

EXPRESSION OF CD34 IN AMELOBLASTOMA AND ODONTOGENIC KERATOCYST

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ABSTRACT

Objectives: To evaluate the expression of CD34 in Ameloblastoma and Odontogenic keratocyst (OKC) in various age groups and to determine the difference in the biological behavior of the lesions according to CD 34 expression.

Materials and Methods: This was a comparative cross sectional study of total 80 cases, 40 each of ameloblastoma and OKC which were retrieved from histopathology lab of Peshawar Medical College, Peshawar (PMC) and Pakistan Institute of Medical Sciences, Islamabad (PIMS). The study was conducted at the laboratory of Peshawar Medical College & Peshawar dental college. Mean vascular density was determined. A descriptive analysis was done using SPSS version 21.

Results: Mean microvessel density was significantly higher in ameloblastoma as compared to odontogenic keratocyst. Relationship between CD34 expression in ameloblastoma and odontogenic keratocyst was statistically significant (p-value 0.01).

Conclusion: This study concludes that CD34 expression is higher in ameloblastoma than odontogenic keratocyst. This is suggestive of more aggressive behavior of ameloblastoma as compared to odontogenic keratocyst and treatment may be modified according to CD34 evaluation. This study also concludes that in case of Ameloblastoma CD34 expression is dependent on different age groups.

Key words: Ameloblastoma, Odontogenic keratocyst, CD34, Mean Vascular Density (MVD), Angiogenesis

INTRODUCTION

Ameloblastoma is a locally invasive benign tumor which makes up less than 1% of head and neck tumors and has a tendency for malignant transformation^{1,2}. It has predilection for male with occurrence of majority of cases (80%) in mandible².

Odontogenic keratocyst (OKC) is a developmental cyst with a high recurrence rate. It resembles Ameloblastoma in its aggressive behavior³. It is the third most prevalent odontogenic cyst^{4,5}. Male predominance and peak incidence in the third decade of life is documented with an age range of 8 to 82 years⁶.

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Role of angiogenesis in inflammatory and neoplastic disorders, depicting behavior of these lesions is well known. The number, cross-sectional area and diameter of the vessels are determined according to the activity and metabolic needs of the lesion and is used to measure angiogenesis.

Mean vascular density which is measure of angiogenesis can predict the growth of the tumor, metastasis and patient's survival and this value is related to the aggressiveness of the tumor. Since neoplastic tissues require oxygen and nutrients to continue their growth and development, they induce neovascularization. The growth of the tumor also depends on protein molecules expressed in endothelial cells in addition to the increase in the number of blood vessels⁷.

Number of tumor markers such as CD34,

CD105, CD10, CD31 and Ki67 are used for detection of angiogenesis in different cysts and tumors and hence for prognostic evaluation and selection of treatment modalities for ameloblastoma and OKC. Among all CD34 has been found to be the most potent and highly specific for aggressiveness in terms of angiogenesis. It is a transmembrane glycoprotein which shows high specificity for endothelial cells of blood vessels⁸.

Numerous studies have focused the expression of RANK, RANKL, and OPG in odontogenic diseases, such as ameloblastoma and the role of regulatory osteoclast in the development of odontogenic lesions. According to Silva et al, ameloblastoma has higher levels of RANK and RANKL expression compared to odontogenic keratocysts. This finding strengthens the observation that ameloblastoma has high recurrence rate and a higher level of bone invasiveness than odontogenic keratocysts⁹.

The present study is designed to compare the biological behavior in terms of angiogenesis (aggressiveness) of the two lesions which may lead to better management according to their behavior. The treatment may include follow up of all those cases which are highly positive for CD34 expression.

MATERIALS AND METHODS

This was a comparative cross sectional study. The study consisted of already diagnosed formalin fixed paraffin embedded tissue sections of 40 ameloblastoma and 40 odontogenic keratocyst of both genders. Incisional and excisional biopsies of ameloblastoma and OKC were collected from the histopathology labs of Peshawar Medical College & Pakistan Institute of Medical Sciences (PIMS). Samples with excessive hemorrhage and insufficient tissue were excluded from the study.

After microscopic reconfirmation of all the cases, slides showing maximum vessels from each case of ameloblastoma and OKC were selected for immunohistochemistry (IHC). At least three slides from each selected case were prepared. Six slides (3+3) from representative blocks, each of 4-5 microns thin sections were made. One slide of each case was stained with H&E and one stored for IHC and one was kept as a reserve.

Slides for IHC were divided into 8 batches. Capillary hemangioma (pyogenic granuloma) was taken

as positive control. One slide of positive control was kept for each batch. Immunohistochemistry was carried out for CD34 with DAKO Envision system monoclonal antibody (CD34 clone: M7165 A/S, Glostrup, and DAKO, Denmark).

Mean vascular density (MVD) was assessed by evaluating the stained slides by the method proposed by Hosseini et al (9). Four spots showing maximum vascularization (known as the hot spot) were selected under low magnification. At ($\times 40$) magnification microvessel density was counted in each spot. The mean number of blood vessels in the four selected regions were considered as the MVD. Cases were then scored as:

- Score 1+ = Low (0- 19.9),
- Score 2+ = Moderate (20-29.9)
- Score 3+ = High (More Than 40).

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 21. For continuous variable such as age, the findings were presented as mean and standard deviation. To compare age groups with expressions, Fisher exact test was applied. Independent T test was applied to compare mean values of independent variables.

RESULT

Ameloblastoma patients ranged in age from 12 to 80, with a mean age of 36.13 ± 12.8 . The mean age in OKC was 34.15 ± 12.5 with a range of 14 to 64 years. Age groups of 21 to 40 years had the highest number of patients diagnosed with ameloblastoma and OKC followed by 41-60 years age group (Table 1).

Low MVD was found in 2.5% cases of age group of ≤ 20 years. There was no case of moderate MVD while 5% of cases showed high MVD in this age group. The age group 21-40 years showed 17.5% cases of low, 20% cases of moderate and 7.5% cases of high MVD. In the age range of 41 to 60 years, 3% of cases were low, 25% were moderate, and 10% were of high MVD. No case of low MVD was found in the age group of 61+ years. However, 2.5% of cases in the same age group were found in both the moderate and high MVD categories. High MVD score was also identified in 2.5% cases of not specified age group. Relationship between CD34 expression in ameloblastoma and age groups was statistically significant (p-value 0.004) (Table 2).

The age group of ≤20 years showed 5% cases of low, 5% cases of moderate and 2.5% cases of high MVD while 22.5% cases of low, 17.5% cases of moderate and 5% cases of high MVD was observed in the age range of 21-40 years. There were 12.5% cases of low, moderate and high MVD in the age group of 41 to 60 years. In the age group of 61+ years there were 2.5% cases each of low and moderate and no case of high MVD. Expression of CD34 in OKC revealed insignificant relationship with age groups (p-value 0.3) (Table 3).

Expression of CD34 was observed in all cases of ameloblastoma. Low MVD was found in 11 cases

Table 1: Age distribution in ameloblastoma and odontogenic keratocyst cases

Age	Ameloblastoma	Odontogenic keratocyst
≤20	03(7.5%)	04(10%)
21-40	18(45%)	20(50%)
41-60	17(42.5%)	13(32.5%)
61+	02(05%)	02(05%)
Not specified	-	01(2.5%)
Total	40(100%)	40(100%)

Categorical data: Fischer Exact test applied

(27.5%), moderate in 19 cases (47.5%) and high in 10 cases (25%). Among all cases of OKC, 17 cases (42.5%) exhibited low, 15 cases (37.5%) moderate and 08 cases (20%) high expression of CD34 (Table 4).

Mean MVD for ameloblastoma was 26.71±12.84 with 3 and 68 as lowest and highest values and 20.90±12.61 for OKC with 0 and 48 as their minimum and maximum values. Relationship between CD34 expression in ameloblastoma and OKC was statistically significant (p-value 0.01) and is shown in table 5.

DISCUSSION

The present study consisted of total 80 cases of ameloblastoma and OKC (40 each). The study found

Table 4: Expression of CD34 in Ameloblastoma and OKC

CD34 expression	Ameloblastoma	Odontogenic Keratocyst
Low	11(27.5%)	17(42.5%)
Moderate	19(47.5%)	15(37.5%)
High	10(25%)	08(20%)
Total	40(100%)	40(100%)

Categorical data: Fischer Exact test applied

Table 2: Expression of CD34 in Ameloblastoma with Respect to Age Groups

Age	MVD SCORE				P value
	Low	Moderate	High	Total	
≤20	01(2.5%)	-	02(05%)	03(7.5%)	0.004*
21-40	07(17.5%)	08(20%)	03(7.5%)	18(45%)	
41-60	03(7.5%)	10(25%)	04(10%)	17(42.5%)	
61+	-	01(2.5%)	01(2.5%)	02(05%)	
Total	11(27.5%)	19(49.5%)	10(25%)	40(100%)	

Categorical data: Fischer Exact test applied

Table 3: Expression of CD34 in OKC with Respect to Age Groups

Age	MVD SCORE				P value
	Low	Moderate	High	Total	
≤20	02(05%)	02(05%)	01(2.5%)	05(12.5%)	0.39
21-40	09(22.5%)	07(17.5%)	02(05%)	18(45%)	
41-60	05(12.5%)	05(12.5%)	05(12.5%)	15(37.5%)	
61+	01(2.5%)	01(2.5%)	-	05(12.5%)	
Total	17(42.5%)	15(37.5%)	08(20%)	40(100%)	

Categorical data: Fischer Exact test applied

Table 5: Comparison of CD 34 expression in Ameloblastoma and odontogenic Keratocyst cases

	N	Minimum	Maximum	Mean	Standard deviation	p-value
MVD AM	40	3	68	26.72	12.844	0.01*
MVD OKC	40	0	48	20.90	12.767	

Numerical data: Independent t-test applied

second and third decades of life to be the most common age groups for developing ameloblastoma and OKC, with an average age of 36.13 and 34.15 years. The findings corroborated other previously published research^{4,10,11}. The present study found significant relationship of CD 34 expressions in Ameloblastoma but not in OKC with different age groups.

In the present study, significant difference between MVD of ameloblastoma and OKC (Table 5) suggests high aggressive nature of ameloblastoma as compared to OKC. This is similar to available literature where higher angiogenesis has been reported in ameloblastoma as compared to OKC which might reflect higher tissue metabolism, more aggressive biologic behavior, greater recurrence, growth rate and cellular metastasis¹². Alaeddini et al evaluated and compared angiogenesis in odontogenic keratocyst, dentigerous cysts and ameloblastoma using a monoclonal antibody against CD34 and found a statistically significant difference in the mean MVD showing maximum score in ameloblastoma followed by OKC¹³. Kumamoto et al reported same results but the methodology was different as they used VEGF to measure MVD. Results of the current study are not in

agreement with the study of Gadbail et al as he could not find difference in MVD of ameloblastoma and OKC. This contrast in the results may be explained on the basis of genetic variability and difference in methodology (CD105 was used)¹⁴.

Number of treatment options are available to treat both these aggressive lesions, each having its own drawback which make recurrence for these lesions unavoidable; however this risk can be reduced if not completely avoided by carrying out proper initial treatment. Based on molecular studies, targeting the SMO receptor and suppressing the SHH signalling cascade (essential for vasculogenesis and angiogenesis) has been suggested for treatment of the lesions showing high expression of CD34 as aggressiveness is sturdily related to proliferation of epithelial cells. Similar studies on pancreatic cancers shows reduction of pancreatic cell growth by inhibiting SHH signaling pathway which supports novelty or modification in the treatment approach of odontogenic lesions (AM & OKC). This may include assesment of MVD and hence aggressiveness in terms of angiogenesis along with post operative follow-up^{15,16}.

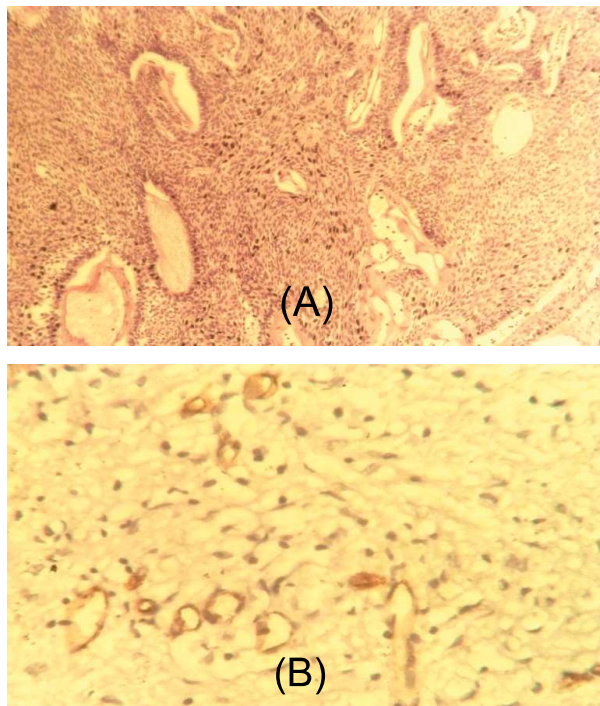


Fig 1: Photomicrograph of Ameloblastoma with H&E staining (A) and immunohistochemical staining (B) with CD34 of moderate MVD in blood vessels (magnification x 100)

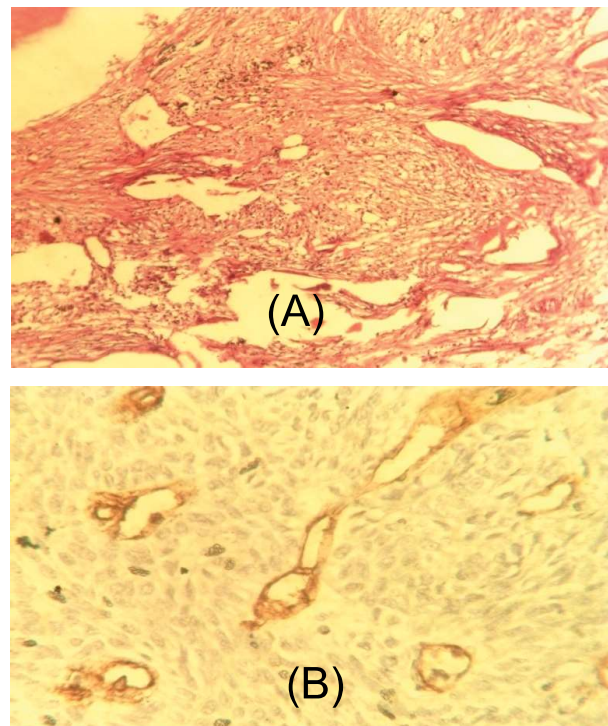


Fig 2: Photomicrograph of OKC with H&E staining (A) and immunohistochemical staining (B) with CD34 of moderate MVD in blood vessels (magnification x 100).

CONCLUSION


This study concludes that CD34 is expressed more in ameloblastoma than OKC. The statistically substantial difference in their expression indicates that ameloblastoma is more aggressive than OKC and treatment may be modified accordingly.

RECOMMENDATIONS

It is recommended that ameloblastomas and OKCs should be evaluated for CD34 expression with immunohistochemical method for prediction of their biological behavior, prognosis and management of patients.

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