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Evaluation of Microvessel Density in Odontogenic Keratocyst, Dentigerous Cyst and Radicular Cyst

Premkumar, Maya Ramesh, Mathew Jacob, B Sekar, K. Indrapriya Dharshini, Diana Prem

Department of Oral Pathology and Oral Microbiology, JKKN Dental College Salem, Vinayaka Missions Sankarachariyar Dental College (Deemed To Be University), Vinayaka Missions Research Foundation, Salem, Tamilnadu, India.

ABSTRACT

Introduction: Odontogenic cysts are encountered relatively common in dental practice. Based on the new WHO classification, odontogenic cysts are categorized into 2 main groups that imitate their pathogenesis. Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels and is a complex multi-stage process including degradation of the extracellular matrix, proliferation and migration of endothelial cells, capillary differentiation and anastomosis CD34 molecule is a cluster differentiation molecule present on certain cells within the human body.

Objectives: Hence; the present study was conducted for analyzing the immunohistochemical expression of CD34 in odontogenic keratocyst, Radicular cyst and Dentigerous cyst.

Methods: A total of 30 cases were included and were divided as follows: 10 cases of Periapical cyst, 10 cases of dentigerous cyst and 10 cases of Odontogenic cyst. The biopsy was obtained with the patient's consent under local anaesthesia. The specimens were immediately fixed in 10% neutral buffered formalin, processed and embedded in paraffin wax. Serial sections of4 µm were obtained from both the archival material and also from the cases. One group of sections was stained for routine haematoxylin and eosin examination to confirm the clinical diagnosis and also for comparison. The other group of sections was used for immunohistochemical study using mouse monoclonal Anti-vimentin antibody. The histomorphometric analysis of blood vessels was carried out by Moticplus software was attached with an Olympus BX41 microscope. The histomorphometric analysis of blood vessels was carried out by Image J software.

Results: In the periapical cyst mean MVD was found to be 43.40 ± 6.851. Among the dentigerous cyst, mean MVD was 38.40 ± 17.141. P-value was found to be 0.30 found to be significant. In the odontogenickerato cyst mean MVD was found to be 57.20±27.59. Among the dentigerous cyst mean MVD was 38.40 ± 17.141. The mean difference in MVD between periapical cyst and odontogenickerato cyst was 18.800.

Conclusion: CD34 expression act as an important role in determining the microvessel density of odontogenic cysts and it might relate to the invasive growth of the odonto genickeratocysts for its clinical aggressive behaviour.

Key Words: Cyst, Dentigerous, Keratocyst, Microvessel density, Odontogenic, Radicular cyst

INTRODUCTION

A cyst according to Kramer 1974 is defined as "a pathological cavity which may or may not is lined by epithelium and is filled with solid, semisolid or gaseous material but not pus". Most cysts in the jaw, with rare exceptions, are epithelial lined cysts and usually derived from odontogenic apparatus and remnants. These odontogenic cysts are encountered relatively common in dental practice. Based on the new WHO classification, odontogenic cysts are categorized into 2 main groups that imitate their pathogenesis. These are inflammatory cysts, such as radicular cysts, and developmental cysts, such as dentigerous cyst and odontogenic keratocyst.[1-3]

Generally, odontogenic cysts show sluggish growth and have an affinity towards expansion. Despite their benign biological behaviour, these cysts can reach a significant size, if not diagnosed on time and treated properly. The commonly encountered odontogenic cysts in all diagnostic oral pathology departments around the world include radicular cysts, dentigerous cysts, and odontogenic keratocyst. Radicular cysts are the quite common cysts of the jaw, which have been cat-

Corresponding Author:
Dr. Premkumar, Department of Oral Pathology and Oral Microbiology, Vinayaka Missions Sankarachariyar Dental College, Sankakiri, Main road, Salem-636308, Tamilnadu - 638183, India; E-mail: premkumartamil@gmail.com

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egorized as inflammatory cysts originating from epithelial cell rests of Malassez, secondary to pulpal necrosis. Dentigerous cysts enclose the crown of unerupted teeth and are attached to the neck of the teeth, it is believed to develop from a tooth follicle. Odontogenickeratocysts are clinically aggressive cystic lesions assumed to originate from dental lamina or its remnants. The most characteristic clinical aspect of keratocyst is its high frequency of recurrence.[4]

Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels and is a complex multi-stage process including degradation of the extracellular matrix, proliferation and migration of endothelial cells, capillary differentiation and anastomosis CD34 molecule is a cluster differentiation molecule present on certain cells within the human body.[5]

Cells expressing CD34 (CD34+cell) are normally found in the subset of mesenchymal stem cells, endothelial progenitor cells, umbilical cord and bone marrow as hematopoietic cells, normally shows stronger staining with endothelial cells. However, some researchers believe that CD34 cannot be used to distinguish between the newly formed blood vessels and the old host ones.[6-8]

Hence; the present study was conducted for analyzing the immunohistochemical expression of CD34 in odontogenic keratocyst, Radicular cyst and Dentigerous cyst.

MATERIALS AND METHODS

The present study was conducted in the Department of Oral Pathology and Oral Microbiology for assessing the immunohistochemical expression of CD34 in odontogenic keratocyst, Radicular cyst and Dentigerous cyst. A total of 30 cases were included and were divided as follows:

1) 10 cases of Periapical cyst
2) 10 cases of Dentigerous cyst
3) 10 cases of Odontogenic cyst

The biopsy was obtained with the patient's consent under local anaesthesia. The specimens were immediately fixed in 10% neutral buffered formalin, processed and embedded in paraffin wax. Serial sections of4 µm were obtained from both the archival material and also from the cases. One group of sections was stained for routine haematoxylin and eosin examination to confirm the clinical diagnosis and also for comparison. The other group of sections was used for immunohistochemical study using mouse monoclonal Anti-vimentin antibody.

Staining techniques with immunohistochemical (IHC) means allow for the conception of antigens via the sequential presentation of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody and an enzyme complex with a chromogenic substrate with interposed washing steps. The enzymatic initiation of the chromogen results in an evident reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and help in the differential diagnosis of pathophysiological courses, which may or may not be related to a specific antigen.

Specimen Preparation

Routine processing of all the specimens was done with the help of alcohol, xylene etc and finally embedded in paraffin. Sectioning was done using rotary microtome and sections of 4 microns thick were cut and carefully fixed on the positively charged poly-L-Lysine coated microscopic slides. The sections were dried at room temperature (37°C) followed by 1-hour incubation at 60°C. The tissue was de-paraffinized by giving 2 dips lasting 10 minutes each in fresh xylene. Rehydration of tissue was carried out by giving 2 dips for 10 minutes each in absolute alcohol and placed in the distilled water bath and not allowed to dry. The histomorphometric analysis of blood vessels was carried out by Moticplus software was attached with an Olympus BX41 microscope. The histomorphometric analysis of blood vessels was carried out by Image J software.

RESULTS

The microvessel density (MVD) was compared between the dentigerous cyst and periapical cyst. In the periapical cyst mean MVD was found to be 43.40 ± 6.851 and the S.D error of the mean was found to be 2.166. Among the dentigerous cyst, mean MVD was 38.40 ± 17.141 and S.D error of the mean was 5.421. The mean difference in MVD between the periapical cyst and dentigerous cyst 5.000, CI of mean difference was -7.264 to 17.264. Based on the student 't' test was 0.857 (Table 1, 1a). P-value was found to be 0.30 found to be significant.

The MVD was compared between the dentigerous cyst and odontogeni-ckerato cysts. In the odontogeni- ckerato cyst mean MVD was found to be 57.20±27.595 and the S.D error of the mean was found to be 8.726. Among the dentigerous cyst mean MVD was 38.40 ± 17.141 and S.D error of the mean was 5.421. The mean difference in MVD between a periapical cyst and odontogeni-ckerato cyst was 18.800. CI of mean difference was -2.78 to 40.383. Based on the student 't' test was 1.830. P-value found to be 0.49 is found to be significant (Table 2).

The MVD was compared between the periapical cyst and odontogeni-ckerato cyst. In the odontogeni-ckerato cyst mean MVD was found to be 57.20 ± 27.595 and theS.D error of the mean was found to be 8.726. Among the periapical cyst mean MVD was 43.40 ±6.851 and S.D error of the mean

was 2.166. The mean difference in MVD between the periapical cyst and odontogenickerato cyst 13.800. CI of mean difference was -5.090 to 6.206, based upon the student 't' test is 1.535. P-value was found to be 0.44 is found to be significant (Table 3, 4).

The MVD was compared between a dentigerous cyst, periapical cyst and odontogeni-ckerato cyst. In the dentigerous cyst, mean MVD was found to be 38.40 ± 17.141 and the S.D error of the mean was 5.421. In the periapical cyst, mean MVD was 43.40 ± 6.851 and the S.D error of mean was2.166. In the odontogeni-ckerato cyst the mean MVD was 57.20 ± 27.595 and S.D error of mean 8.726. Comparison between the three cysts, based on ANOVA test. F-value was found to be 2.581. P-value was found to be 0.47 (Table 5).

DISCUSSION

In the current study of microvessel density in odontogeni-ckeratocyst, radicular cyst and dentigerous cyst it was seen that microvessel density of odontogeni ckeratocyst was higher than radicular and dentigerous cyst which is a form suggests higher angiogenesis in odontogeni ckeratocyst lesion with higher aggressive behaviour, in comparison to the periapical and dentigerous cyst. Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels and in cancer, which is a complex multi-stage process including degradation of the extracellular matrix, migration and proliferation of endothelial cells, anastomosis and capillary differentiation. Some research has been done on angiogenesis in melanoma, squamous cell carcinoma, and salivary gland tumours. Different markers like CD34, CD31, CD105 and antibodies like VEGF, Bfgf, Tek2 are used to measure microvessel density in each microscopic field. In comparison to antibodies, the markers are more practical for the measurement of microvessel density.[9-13]

CD34 staining is stronger with a lower error rate in comparison to CD31. Sialomucin CD34 is a cell surface 110-120 KD monomeric glycoprotein and is a pan endothelial marker of endothelial cells and generally displays stronger staining with endothelial cells. However, some researchers believe that CD34 cannot be used to distinguish between the newly formed blood vessels and the old host ones but CD34 has the main role in the evaluation of microvessels density in tumours. Until now, few studies have reported on the role of angiogenesis in odontogenic lesions and little has been known on the role of angiogenesis in odontogenic cysts and tumors.[14,15]

In our study, we measured the microvessel density and its role on the clinical behaviour of odontogenic lesions and to determine whether microvessel density has a more prominent role in clinical behaviour of odontogenic lesions. In the study, we used 30 cases of odontogenic cysts which included 10 cases of odontogeni ckeratocysts, 10 cases of the radicular cyst, 10 cases of periapical cysts. Sections were stained with CD34 and assessed for the microvessel density in connective tissue wall of cysts. Different studies have reported the effect of matrix metalloproteinase expression and its inducers in the connective tissue of follicular cyst. However, it has been reported to have a higher expression in keratocyst and dentigerous cyst. Meanwhile, some previous studies have reported a positive relationship between matrix metalloproteinases inducers and the vascular density. The presence of matrix metalloproteinases is essential for angiogenesis, and its higher expression in odontogeni-ckeratocyst may be involved in a higher rate of angiogenesis and its greater clinical invasive behaviour.[16]

In the current study, the microvessel density of keratocystic Odontogenic tumour was higher than follicular cyst which explains higher recurrence rate and aggressive behaviour of keratocysti-codontogenic tumor in comparison to the follicular cyst. Also, it is suggested that higher need of tumoral tissue in odontogeni-ckeratocystto nutritional substances and oxygen than follicular cyst was seen. Gadbil et al. studied the relationship between the proliferative activity of epithelial cells in keratocysti-codontogenictumor and follicular cyst and normal oral mucosa with angiogenesis using CD 105 marker. They reported higher CD 105 expression in the keratocysti-codontogenic tumor in comparison to dentigerous cysts and concluded that keratocysti-codontogenic tumour stroma was involved in the observed neoplastic behaviour.[19] The results of study Gadbi et al. are similar to our result. But periapical cysts are included in our study along with keratocysts and follicular cysts.[17-19]

Although the number of blood vessels is higher in multicystic Camelo blastoma, they are smaller in size and anastomosis was seen. In tumours, the number of blood vessels seemed bigger. However, it is superior to use histomorphometry technique to assess the vessel area and diameter of blood vessels to assess the role of vessel area effect in aggressive behaviour of odontogenic lesions. However, Gadbil et al. reported a bigger vessel area in keratocystic Odontogenic tumour in comparison to dentigerous cysts.[19]

When selecting the paraffin blocks of the follicular cyst, keratocystic-odontogenic tumors and periapical cyst, the samples with severe inflammation were excluded from the study because inflammation can affect the microvessel density. However, in the periapical cyst, there were areas of stromal inflammation with a focal increase of microvessel density. This may be because inflammatory cells need nutritional substances for their activities. by Graziani et al assessed the association between inflammation and angiogenesis in radicular cysts using VEGF antibody. They presented an upsurge in microvessel density with an increase in inflammation.[20-22]

The result of the above-mentioned study is similar to the present study in which the periapical cyst which has more blood vessels when compared to the follicular cyst. So the microvessel density is higher in periapical cyst when compared to the dentigerous cyst.

CONCLUSION

In the current study, microvessel density in odontogeni ckeratocyst, was higher than radicular and dentigerous cyst which suggests higher angiogenesis in odontogeni ckeratocyst lesion with higher aggressive behaviour. The authors concluded that CD34 expression act as an important role in determining the microvessel density of odontogenic cysts and it might relate to the invasive growth of the odontogeni ckeratocysts for its clinical aggressive behaviour.

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Authors contribution

1. Dr. Premkumar- Investigation
2. Dr. Maya Ramesh - Investigation
3. Dr. Mathew Jacob - Data collection
4. Dr. B Sekar - Manuscript Preparation
5. Dr. K. Indrapridharshini- Editing
6. Dr. Diana Prem- Statistical Analysis

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Table 1: Comparison of sex ratio between OKC, Periapical cyst and Dentigerous cyst

	Group	OKC	Periapical Cyst	Dentigerous cyst	Total	Chi-Square Value	P-Value
Sex	Male	6	6	5	17	.271	0.873
	Female	4	4	5	13		

Table 1 (a): Comparison of sex ratio between OKC, Periapical cyst and Dentigerous cyst

Group	Mean	F Value	P-Value
OKC	32.4		
Periapical Cyst	27	0.618	0.546^{NS}
Dentigerous cyst	28.8		

Table 2: Comparison of mean microvessel density (MVD) between the periapical cyst and dentigerous cyst

Group	Mean±SD	Std Error of Mean	Mean difference	CI of Mean Difference	Test value	P-value
Periapical Cyst	43.40±6.851	2.166	5.000	-7.264 to 17.264	0.857	0.030*
Dentigerous Cyst	38.40±17.141	5.421				

Table 3: Comparison of mean microvessel density between OKC and dentigerous cyst

Group	Mean±SD	Std Error of Mean	Mean difference	CI of Mean Difference	Test value	P-value
OKC	57.20±27.595	8.726	18.800	-2.783to 40.383	1.830	0.049*
Dentigerous cyst	38.40±17.141	5.421				

Table 4: Comparison of mean microvessel density between OKC and Periapical cyst

Group	Mean±SD	Std Error of Mean	Mean difference	CI of Mean Difference	Test value	P-value
OKC	57.20±27.595	8.726	13.800	-5.090 to 6.206	1.535	0.044*
Periapical Cyst	43.40±6.851	2.166				

Table 5: Comparison of mean microvessel density between OKC, Periapical cyst and dentigerous cyst

Group	Mean ± SD	Std Error of Mean	F value	P-value
OKC	57.20 ± 27.595	8.726		
Periapical Cyst	43.40 ± 6.851	2.166	2.581	0.047*
Dentigerous cyst	38.40 ± 17.141	5.421		