DOI: 10.7860/JCDR/2021/49261.15423

Original Article

Dentistry Section

Expression of Matrix Metalloproteinase-9 in Different Histopathological Variants of Ameloblastoma: A Cross-sectional Study

REVATI SHAILESH DESHMUKH[1], PRIYA NIMISH DEO[2], SUREKHA LAXMAN CHAVAN[3], PRASAD KANGO[4]

ABSTRACT

Introduction: Ameloblastomas are benign and the most common odontogenic neoplasms with many histopathological subtypes depending on the predominant pattern. They are known for their aggressive behaviour. As ameloblastomas have a high rate of recurrence, it is necessary to understand the biological behaviour of these neoplasms. Matrix Metalloproteinase-9 (MMP-9) is an enzyme that belongs to Metalloproteinases family and is known to degrade the Extracellular Matrix (ECM) and facilitate tumour progression. Evaluation of the expression of MMP-9 in ameloblastomas could contribute in understanding its biological behaviour.

Aim: To analyse the expression of MMP-9 in different histopathological variants of ameloblastoma.

Materials and Methods: A cross-sectional observational study was done in the Department of Oral Pathology and Microbiology, Bharati Vidyapeeth Deemed to be University, Dental College and Hospital, Pune, India. A total of 30 cases were selected for this study. The study was carried out for a duration of two years from April 2018 to April 2020. The MMP-9 expression was studied by immunohistochemical staining. The statistical comparison was done using Chi-square test between groups, p-value <0.05 was considered significant. The data was statistically analysed using Statistical Package for Social Sciences (SPSS) version 22.0, IBM Corporation, USA for MS Windows.

Results: A total of 30 paraffin embedded archival tissue blocks were selected for this study. Among them nine cases were of Plexiform Ameloblastoma, eight of Unicystic Ameloblastoma, three of Acanthomatous Ameloblastoma, four of Desmoplastic Ameloblastoma and six were of Follicular Ameloblastomas. The MMP-9 showed variable expression in different histopathological subtypes of ameloblastoma. This difference was statistically significant between Plexiform and Acanthomatous as well as Plexiform and Follicular variants (p<0.05). A 66.7% (6 out of 9 samples) of Plexiform Ameloblastoma showed intense staining for MMP-9.

Conclusion: Expression of MMP-9 varies in different histopathological variants of ameloblastoma and may not have an association with biological behaviour and aggressiveness.

Keywords: Follicular, Immuohistochemistry, Plexiform

INTRODUCTION

Ameloblastomas are benign but locally invasive odontogenic neoplasms arising from the odontogenic epithelium. They account for 1% of all oral tumours and about 18% of odontogenic tumours. They generally occur in the third to fifth decades of life with nearly equal gender predilection and are more common in mandible than the maxilla [1]. In the mandible they have a predilection for the molar-ramus area [2].

Clinically, ameloblastomas are classified into solid/multicystic, unicystic and peripheral types. On the basis of the pattern in which the tumour cells are arranged they may be divided into follicular, plexiform, acanthomatous, granular and basal cell types [3]. Rare subtypes of ameloblastomas include haemangio-ameloblastoma, melano-ameloblastoma, clear cell ameloblastoma, papilliferous Ameloblastoma, kerato-ameloblastoma, pituitary ameloblastoma which were not included in this study.

Majority of ameloblastomas are slow growing, painless swellings of the jaws. They do not have metastatic potential [4]. They grow by expansion and local invasion [5]. Matrix metalloproteinases are enzymes that have proved to play a role in local invasion.

The MMPs are also known as matrixins and constitute a family of zinc and calcium-dependent proteolytic enzymes. They degrade ECM macromolecules such as collagens, gelatins, tenascin, fibronectin and laminin at physiological pH [6]. A total of 25 members of the MMP family have been reported [7]. They are divided into six groups – collagenases, gelatinases, stromelysins, matrilysins, membrane type MMPs and other non classified MMPs [8]. The MMP-9 was first described as 92 kDa type IV collagenase or gelatinase B [9]. The MMP-9 is produced by different types of cells namely epithelial cells, fibroblasts, keratinocytes, dendritic cells, osteoblasts, macrophages, granulocytes, T cells and mast cells. It is expressed as a pro-enzyme and its activation to a smaller mature form depends on the removal of the prodomain at the extracellular space [10].

The MMPs play role in both physiological and pathologic processes. In adult tissues, their expression and activity is normally quite low. It increases notably in many pathological conditions which may leads to unwanted tissue destruction, such as inflammatory diseases, tumour growth and metastasis [11]. Under physiological conditions, these enzymes play a key role in ECM regulation during embryogenesis and tissue remodelling [6]. MMPs like 1, 2, 3 and 9 participate in early tooth development [12]. During tooth development, MMP-9 plays a role in resorption of the bone, degradation of the basement membrane of the reduced enamel epithelium and of the ECM related with the erupting tooth. Many studies indicates that MMP-9 binds to amelogenin or both are co-expressed in ameloblasts during the development of the dental germ [7]. It has been noted that there is an abnormal expression of MMP-1, 2 and 9 in many aggressive tumours [6].

Taking into consideration the hypothesis that immunohistochemical expression of MMP-9 in variants of ameloblastoma for the assessment of aggressiveness can have diagnostic, therapeutic and prognostic implications, this study was deemed necessary. This study is an attempt to analyse and compare the expression of MMP-9 for the assessment of aggressiveness in different histological variants of ameloblastoma. The objectives were to evaluate and compare the expression of MMP-9 in various histological subtypes of

ameloblastoma. A new dimension (novelty) in understanding a precise pattern of MMP-9 expression and its role in the biological behaviour may assist in a better treatment planning.

MATERIALS AND METHODS

The present cross-sectional observational study was carried out in the Department of Oral Pathology and Microbiology, Bharati Vidyapeeth Deemed to be University, Dental College and Hospital, Pune, India. The study was carried out for a duration of around two years from April 2018 to April 2020. Ethical clearance was obtained from the Institutional Ethics Committee, Bharati Vidyapeeth Deemed to be University, Dental College and Hospital, Pune, Oral Pathology and Microbiology- Br VI/ ECR/328/Inst/MH/2013/RR-16 dated 28-03-2018. This was a pilot study and we used Browne's thumb rule method (Browne RH 1995) to decide the sample size (n=30) for the present study [13].

Inclusion criteria: A total of 30 archival tissue blocks of histopathologically confirmed ameloblastoma were selected. Nine cases of plexiform ameloblastoma, eight cases of unicystic ameloblastoma, three cases of acanthomatous ameloblastoma, four cases of desmoplastic ameloblastoma and six cases of follicular ameloblastoma available from the archives were included in the study.

Exclusion criteria: The sixth variant being granular cell ameloblastoma was not available in the archives, hence not included. Insufficient tissue samples and poorly preserved specimens were also excluded from the study.

Haematoxylin and Eosin (H&E) stained slides were reviewed to confirm the diagnosis. Immunohistochemical study using an antibody to MMP-9 protein was performed on 4 µm sections on polylysine coated slides. The sections were made with a semiautomatic rotary microtome. Dewaxing of the tissue sections was done in xylene and dehydration in graded ethanol.

Antigen retrieval was performed by heat induced epitope retrieval technique using a microwave. Primary antibody, MMP-9-EP127 rabbit monoclonal antibody (PathnSitu) was used. Formalin fixed paraffin embedded tissue of breast cancer was taken as the positive control.

Evaluation of Staining

The slides were viewed under 10x and 40x magnification with a binocular microscope. All areas in each section of the slide were evaluated. Brown staining was considered as positive for the expression of MMP-9. Nuclear as well as cytoplasmic staining was considered. The expression of MMP-9 was assessed by two examiners individually to determine the intensity and localisation of staining. The intensity of MMP-9 staining was assessed using a criteria indicated by de Oliveira MD et al., as [14]:

0- No staining

1- Moderate staining greater than background staining

2- Intense staining

STATISTICAL ANALYSIS

The results were tabulated and statistically analysed using Statistical Package for Social Sciences (SPSS) version 22.0, IBM Corporation, USA for MS Windows. The evaluation of MMP-9 expression in between the groups was done using Chi-square test. In the entire study, the p-value less than 0.05 were considered to be statistically significant.

RESULTS

A total of 30 samples of ameloblastoma were selected for the study. Out of the 30 samples studied, MMP-9 showed a varied expression in different histologic subtypes of ameloblastoma. This expression ranged from no staining to intense staining. Immunoreactivity was seen in epithelial as well as in the stroma. Immunostaining was mostly seen in the ameloblast like cells and stellate reticulum like cells.

Expression of MMP-9 in different histologic subtypes of ameloblastoma is summarised in [Table/Fig-1]. The intergroup statistical comparison of MMP-9 is summarised in [Table/Fig-2].

Variables	Group 1 (n=9) Plexiform ameloblastoma		Group 2 (n=8) Unicystic ameloblastoma		Group 3 (n=3) Acanthomatous ameloblastoma		Group 4 (n=4) Desmoplastic ameloblastoma		Group 5 (n=6) Follicular ameloblastoma	
MMP-9 expression	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
No staining	2	22.2	2	25.0	0	0.0	1	25.0	1	16.7
Moderate staining	1	11.1	3	37.5	3	100.0	2	50.0	5	83.3
Intense staining	6	66.7	3	37.5	0	0.0	1	25.0	0	0.0
Total	9	100.0	8	100.0	3	100.0	4	100.0	6	100.0

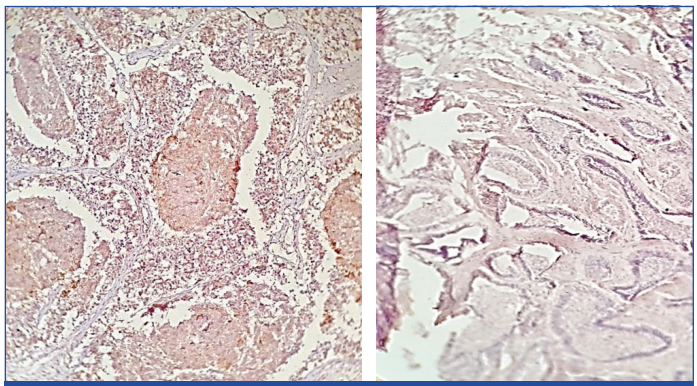
[Table/Fig-1]: Inter-group distribution of expression of MMP-9 across five study groups.

	Inter-group statistical comparisons				
	Group 1 vs Group 2	**Group 1 vs Group 3**	**Group 1 vs Group 4**	**Group 1 vs Group 5**	**Group 2 vs Group 3**
p-value	0.378**	0.018*	0.257**	0.013*	0.179**
	Group 2 vs Group 4	**Group 2 vs Group 5**	**Group 3 vs Group 4**	**Group 3 vs Group 5**	**Group 4 vs Group 5**
p-value	0.894**	0.164**	0.350**	0.999**	0.375**

[Table/Fig-2]: Inter-group statistical comparison of expression of MMP-9.
Chi-square test- the p-value <0.05 were considered to be statistically significant; NS**: Non significant

[Table/Fig-2] shows the intergroup statistical comparison between different variants of ameloblastoma. Out of all the subgroups, statistically significant difference in the expression of MMP-9 is noted between plexiform and acanthomatous ameloblastoma where the p-value is 0.018 (less than 0.05). Also, between the plexiform and follicular ameloblastoma sub groups statistically significant difference was seen in the expression of MMP-9, where that p-value is 0.013 (less than 0.05). Breast cancer tissue was taken as positive control for this study [Table/Fig-3].

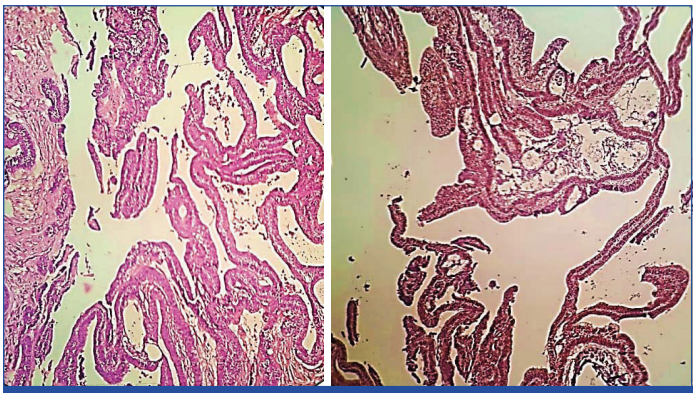
Out of the 6 samples of follicular ameloblastoma, 1 (16.7%) showed no staining, 5 (83.3%) had moderate staining and none had intense staining. [Table/Fig-4] shows the expression of MMP-9 in follicular ameloblastoma.



[Table/Fig-3]: Positive staining of MMP-9 in breast tissue which served as control (10x).
[Table/Fig-4]: Photomicrograph of Follicular Ameloblastoma showing immune-reactivity to MMP-9 (10x magnification). (Images from left to right)

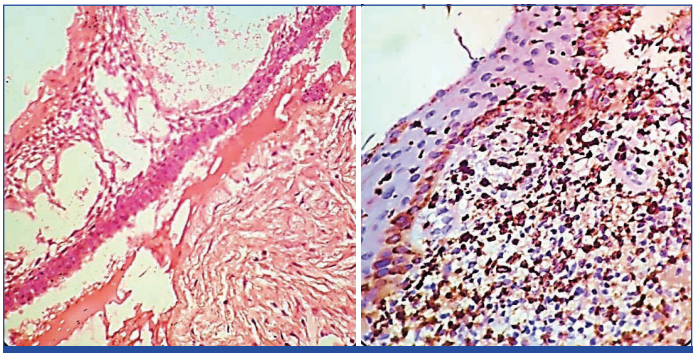
Majority of samples of plexiform ameloblastoma [Table/Fig-5], showed an intense staining for MMP-9. Out of the 9 samples of plexiform ameloblastoma, 2 (22.2%) had no staining, 1 (11.1%) had moderate staining and 6 (66.7%) had intense staining. MMP-9 expression was seen in the ameloblast like cells as well as the stroma [Table/Fig-6]. Out of 4 samples of desmoplastic ameloblastoma, 1 (25.0%)

had no staining, 2 (50.0%) had moderate staining and 1 (25.0%) had intense staining. Haematoxylin and Eosin stained slides of unicystic ameloblastoma is shown in [Table/Fig-7].



[Table/Fig-5]: Photomicrograph of Plexiform Ameloblastoma (Haematoxylin and Eosin staining) (10x magnification).
[Table/Fig-6]: Photomicrograph of Plexiform Ameloblastoma showing immunoreactivity to MMP-9 (4x magnification) (Images from left to right)

Out of 8 samples of unicystic ameloblastoma studied, 2 (25.0%) had no staining, 3 (37.5%) had moderate staining and 3 (37.5%) had intense staining. The MMP-9 expression seen in the epithelium and stroma [Table/Fig-8]. All the 3 samples of acanthomatous ameloblastoma studied (100%) showed moderate staining.



[Table/Fig-7]: Photomicrograph of Unicystic Ameloblastoma (Haematoxylin and Eosin staining) (40x magnification).
[Table/Fig-8]: Photomicrograph of Unicystic Ameloblastoma showing immunoreactivity to MMP-9 (40x magnification). (Images from left to right)

DISCUSSION

Ameloblastoma is a tumour which arises from the odontogenic epithelium. It is a slow growing, locally invasive tumour with a peak incidence in the third to fourth decades of life and with a male to female ratio of 1:1. Ameloblastomas are classified into solid/multicystic type, extraosseous/peripheral type and unicystic type, based on the location and the type of presentation. The solid/multicystic types are the most common and aggressive, whereas the unicystic and peripheral ameloblastomas are less common and less aggressive variants [1]. The most common histological variants of ameloblastomas are the follicular and plexiform patterns. The less common ones are the granular cell, desmoplastic and basal cell [15]. It is presently accepted that there is no association between the histological pattern and tumour behaviour on the prognosis [16].

One of the pre-requisite for tumour progression is the invasion of surrounding healthy tissues by tumour cells. Identification of invasive activities in ameloblastomas is less studied and may be helpful to anticipate their biological behaviour [17]. The MMPs mediate ECM breakdown which is essential for cellular migration and is a key element in the multistage process of tumour invasion and metastasis [18]. As a preface to invasion, they can bring about a breach in the basement membrane by cleaving type IV collagen [17].

The MMP-9 is considered to play an important role in bone resorption and is closely related with several osteodestructive pathologies [19]. It is present in a quiescent form and once activated, it causes degradation and increases the metastasis of tumour cells [20]. The MMPs trigger the release of mitogens, leading to proliferation of ameloblastoma cells [21]. According to the results of this study, a variable expression of MMP-9 was seen in the tumour cells as well as the stroma in different histopathological subtypes of ameloblastoma. This variability in the expression of MMP-9 could be correlated to the amount of degradation of the basement membrane which has high content of Type IV collagenase. The degradation of the basement membrane by MMP-9 could lead to local invasion which may be directly proportional to the biological behaviour of the tumour and therefore aggressiveness.

Henriques AC et al., quoted numerous factors which play a role in the aggressive behaviour of ameloblastomas. They include increased potential for proliferation, variations in the expression of tumour suppressor genes and the aberrant expression of the cell cycle regulating proteins, adhesion molecules and MMPs and their inhibitors [22]. According to de Vicente JC et al., MMP-2 and MMP-9 play a role in tumour growth and angiogenesis. This suggests an association of these gelatinases with aggressive behaviour and uncertain clinical outcome in some human neoplasms [23].

The results of this study are in accordance with the study conducted by Florescu A et al., they observed variable intensity, both in the neoplastic epithelium and in the stroma that surround the ameloblastic proliferations. The expression was detected in 13 out of 17 cases [24].

According to the study conducted by Anne R et al., all samples showed positive MMP-9 expression with moderate to strong intensity [19]. In a study conducted by Da Silva AD et al., MMP-9 was detected in tumour cells and stromal cells in all the samples. A higher MMP-9 expression was seen in the stroma of solid ameloblastoma than in unicystic ameloblastoma which was statistically significant [25]. Pinheiro JJ et al., studied the local invasiveness of ameloblastoma by detecting the MMPs by immunohistochemistry and concluded that MMPs may contribute to the local invasiveness [26]. Indirapriyadarsini K et al., studied the immunohistochemical expression of MMP-9 in ameloblastoma along with osteonectin and Ki-67. All 20 cases showed positive staining for MMP-9 with different intensities which showed that increased expression of MMP-9 may play a role in the aggressive nature of this neoplasm [6]. According to a systematic review and meta-analysis conducted by Zhou YM et al., expression of MMP-9 in the follicular type is lower than other subgroups of ameloblastoma [18].

In the present study, all the histological groups showed a variable staining intensity and pattern. In plexiform ameloblastoma, 6 samples (66.7%) showed intense staining whereas In unicystic ameloblastoma 6 samples (75%) showed moderate to intense staining. In follicular ameloblastoma, 5 samples (83.3%) had moderate staining. All the 3 samples (100%) of acanthomatous ameloblastoma showed moderate staining. In the desmoplastic samples, 2 samples (50%) had moderate staining and 1 sample (25%) had intense staining. Increased expression of MMP-9 suggests increased degradation of the ECM, increased spread (local invasion) and therefore aggressiveness.

Limitation(s)

The study needs to be done on a larger sample size and use of fresh tissue may give an insight into the biological behaviour. It is suggested that as far as possible uniform groups of samples should be considered.

CONCLUSION(S)

The present study showed a variable expression of MMP-9 in different histopathological subtypes of ameloblastoma, which may suggest that MMP-9 plays a role in local invasion/spread. Difference in the expression of MMP-9 may be related to the biological behaviour of ameloblastomas wherein acanthomatous and desmoplastic variants showed equivocal expression thus rethinking the aggressive behaviour

of acanthomatous and desmoplastic ameloblastoma. This could lead to two different schools of thoughts metaplasia could be observed in acanthomatous ameloblastoma related to its transformation into cancerous tissue which may be the reason for its aggressiveness and excessive collagenisation in desmoplastic ameloblastoma may be another reason for its aggressive behaviour.

This study shows that there is higher expression of MMP-9 in plexiform and follicular ameloblastomas indicating their aggressiveness which in turn suggests that the histological pattern and tumour behaviour or aggressiveness may not be related.

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PARTICULARS OF CONTRIBUTORS:
1. Professor and Head, Department of Oral Pathology and Microbiology, Bharati Vidyapeeth Deemed to be University, Dental College and Hospital, Pune, India.
2. Assistant Professor, Department of Oral Pathology and Microbiology, Bharati Vidyapeeth Deemed to be University, Dental College and Hospital, Pune, India.
3. Associate Professor, Department of Oral Pathology and Microbiology, Bharati Vidyapeeth Deemed to be University, Dental College and Hospital, Pune, India.
4. Private Practitioner, New Dental Clinic, Solapur, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Revati Shailesh Deshmukh,
Professor and Head, Department of Oral Pathology and Microbiology,
Bharati Vidyapeeth Deemed to be University, Dental College and Hospital,
Pune, Maharashtra, India.
E-mail: rvt_deshmukh@yahoo.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Mar 03, 2021
- Manual Googling: Jun 18, 2021
- iThenticate Software: Aug 14, 2021 (14%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Mar 04, 2021**
Date of Peer Review: **Jun 01, 2021**
Date of Acceptance: **Jul 17, 2021**
Date of Publishing: **Sep 01, 2021**