CD10 EXPRESSION IN AMELOBLASTOMA VARIANTS

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ABSTRACT

Although benign ameloblastomas tend to behave aggressively with a high rate of recurrence. CD10 expression can help in predicting the behavior of these tumors. The aim of this study was to determine CD10 expression in variants of ameloblastomas. The data of thirty already diagnosed cases of ameloblastomas were taken. They were examined microscopically for selecting the sections with maximum epithelial content for immunohistochemical staining for CD10. A semi-quantitative scoring for determining the expression was used.

In the present study, the follicular variant of ameloblastoma was the commonest (76.7%) followed by unicystic variant (13.3 %) and plexiform variant (10 %). In the epithelial component CD10 expression was strongly positive in (76.2 %) and (80 %) showed moderate positivity. All the three cases of plexiform variant showed strong positivity. In unicystic variant (9.5%) were strongly positive and (20 %) were moderately positive. In the stromal component 50 % cases of follicular variant were strongly positive and 85.7 % were moderately positive. In the plexiform variant 25 % were strongly and 14.3 were moderately positive. In the unicystic variant strong CD10 positivity was seen in 25 % of cases. The present study concludes that all variants of ameloblastomas express CD10 positivity which may indicate their biological behavior.

Key words: *Ameloblastoma, CD 10, Follicular ameloblastoma, Unicystic ameloblastoma, Plexiform ameloblastoma*

INTRODUCTION

Ameloblastoma is a benign but locally aggressive tumor with a high tendency to recur.[1,2] Globally ameloblastoma is the most common neoplasm of odontogenic origin with worldwide incidence of about 2.41 per one million populations.[3] Recently a Karachi based Pakistani study of tumors of odontogenic origin revealed that 24.8 % of all the tumors included in the study were ameloblastomas.[4]

Ameloblastoma commonly occurs in the posterior mandible (80%) and to a lesser extent in the posterior maxilla (20%).[5,6] It usually affects adults in the 4th - 5th decades of life.[7] The etiology is not known.[8] It may arise from rests of dental lamina, from a developing enamel organ, from the epithelial lining of an odontogenic cyst, or from the basal cells of the oral mucosa.[9]

According to WHO ameloblastomas are classified into solid/multicystic, extra osseous/peripheral, desmoplastic and unicystic variants. The multicystic variant histologically is further classified into follicular and plexiform type. The follicular type is subdivided into spindle, acanthomatous, granular and basal types.[10]

CD10 is 90-110 kDa transmembrane enzymatic glycoprotein. It is also known as neprilysin, common acute lymphoblastic leukemia/lymphoma antigen (CALLA) and neutral endopeptidase (NEP). CD10 causes cleavage and inactivation of neuropeptides and peptide hormones at the amino terminus to hydrophobic residues within the peptides sequences, leading to decrease cellular response to local peptide hormones.[11,12]

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It is also present on the surface of many other cell types such as lymphoid precursor cells, germinal center B lymphocytes, kidney and lung tissues and stromal myoepithelial cells of normal breast tissue.[13,14] The role of CD10 is vital in tumor stromal reactions originating from these tissues. Its expression in stromal cells of these tumors is associated with biological aggressiveness.[15,16]

The aim of the present study was to compare the expression of CD10 in variants of ameloblastoma to explore the possible utility of marker for proper diagnosis, selection of modality of treatment, evaluation of prognosis and long term follow up.

MATERIALS AND METHODS

The study consisted of already diagnosed, formalin fixed, paraffin embedded (FFPE) tissue sections of thirty cases of ameloblastoma of different age and sex groups. Non-probability consecutive sampling technique was acquired for retrieval of cases from Department of Pathology, Peshawar Medical College, City Medical Laboratory, Peshawar and Pakistan Institute of Medical Sciences, Islamabad in 06 months from August 2016 to February 2017.

Hematoxylin and eosin (H&E) stained slides of available blocks were reviewed. Diagnosis was reconfirmed and blocks with adequate tissue were selected for immunohistochemistry (IHC). Three slides with 4 to 5-micron thin sections were made. One was stained with H&E, another kept for immunohistochemistry and the third one kept as reserve. For control slides of tonsillar tissue were used.

Deparaffinization of FFPE before staining was carried out. Antigen retrieval was done by inserting slides in citrate buffer and then heating in microwave oven at 95-100° C for 20 minutes. Slides were allowed to cool at room temperature for 15-20 minutes. Then they were rinsed with distilled water and phosphate buffer saline (PBS). Peroxidase blocking solution was added to the sections of the slides incubated for 10 minutes at room temperature. Rinse in PBS for 6 minutes. Primary DAKO monoclonal antibody (CD10 clone 56C6) was applied to sections on the slides and incubated for 60 minutes in humidified chamber at room temperature. After 1 hour, the slides were washed again. Biotinylated secondary antibody was applied to the sections and incubated for 30 minutes at room temperature. Then rinsed in PBS for 6 minutes. Chromogen/substrate was applied, and sections were incubated in peroxidase substrate solution to reveal the color of the antibody.

Epithelial and stromal CD10 was scored according to the method described by Iwaya et al as follow; 0: equivalent to the negative control, 1: weak cytoplasmic stain, 2: moderate stain, 3: intense stain. The percentage of stained cells was also scored on a semi quantitative 4-point scale as: 0: < 10%, 1: 10-25%, 2: 25-50%; 3: >50%. Then, combining the score of staining intensity and percentage of stained cells a score of 0-1: negative, 2: +, 3-4: ++ and 5-6: +++. The score was evaluated by two histopathologists independent of each other.[17]

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 19. Statistical results were given as a mean and standard deviation for continuous variables was calculated. Chi square test was used to compare categorical variables for example gender, site and ameloblastoma variants with expression of marker. Fisher's exact test was applied where values were less than 5. Probability value of less than and equal to 0.05 (P ≤ 0.05) was considered statistically significant.

RESULTS

The study consisted of already diagnosed thirty cases of ameloblastoma. Male to female ratio was 1:1 (Table 1). The age range was 6 - 80 years with a mean age of 32.3 (S.D±17.1). Maximum patients were diagnosed with ameloblastoma in the age range of 21-40 years followed by 41-60 years (Figure 1). Follicular ameloblastoma (FA) was the most common (76.7%) followed by unicystic ameloblastoma (UA) 13.3% and plexiform ameloblastoma (PA) 10%. In epithelial cells CD10 marker showed strong positive expression in (76.2%) cases of FA and (80%) showed moderate positivity while in (9.5%) cases of UA CD10 was strongly positive and (20%) was moderately positive. All the 3 PA cases were strongly positive (100 %) for CD10 marker with a p-value ≥ 0.05 (Table 2). The stroma of (50%) cases of FA showed strong positivity while moderate positivity was seen in (85.7%) of follicular type. In the stromal component CD10 positivity was observed in (25%) cases of UA. The stromal component of PA (25%) was strongly positive and PA (14.3%) was moderately positive for CD10 in tumor tissue respectively with a p-value ≥ 0.05 (Table 3).

DISCUSSION

In the present study the commonest age group of occurrence of ameloblastoma was second and third de-
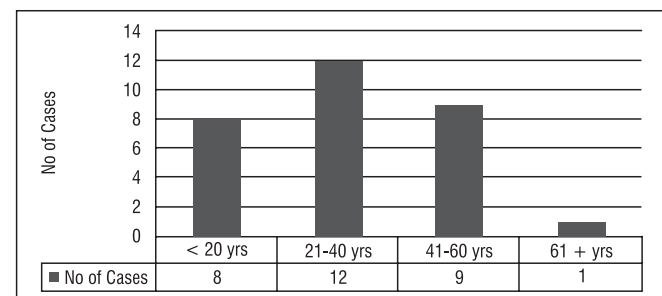

Fig 1: Age distribution in ameloblastoma cases

	< 20 yrs	21-40 yrs	41-60 yrs	61 + yrs
No of Cases	8	12	9	1

TABLE 1: FREQUENCY OF AMELOBLASTOMA ACCORDING TO GENDER AND SITE

Histological variants	Gender		Site			
	Male	Female	Maxilla	Mandible	Alveolar bone	Hard palate
Follicular (76.7%)	11(47.8%)	12(52.1%)	04(17.3%)	17(73.9%)	01(4.3%)	01(4.3%)
Unicystic 13.3%)	03 (75%)	01 (25%)	0.0	04(100%)	0.0	0.0
Plexiform (10%)	01(33.3%)	02(66.6%)	0.0	03(100%)	0.0	0.0

TABLE 2: EPITHELIAL CD10 EXPRESSION IN HISTOLOGICAL VARIANTS OF AMELOBLASTOMA

Histological Variants	Epithelial CD10 Expression				Total n (%)	p value
	Negative n (%)	Weak positive n (%)	Moderate positive n (%)	Strong positive n (%)		
Unicystic	01 (33.3)	0.0	01 (20)	02 (9.5)	04 (13.3)	
Follicular	02 (66.7)	01 (100)	04 (80)	16 (76.2)	23 (76.7)	≥ 0.05
Plexiform	0.0	0.0	0.0	03 (14.3)	03 (10)	
Total	03 (100)	01 (100)	05 (100)	21 (100)	30 (100)	

TABLE 3: STROMAL CD10 EXPRESSION IN HISTOLOGICAL VARIANTS OF AMELOBLASTOMA

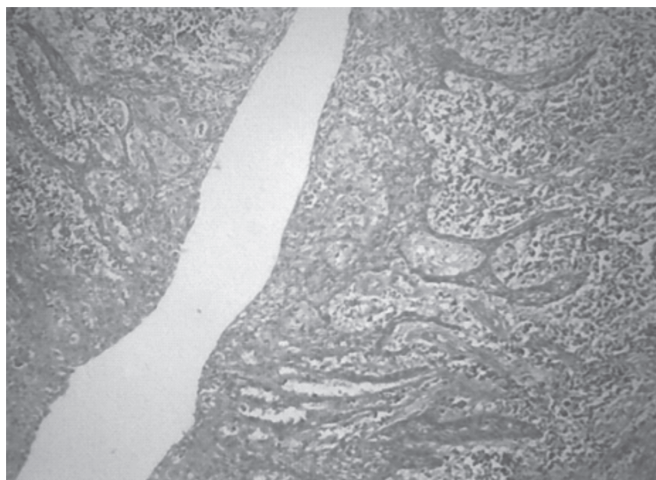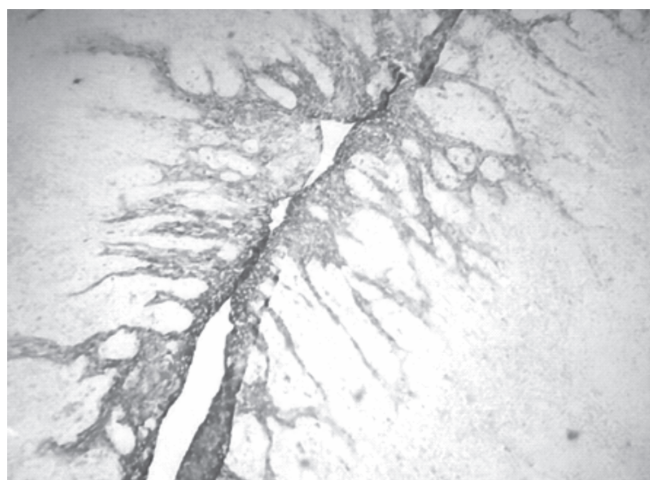| Histological Variants	Stromal CD10 Expression				Total n (%)	p value
	Negative n (%)	Weak positive n (%)	Moderate positive n (%)	Strong positive n (%)		
Unicystic	01 (11.1)	02 (20)	0.0	01 (25)	04 (13.3)	
Follicular	08 (88.9)	07 (70)	06 (85.7)	02 (50)	23 (76.7)	≥ 0.05
Plexiform	0.0	01 (10)	01 (14.3)	01 (25)	03 (10)	
Total	09 (100)	10 (100)	07 (100)	04 (100)	30 (100)	

Fig 2: Plexiform ameloblastoma (H&E-10x)

Fig 3: Plexiform ameloblastoma. CD10 immunoreactivity in epithelial and stromal cells (10x)

cades of life (40%) with a mean of 32.3 years and only one case above 60 years of age (Figure 1). The results were similar to other studies in literature.[18,19]

In our study the male to female ratio was 1:1. This is in correlation with another international study from Netherland.[20] However one of the recently published work from Karachi Pakistan shows male predominance.[4] This male predominance may be due to a large sample size and may reflect regional differences.

In this study mandible was the commonest primary site of ameloblastoma (80%) followed by maxilla (13%). There was an equal involvement (3.3%) of hard palate and alveolar bone. The results are comparable with other internationally published studies.[21]

In our study, FA was the most common histological variant (76.7%) followed by UA (13.3%) and PA (10%). These findings are consistent with most of the published data.[22,23] However in one of the study conducted in Thailand comprising of twenty six cases, PA was the most common type accounting for fifteen cases (57.6%) while other studies showed UA to be the commonest histological variant.[24,25] This might be due to variable geographical distribution, cultural and genetic differences.

According to our study three out of four of the UA revealed staining of epithelial cells for CD10. This finding is in agreement with the study conducted by Tadbir et al in Iran which observed weak to moderate staining of epithelial CD10 in twenty nine cases of UA.[15] However it is in contrast to the other study performed in Iran that concluded negative staining in epithelium of all UA samples.[26] This incongruity might be due to a very small sample size as only two cases of UA were included in that study.

Among MA sixteen out of twenty-three follicular variants and all three cases of PA showed strong CD10 positivity in epithelium. However, Tadbir concluded strong CD10 expression in all eleven cases of MA.[15]

In our cases twenty-three follicular variants of ameloblastoma two were found in association with dentigerous cyst. These two cases also showed strong positivity for epithelium in follicular part as compared to dentigerous cyst epithelium which was weakly positive. This indicates a possible neoplastic transformation of epithelial lining of the cyst into ameloblastoma. These results are in accordance with the study conducted by Masloub et al in Egypt.[27]

In stroma the present study showed that both unicystic and multicystic ameloblastomas were immunoreactive for CD10 marker. In most of the MA six out of twenty-three follicular variants was moderately positive while two out of twenty-three were strongly positive for CD10. In FA Ahlem concluded moderate positivity in six out of eighteen cases and strong positivity in one out of eighteen cases. In our study plexiform variant showed moderate positivity in one case while the other showed strong positivity. Whereas Ahlem observed intense positivity in only one case of PA and moderate positivity in two cases of PA.[28] These results are also consistent with the study reported by Iezzi et al in which positivity was calculated in 60 % of the cases while our study concluded positivity in 70% of the cases for CD10 in stromal cells. In our study only one case of UA showed strong stromal positivity which is in disparity to the work of Iezzi and Anjum. The above indicates the possibility of aggressive behavior of UA in our population. According to them UA were weakly positive for CD10 in the stroma.[29,30]

CONCLUSION

This study concludes that the commonest age for the prevalence of ameloblastoma is second and third decades of life with no gender predilection. Mandible was the most affected site. The most common histological variant in this study was follicular ameloblastoma.

It is concluded that irrespective of histological type, a positive CD10 expression was found in both epithelial and stromal components in majority of ameloblastomas included in our study. The strong CD10 positivity (75%) in all components of unicystic ameloblastoma points at its aggressive behavior. Based on our study it is recommended that all ameloblastomas should be evaluated for CD10 expression with immunohistochemical method for prediction of their biological behavior, assessment of prognosis and management of patients.

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3	**Muhammad Mumtaz Khan:**	Final approval of the version to be published.
4	**Nasiha Bashir:**	Critical review of the work.
5	**Naveed Sharif:**	Analysis and interpretation of the data.