoplasm and the rest of the section stained blue.

Statistical Analysis of Immunohistochemical results (microvessel density MVD):

It was found that, RC demonstrated a mean value of 18.10 (± 8.38). DCs showed mean value of 12.38 (± 5.42) and OKC demonstrated a mean value of 17.88 (± 7.11). Radicular Cysts demonstrated the highest value followed by OKCs. The lowest value demonstrated by DCs. On comparing of the three groups by one-way analysis of variance, no statistically significant difference was found ($P=0.054$) ($P<0.05$).

According to the t-test, we did not observe a significant difference in microvessel density (MVD) between the RC and OKC ($P = 0.46$). On the other hand, the difference between dentigerous cyst and both radicular cyst ($p=0.017$) and odontogenic keratocyst ($P=0.012$) was statistically significant ($P < 0.05$).

When DCs and OKCs were divided into inflamed and non-inflamed cases, it was observed that the inflamed lesions showed higher mean number of microvessel density as they demonstrated $15.13 \pm (6.23)$ and $23.01 \pm (5.08)$. By comparing the inflamed and non-inflamed cysts, there was a statistically significant difference between inflamed and non-inflamed DC ($P=0.014$) and between

hypothesis that the more aggressive behavior of odontogenic keratocysts is related at least, partly, to distribution of mast cells. However, their pathogenesis and mechanism of expansion and enlargement have not been evaluated⁽³¹⁾.

The aim of this study was to determine the density of microvessels and MCs in odontogenic cysts. Correlate the microvessel density with their corresponding mast cells density in the three types of cysts, in order to detect their possible role in the variable behavior of these odontogenic cysts.

humidifying chamber, followed by incubation with secondary biotinylated antibodies and streptavidin for 15 min each. Diaminobenzidine was applied to produce brown staining followed by counterstaining with Mayer's hematoxylin. After each step, the slides were put in phosphate-buffered solution (PBS). For the negative control, the primary antibody was eliminated and replaced with PBS.

For immunohistochemical and histochemical counting using the microvascular count technique, according to the method suggested by Weidner et al.⁽³³⁾. MVD and MCD was assessed as the mean number of microvessels and MCs per high power field. The field size for 400 magnification (40 objectives and 10 ocular) was approximately 0.18 mm^2 . Scores of overall CD105 and Toluidine blue expression were represented as mean density/ $\text{mm}^2 \pm \text{SD}$ for quantitative variables using SPSS (Statistical package for Social Sciences) software. Comparisons among the experimental groups were done using One-way analysis of variance test (ANOVA) ($p<0.05$). To compare the number of microvessels and MCs between inflamed and non-inflamed DCs and OKCs, independent t-test was used. Pearson correlation coefficient test was used to determine the correlation between MVD and MCD. A p-value of <0.05 was considered statistically significant

inflamed and non-inflamed OKC ($P= 0.0001$). In addition, there was statistically significant difference by comparing between inflamed DC and inflamed OKC ($P=0.013$). On the other hand, there was no statistically significant difference by comparing between non-inflamed DC and non-inflamed OKC ($P=0.078$).

Statistical Analysis of Histochemical results (mast cell density MCD):

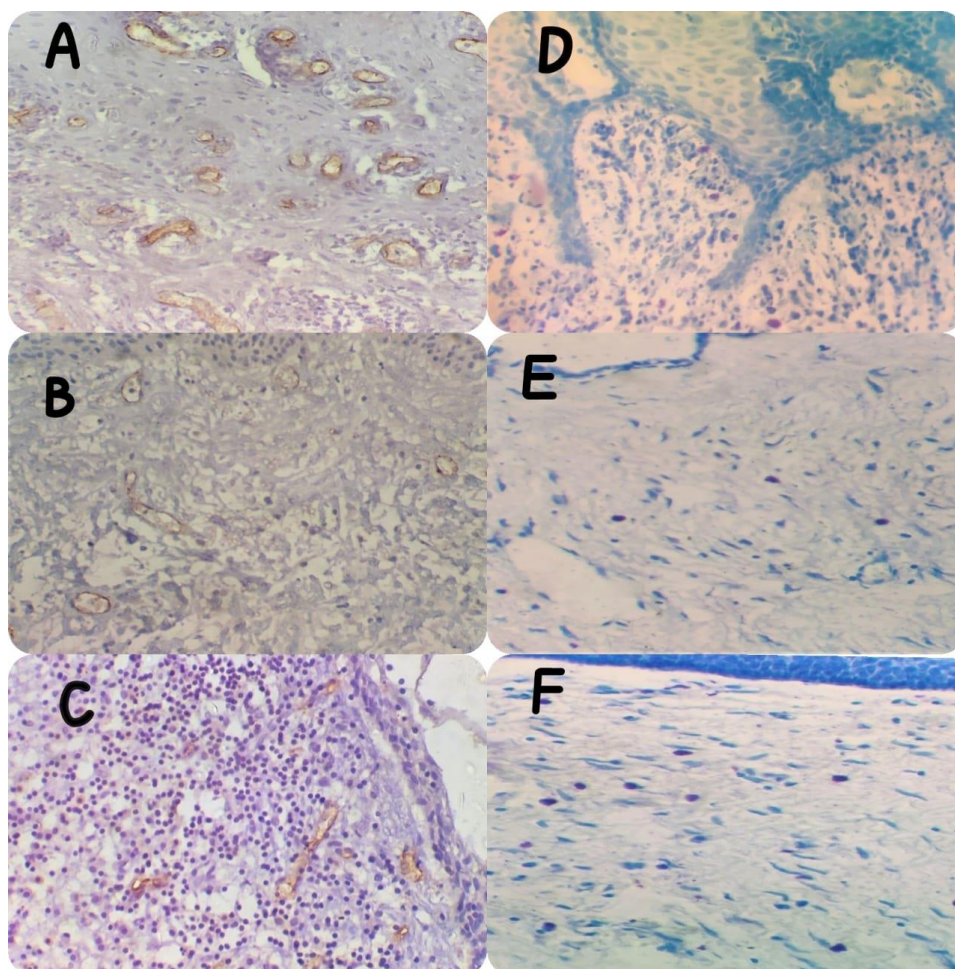
It was found that, the study cases RCs, DCs and OKCs demonstrated a mean mast cells value of $11.30 \pm (6.63)$, $7.19 \pm (1.97)$ and $10.28 \pm (4.39)$ respectively. OKCs and RCs demonstrated a higher mean value than DCs. According to the t-test, there was no significant difference observed in mast cell density (MCD) between the RC and OKC ($P=0.623$), but the difference between RC and DC ($P=0.019$); and between DC and OKC ($P=0.028$) was statistically significant ($P<0.05$). It was observed that, subgroups of cysts (inflamed and non-inflamed cases), the inflamed lesions demonstrated higher mean number of mast cells as inflamed DC and inflamed OKC showed mean value of $8.22 \pm (2.11)$ and $11.95 \pm (8.44)$. On the other hand, the non-inflamed lesions observed the lower mean value of mast cell density as non-inflamed DC and non-inflamed OKC showed $5.25 \pm (0.97)$ and $10.56 \pm (4.27)$ respectively.

There was a significant difference by comparing between inflamed and non-inflamed DC ($P=0.012$), but by comparing between inflamed and non-inflamed OKC ($P=0.351$) there was no statistically significant difference.

By comparing between inflamed DC and inflamed OKC (P=0.24) there was no significant difference. On the other hand, there was a

significant difference between non- inflamed DC and non- inflamed OKC (P=0.023).

Fig.(1): A photomicrograph showing CD 105 positive immunoreactivity in A) radicular cyst B) dentigerous cyst C) odontogenickeratocyst (ABC X400) and mast cells in connective tissue wall of D) radicular cyst E) dentigerous cyst F) odontogenickeratocyst stained by Touilidine blue (X400).



odontogenickeratocyst (ABC X400) and mast cells in connective tissue wall of D) radicular cyst E) dentigerous cyst F) odontogenickeratocyst stained by Touilidine blue (X400).

STUDIED GROUPS	NUMBERS	MICROVESSELS (MVD)	DENSITY	MAST CELLS (MCD)	DENSITY
RC	15	18.10 ± 8.38		11.30 ± 6.63	
DC	15	12.38 ± 5.42		7.19 ± 1.97	
OKC	15	17.88 ± 7.11		10.28 ± 4.39	

Table (1): Microvessel and Mast cell density in odontogenic cysts.

SUBGROUPS	NUMBERS	MVD	MCD
DC INF	8	15.13 ± 6.23	8.22±2.11
DC NON	7	9.25 ± 1.32	5.25±0.97
OKC INF	8	23.01 ± 5.08	11.95 ± 8.44
OKC NON	7	12.01 ± 3.55	10.56 ± 4.27

Table (2): Microvessel and Mast cell density in subgroups of dentigerous cysts and odontogenickeratocyst as inflamed and non inflamed cysts.

Discussion:

Odontogenic cysts (OCs) are derived from the epithelium, which is associated with the dental apparatus, yet, and despite this, our knowledge of their pathogenesis is at best rudimentary and some of them have different biologic behavior⁽³⁴⁾. OKC is different from those of other more common cysts such as radicular and dentigerous cysts as it has a more aggressive behavior⁽⁶⁾. Angiogenesis is one of the well-known stromal factors contributing in lesion progression. Recently, various angiogenic factors have been implicated in the growth of cysts⁽³⁵⁾. Browne et al⁽³⁶⁾ were pioneers to propose that the connective tissue wall of OKC may have an important role in the pathogenesis of this lesion.

CD105 is considered as an ideal marker for angiogenesis which detects vessels for its quality as well as its quantity. Moreover, CD105 has higher accuracy and specificity to bind newly formed blood vessels. So, several studies emphasize the involvement of CD105 in blood vessel formation and its utility as a powerful marker for the quantification of microvessels in many types of tumors and lesions^{37, 38, 39}.

In this work, it was found that the highest mean MVD in the studied lesions was observed in RCs followed by OKCs and then DCs. On comparing the MVD in RCs and OKCs, a slightly higher MVD was recorded in RCs compared with OKCs, but without significant differences. However, there was a significant difference in MVD between RCs and DCs and between OKCs and DCs. These findings were in agreement with Kouhsoltani et al.⁴⁰ who found that the highest mean numbers of microvessels was showed in RC. The higher MVD in RCs seen in the present study might be related to the presence of an exuberant inflammatory infiltrate in these lesions, because, according to Graziani et al.⁴¹, Nonaka et al.⁴² and Tete et al.⁴³ inflammatory cells can exert angiogenic activity in these cysts. In addition, the high MVD in OKCs in our work suggest that it is aggressive lesion that requires a larger number of blood vessels. In OKC, large concentrations of blood vessels not only were detected in areas of significant inflammation, but also in areas where significant inflammation was absent.

When microvessel density in OKCs compared with DCs, a higher MVD was found in the former. This result was in agreement with those reported by Jamshidi et al⁴⁴, and Seifi et al.⁴⁵. They observed higher MVD in OKCs compared with DCs. So, the increase in angiogenesis in OKC compared to dentigerous cyst can indicate higher tissue metabolism in OKC and may indicate more aggressive biologic behavior in this cyst. Some authors stated that it might be possible to control the aggressive behavior of OKC with anti-angiogenic treatments⁴⁴. Our result coincide also with Gadbaill et al.⁴⁶ and Alaeddini et al.¹³ reported that angiogenesis has an important role in the biologic behaviors of odontogenic keratocysts.

Our result contradict with that of De Andrade et al.⁴⁷ who showed that microvessel count was higher in dentigerous cyst than in OKCs and the differences in the biologic behavior of these lesions do not appear to be associated with the angiogenic index. This difference in the result might be attributed to the differences in methodology such as counting method and number of samples.

Although OKCs and DCs are developmental in origin, however, the microscopic examination of these cysts showed some inflammatory changes present in its wall³⁴. In the current work odontogenic cysts were compared for the presence of inflammation in their connective tissue wall. It was found that inflamed OKCs and DCs showed higher mean numbers of microvessels compared to their non-inflamed counterparts with statistically significant difference. This was in accordance with Kouhsoltani et al.⁴⁰ who thought that the inflammatory reactions increase angiogenic activity in odontogenic cysts. They stated that inflammatory cells are generally considered to be related to angiogenesis in any lesions.

From the time of their discovery by Paul Ehrlich, mast cells have been of interest because of their characteristic granules⁴⁸. Mast cells have already been reported to be associated with odontogenic cysts and tumors, especially radicular, dentigerous and odontogenic keratocyst^{16, 30, 49, 50}.

In the present study, mast cells were found in connective tissue wall of the studied cysts. This was in agreement with Anandani et al.⁵¹, who concluded that mast cells may play a role in the pathogenesis of odontogenic cysts as an elevated number of mast cells were found in the connective tissue capsule of all odontogenic cysts they studied.

In the present study, maximum infiltration of mast cells was distinguished in OKCs followed by RCs and then DCs. These results were in accordance with the results of Smith et al.⁵⁰, Anandani et al.⁵¹, Rodini et al.⁵² and Chatterjee et al.⁵³.

Chatterjee et al⁵³ stated that the greater concentration of mast cells in OKC than dentigerous and radicular cyst suggests an increased breakdown of its capsular matrix. This hypothesis was supported by the nonkeratinization of epithelium that has been shown in some areas of OKC were at places where MCs are predominant, which causes a transport of breakdown matrix products into the cystic lumen, and consequently can determine an elevated osmolality of the cystic fluid. This hypothesis partly explains the greater aggressiveness of OKC compared to other odontogenic cysts.

Contradicting our results, a study performed by Kouhsoltani et al.⁴⁰ using immunohistochemistry has found that MCs was higher in RCs followed by OKCs and finally DCs. This difference could be attributed to the mast cell anti-tryptase antibody that has been used as a marker for mast cell activation which also stains degranulated mast cells.

In 2010 a study accomplished by Debta P et al.⁵⁴ using toluidine blue staining showed that number of mast cells was more in radicular cyst in comparison OKC and followed by dentigerous cyst. This may be associated with more inflammation in the connective tissue stroma of radicular cyst of their studied group.

On comparing between inflamed and non inflamed types of dentigerous cyst and odontogenic keratocyst it was found that MCs were more frequently observed in OKC compared to DC, which indicate that mast cells may play a role in the aggressive behavior of OKCs.

In our work, inflamed OKCs and DCs showed higher mean numbers of MCs compared to non-inflamed counterparts, which indicate the involvement of MCs in the inflammatory

reactions in odontogenic cysts. From our results, there was statistically significant difference by comparing between inflamed and non-inflamed DC, which indicate that mast cells were involved mainly in inflammatory reactions of these cysts. On the other hand, by comparing between inflamed and non-inflamed OKC there was no statistically significant difference, which indicate that mast cells play an important role in its aggressive behavior as in case of OKCs there were large concentrations of mast cells not only detected in areas of significant inflammation, but also in areas where inflammation was absent.

The role of MCs in vasoinductive events has been investigated by many researchers and these cells have been reported to promote angiogenesis in many lesions^{55, 56, 57}. Our results revealed a positive correlation between the numbers of microvessel and MCs density. This demonstrates that the presence of blood vessels is accompanied by the presence of MCs and vice versa. These findings suggested that MCs may be associated with the angiogenesis and development of odontogenic cysts⁴⁰. Our findings also suggested that MCs contribute to blood vessel formation as previously reported by Crivellato et al.⁵⁸ They suggested that the proangiogenic property of MCs is attributed to their capacity of secreting molecules such as VEGF, FGF- 2, IL- 8, and TGFβ.

Conclusion: Increase in angiogenesis of OKC confirms that aggressive lesions require a larger number of blood vessels and highlights the concept of unique clinical behavior of this cyst. In OKCs and DCs cysts, the MVD is closely related to of the inflammatory infiltrate. Mast cells are imperative in the pathogenesis of odontogenic cysts. Mast cells have a role in promoting the growth of odontogenic cysts, particularly OKC.

References

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