Original Article

Immunohistochemical Expression of MMP-9 in Subtypes of Ameloblastoma

Immuno Expression of MMP-9 in Ameloblastoma

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ABSTRACT

Objective: To assess the immune expression of MMP-9 in ameloblastoma and its association with different histological subtypes of ameloblastoma.

Study Design: Analytical / cross-sectional study

Place and Duration of Study: This study was conducted at the Armed Forces Institute of Pathology, Rawalpindi (AFIP) from June 2016 till June 2017.

Materials and Methods: In this study, total of 60 cases of ameloblastoma were included. Parafinembedded blocks of patients of both genders, diagnosed with ameloblastoma were included as experimental samples while necrotic, scarce and autolyzed cases were not included. The tumor was sub-classified histologically on the basis of WHO classified and section were stained with H&E followed by (IHC) staining for MMP-9.SPSS version 20 was used to analyse the final result analyzed using chi-square test.

Results: Out of 60 cases of ameloblastoma, 32 were males and 28 were females. Mean age was 35.6 years with maximum 25 cases(83.3%) from mandible. On histopathological sub classification, 38 cases (63%) were diagnosed as follicular type, 14 cases(23.3%) were plexiform, 8 cases(13.3%) were acanthomatous type. All samples represent variable MMP-9 expression alongwith mild, moderate and strong intensity. Adjacent to the tumoral island invasive font was with highest reactivity. The strong immunoexpression, was seen in 75% acanthomatous and 57% plexiform, which was significantly different from follicular type with only 5.26%. Statistical test on MMP-9($P < 0.05$) provide highest reactivity in acanthomatous typealongwiththe presence of substantial differences within histological variant.

Conclusion: Our results proved the implication of MMP-9 in ameloblastoma growth and as a result their effectiveness in local aggressiveness monitoring in different subtypes of ameloblastoma.

Key Words: Ameloblastoma, MMP-9, odontogenic tumor.

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INTRODUCTION

Ameloblastoma is a benign, slow growing odontogenic tumor of epithelial origin. It exhibits an invasive and aggressive behavior showing unlimited growth invading the surrounding cancellous bone beyond the tumour margin, if left untreated[1,5]. Recommended treatment to avoid massive destruction and recurrence is wide range radical resection extending 1-2cm from the tumor margin. It constitutes 11% of all odontogenicneoplasma.

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Most cases of ameloblastomas are observed in patients between 30 to 40 years of age[1,2]. World Health Organization in 2005 classified ameloblastoma on basis of anatomical location into 1) Multicystic/ Conventional, 2) Peripheral/Extraosseous, 3)Unicystic types[2,3]. Multicytic is the most common type, exhibiting 91% of all cases. Histologically it is further subdivided into follicular, plexiform, acanthomatous, granular, basal cell and desmoplastic type.

Matrix Metalloproteinases (MMPs) belong to a family of structurally related zinc-dependent endopeptidases. They are collectively capable of degrading the basement membrane and nearly all other structural constituents of the Extracellular matrix (ECM) that appears to be critical in tumor cell invasion and metastasis[1,6]. It can control the tumor microenvironment by virtue of processing substrates, including growth factors and their receptors, chemokines, cell adhesion molecules, cytokines, angiogenic factors and apoptotic ligands[7,8]. The MMPs are formed as inactive precursors holding a propeptide and a secretory signal sequence. Proteolytic cleavage of this propeptide is essential for MMP activation. Carrying the label of enzyme, MMP-9 is encoded by MMP-9 gene in human[9]. It breaks down the type IV

collagen, a major component of the basement membrane in human tissues. This collagen allows the tumor cells to spread from the site of the primary tumor, resulting in invasion and metastasis[9,10].

The current study is designed to determine the immunohistochemical expression of Matrix Metalloproteinase-9 in histological subtypes of ameloblastoma for predicting the aggressiveness and behavior of this tumor in our population. Previous studies reveal that this marker directly degrades extracellular matrix(ECM) proteins and accelerates tissue

The rationale of this study is the prevalence of lack of consensus on the most appropriate treatment modality for ameloblastomas. This indicates the absence of evidence based immunohistochemical studies on the diagnosis and management of ameloblastomas. The proposed study was able to measure the aggressiveness, behavior and resulting deformities of this epithelial odontogenic tumor, the outcome of the study may be used in improving and customizing the management of the tumor.

MATERIALS AND METHODS

In this descriptive study thirty(60) paraffin embedded blocks of freshly diagnosed ameloblastomas at AFIP, Rawalpindi were collected along with their demographic data and clinical/ radiographical information. After confirmation of diagnosis, classification according to the World Health Organization (WHO)[2,3] and histopathological subtyping was done on freshly prepared slides. MMP-9 was applied on the tissue according to standard protocol.

Necrosed, scarce and poorly oriented tissues were excluded. The intensity of the stain was measured using criteria described in Immunohistochemical Staining and Scoring section. Final results were analyzed. Immunoreactivity was evaluated and its association with histopathological subtypes was carried out. Immunoreactivity of MMP-9 was evaluated on the criteria described by Alves Pereira[4] using a semi-quantitative analysis of immune stained cells using the following scores: 0 (without any reactivity in parenchymal component), 1 (<10% of positive cells), and 2 (>10% of positive cells).The cellular location of MMP-9 was determined in cytoplasm while for E-Cadherin, it was determined in cell membrane and cytoplasm

Cases showing positive expression were labeled normal while those exhibiting negative and reduced patterns were considered as altered, for statistical analysis. Association between different histological subtypes and MMP-9 was evaluated by Chi-square test. P value value ≥ 0.05 was taken as significant. SPSS version 20.0 was used to inspect the data collected on specifically designed proforma. Parameters like age, gender, site and histological subtypes were narrated by descriptive statistics.

RESULTS

In the present study clinicopathologically out of 60 patients, 32 were male and 28 were female. The mean age ranges from 34.63 +/_ 12.6 years. Most ameloblastomas were arising from right side of the jaw involving 34 cases (56.6%) while the left side was involved in 26 cases (43.3%).The frequency of the site involvement was recorded as 25 cases(83%) for mandible while maxilla was involved in 5 cases (17%). The most common histological type was follicular ameloblastoma 38 cases (63%), followed by plexiform 14 cases (24%) and 8 (13%) were of acanthomatous.

Table No.1: MMP-9 Expression in Different Subtypes of Ameloblastoma

MMP-9 Expression					
S. No	**Histological Sub types of Ameloblastoma**	**Score 0**	**Score 1**	**Score 2**	**Total**
1	Acanthomatous Ameloblastoma	0	2 (25%)	6 (75%)	8
2	PlexifromAmeloblastoma	2 (14.28%)	4 (28.75%)	8 (57%)	14
3	Follicular Ameloblastoma	20 (52.6%)	16 (42%)	2 (5.26%)	38
Total		11	11	8	30
P=0.0038 (significant)					

Immunostain reactivity was assessed by determination of percentage of positive stain cells under 40 x Objective Power with light microscope. The MMP-9 immuno reactivity was observed in a diffused pattern both in parenchymal and stromal cells, only parenchymal cells were observed. MMP-9 reactivity in these cells was found in the cytoplasm of stellate reticulum like angular cells and ameloblastic like columnus cells. Out of 60 cases of ameloblastoma, 38 cases were positively stained while 22 cases appeared negative. Amongst the positively stained, 22 cases scored 1 (36.7%) while 16 cases scored 2 (26.7%).

Expression of MMP-9was foundvariable in different subtypes of ameloblastoma (table-2). According to statistical analysis criteria proposed for MMP-9, amongst 8 cases of acanthomatous, 6 cases scored 2 (75%) as shown in fig-1 and only 2 case showed score 1 (25%).

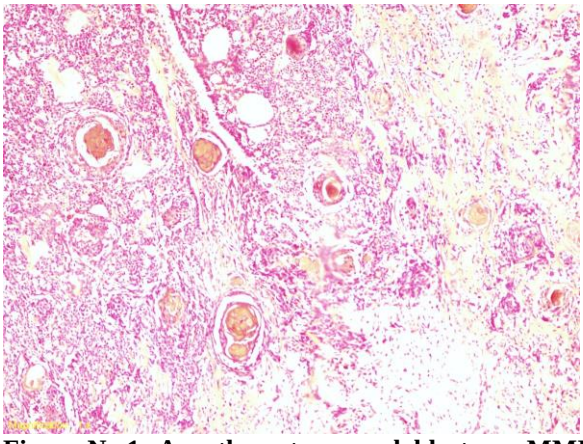

Figure No.1: Acanthomatous ameloblastoma MMP-9 (100x magnification) (Showing strong positive expression in the squamous metaplasia area)

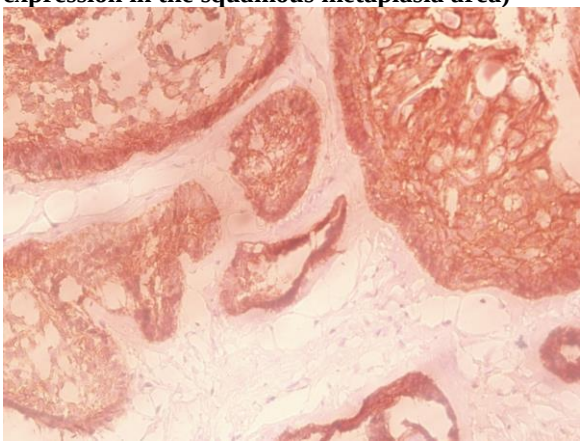

Figure No.2: Plexiform ameloblastoma MMP-9 (400x magnification) (Showing strong positive expression in stellate reticulum like cells and columnar cytoplasm)

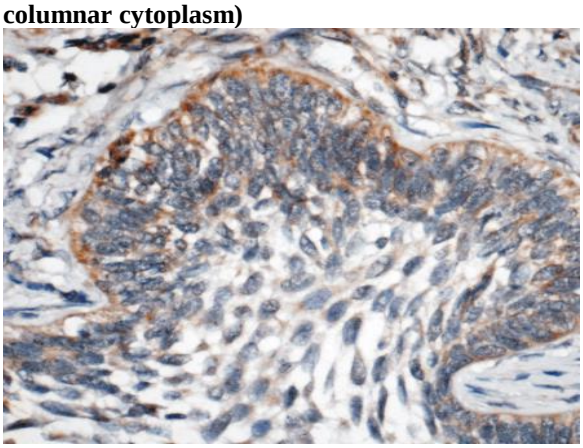

Figure 3: Follicular ameloblastoma MMP-9 stain (400x magnification) (Showing mild positive expression in columnar cells cytoplasm)

The plexiform was diagnosed in 14 cases out of which 2 case showed score 0 (14.28%), 4 cases scored 1 (28.5%) and 8 cases appeared with score 2 (57%) with MMP-9 immunostain as shown in fig-2. The follicular ameloblastomas was diagnosed in maximum cases that is 38in all out of which 20 cases appeared with score 0 (52%), 16 cases exhibited score 1 (42%) as shown in fig-3 and 2 case showed score 2 (5.2%) with MMP-9 immunostain.

DISCUSSION

Ameloblastomas are one of the benign epithelial odontogenic tumors arising of jaw bones. More commonly seen in mandible than in maxilla, these have the tendency to appear in the posterior part of the mandible. Clinically, ameloblastomas are divided into many subtypes which are multicystic, unicystic, peripheral ameloblastomas, malignant ameloblastomas and ameloblastic carcinoma represent unlike clinical behavior and prognosis[3,11].

MMP-9 also called as gelatinase B, a class of enzyme that belongs to zinc-metalloproteinases family and are proficientto degrading basement membrane and nearly entire the structural constituents of ECM. Therefore they are a subject of attention in research field regarding tumor invasion and metastasis.MMP-9 is thought to be found in activated and greater amount in and around the malignant tissue as compared to the benign or normal tissue with maximum expression found in areas of active invasion at the tumor stoma interface[5]. Researches in different parts of world have provided evidences of intrication of MMP-9 involved in tumor cell invasion and bone metastasis[7,8,9] suggested MMP-9 involvement in angiogenesis and tumor growth. According to Stankovic et al. (2010)[15], there was a significant positive association between the tumor size in relation to breast cancer and MMP-9expression. Jordan et al. (2004)[16] correlated the expression of aggressiveness MMP-9 with head and neck tumors. Osteodestructive pathologies are also proved by MMP-9[8] as it playsa vital role in bone resorption.

Several studies conducted to analyze expression of MMP-9 on ameloblastoma[6,8]. Kumamto et al. (2003)[6] has shown correlation between MMP-9 expression and ameloblastoma tumor growth. Pinheiro et al. (2004)[7] proved role of MMP-9 in tumor cell proliferation through the release of mitogens. Qian and Huang (2010)[5] suggested correlation between MMP-9 with osteoclastogenesis caused by ameloblastoma. Anne et al. (2014) and Florescu et al. (2012)[12,13] published detailed studies showing correlation between different histological subtypes of ameloblastoma with their aggressiveness and metastatic potentials.

Our study detected MMP-9 expression in 19 cases (63.3%) out of 30 cases while 11 cases (36.6%) exhibited negative expression. Furthermore, our study reveal presence of MMP-9 in both stromal neoplastic and epithelial compartments of the solid ameloblastoma. Neoplastic epithelium signifies presence of MMP-9 reactivity in both stellate-reticulum like cells and peripheral columnar cells. However, at level of squamous metaplasia areas peak expression

was noticed. Overall small rate and intensity of the MMP-9 positive neoplastic cells in the solid ameloblastomas were seen. This reactivity was detected far more prominent in stromal compartment, being highest expression registered around islands from the invasion front, especially in those cases which extended into the surrounding tissues.

This indicates harmonous result of Florescu et al. (2012)[12] that found 76.5% ameloblastoma with positive MMP-9 immunoreaction. Henriques et al. (2011)[14] also showed 95% positivity in ameloblastoma with MMP-9. However, the research study of Anne et al. (2014)[13] showed all specimen of ameloblastoma showing positive immunoreaction to MMP-9.

Our research study of MMP-9 analysis proved score 1(36.6%) in majority of cases indicating mild positivity, followed by score 2 (26.7%) showing strong positivity. Yoon et al. (2005)[17] also showed that ameloblastoma has mild to strong intensity of MMP-9 expression. The study outcome of Florescu et al. (2012)[12] proved majority cases of ameloblastoma with score 1 (47%), followed by the cases with score 2 (29.5%).Thus detection of constant expression in all specimens may lead to a presumption that MMP-9 plays an indispensable role in development and further progression of ameloblastoma and therefore, can be associated with bone resorption caused by this tumor as well.

Amongst the three variants in our study, lowest reactivity was found in follicular ameloblastoma had the, with score 2 in only 1 cases of follicular type. Highest reactivity in this study was noticed in acanthomatous type. Out of 4 cases, 3 cases (75%) showed score 2 with highest expression at the area of squamous metaplasia followed by plexiform type in which 4 cases (57%) out of 7 cases had score 2. Florescu et al. (2012)[12] observed Acanthomatous type with highest reactivity of MMP-9 at level of squamous metaplasia whereas Anne et al. (2014)[13] noted plexiform with high immune score than follicular type.

Maxillary ameloblastomasbeing thought as more aggressive and at times life threatening[15,16] as compared to mandibular ones. They also show high recurrence rate as compared to mandibular ameloblastoma[1,11]. Our study included 5 cases in maxilla and 25 cases in mandible. While evaluating the MMP-9 expression it was noticed that tumors in maxilla showed more positive MMPK9 expression as compared to mandibular ameloblastomas. In case of mandible only 7cases were strong positive (28%) while in maxilla 3 out of 5 cases were strong positive (60%).Therefore this may indicate aggressive behavior of maxillary ameloblastomas requiring need of more extensive surgery a and long term follow up.

In summary MMP-9 expression was assessed in three different histological subtypes of ameloblastoma i.e. follicular ameloblastoma, plexiform ameloblastoma and acanthomatous ameloblastoma. A varied immune reactivity of MMP-9 protein was observed in these subtypes displaying negative, mild positive and strong positive expressions. Higher MMP-9 expression is evaluated in Acanthomatous and plexiform type than follicular type. Therefore more energetic expression of MMP-9 related toAcanthomatous and plexiform type as well as maxillary ameloblastoma suggests that this protein may participate in proliferation of cell, aongwithexplaination of bone resorption with prognosis marked as underprivileged and greater invasion potential.

Statistical analysis also highlighted significant difference between MMP-9 scores of immune reactivity and the three histological subtypes ($p<0.05$).

CONCLUSION

Expression ofMMP-9by ameloblastoma cells with subtypesvariation.Higher MMP-9 expression in acanthomatous and plexiform ameloblastoma than follicular types, thus being more invasive than follicular ameloblastoma. Hence it may be used in future as a potential markerto serve as an indicator and utilized in monitoring degree of local aggressiveness of ameloblastoma.

Author's Contribution:

Concept & Design of Study:	Farah Farhan
Drafting:	MariaI lyas, Misbah Ali
Data Analysis:	Zainab Akbar, Batool Zahra
Revisiting Critically:	Farah Farhan, MariaI lyas
Final Approval of version:	Farah Farhan

Conflict of Interest: The study has no conflict of interest to declare by any author.

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