Original article

IMMUNOHISTOCHEMICAL EXPRESSION OF MMP2 & TIMP2 IN AMELOBLASTOMA AND THEIR IMPLICATIONS IN BIOLOGIC BEHAVIOUR OF THE TUMOUR

Olajide MA[1], Adeyemi BF[2], Adisa A[2], Kolude B[2], Adebiyi KE[1]

[1]Department of Oral Pathology and Oral Medicine, Faculty of Dentistry, Lagos State University College of Medicine, Ikeja, Nigeria. [2]Department of Oral Pathology /Oral Medicine, Faculty of Dentistry, College of Medicine, University of Ibadan Nigeria.

ABSTRACT

BACKGROUND: Ameloblastoma is a benign aggressive neoplasm of odontogenic epithelium characterized by local invasiveness, propensity for facial deformity, and a high rate of recurrence. Matrix metalloproteinases (MMPs) are proteolytic enzymes that play a central role in the regulation of extracellular matrix complemented by Tissue Inhibitors of Matrix metalloproteinases (TIMPs). MMP-2 has been closely associated with ameloblastoma invasion. TIMP2 acts as a specific inhibitor of MMP-2 or may be involved in its activation.
AIM: To determine and compare the relative immunohistochemical expressions of MMP2 and TIMP2 proteins in the clinical types of ameloblastoma.

METHODS: Formalin-fixed paraffin embedded (FFPE) tissue blocks of 50 ameloblastoma cases were sectioned and stained with antibodies for MMP-2 and TIMP-2. Immunohistochemical staining of stromal and tumour cells were assessed to obtain a combined score of proportion and intensity of staining. Proportion of cases with positive expression of MMP2 and TIMP2 were determined. Mean score and ratios of these proteins were compared within the biological types using the Independent sample t- test.

RESULTS: In all the clinical types of ameloblastoma, the mean MMP-2 expression was higher than the mean expression of the inhibitor, TIMP-2. There was no significant difference in the expression of MMP-2 in both the conventional and unicystic types. TIMP2 expression was significantly higher in the unicystic type than in conventional type (p=0.02). However, MMP2:TIMP2 was significantly higher in the conventional type than in unicystic type (p = 0.01).

CONCLUSION: TIMP-2 is a better predictor of the degree of invasiveness of the clinical types of ameloblastoma than MMP-2. MMP2: TIMP2 ratio also explains the effect of TIMP-2 on MMP-2 and indicates invasiveness in the biologic types of ameloblastoma than MMP-2 alone.

Keywords: Ameloblastoma, Immunohistochemistry, Clinical Types, MMP2, TIMP2

Correspondence address:

Dr. Olajide M.A.
Department of Oral Pathology and Oral Medicine, Faculty of Dentistry, Lagos State University College of Medicine, Ikeja, Nigeria. P.M.B 21266, Ikeja, Lagos, Nigeria
mofoluwasho.olajide@lasucom.edung
Tel: +2348030623759

INTRODUCTION

Ameloblastoma is a benign aggressive neoplasm of odontogenic epithelium.[1] It is the most frequently encountered tumour arising from odontogenic epithelium.[2, 3]

Although classified as benign, its features of biological aggression pose a significant therapeutic challenge.[1,4] Clinical/biological types that have been documented in literature include solid, unicystic, peripheral, sinonasal, malignant ameloblastoma and ameloblastic carcinoma.[1] The WHO 2017 classification of odontogenic tumours has however re-classified ameloblastoma into four clinical types: Conventional, Peripheral, Unicystic and Malignant/metastasizing Ameloblastoma.[5]

Clinically, ameloblastoma presents as a slow growing, locally invasive, painless swelling of the jaw. Usually causing buccolingual jaw expansion, with ability to produce marked deformity and propensity for recurrence, occasional metastasis and malignant transformation. This typical clinical picture characterizes the solid/conventional clinical type.[6] Eversole *et al*[7] first described desmoplastic ameloblastoma as a lesion with unique histological pattern, characterized by presence of islands of ameloblastic columnar cells surrounding spindle shaped stellate reticulum–like cells in a stroma with marked desmoplasia. It became highlighted as a clinical type in the 2005 classification of odontogenic tumours.[8] Investigators also observed that recurrence in cases with desmoplastic ameloblastoma was almost as high as with the conventional ameloblastoma[9,10] with radical therapy being indicated for its treatment.[11,12] It has now been grouped as part of conventional ameloblastoma by the most recent classification[5] However, slight variations exist with other clinical/biologic types. Not all ameloblastoma behave this aggressively and it is important to distinguish between clinical types of ameloblastoma, to be able to give appropriate treatment to patients.

Unicystic ameloblastoma seems to have a less aggressive clinical behaviour and better prognosis than the solid ameloblastoma.[13] Recent evidence, however, indicates that unicystic ameloblastoma can also be destructive and may recur after simple curettage alone, as it is known to have the capacity to expand or perforate jaw cortex.[14]

Peripheral ameloblastoma is the soft tissue counterpart of intraosseous ameloblastoma and is a relatively rare lesion.[15] It appears as an extra osseous soft tissue lesion generally on the gingiva with no bony involvement. It is histologically identical to intraosseous ameloblastoma, but may arise from the surface epithelium or extraosseous remnants of the dental lamina.[16]

Malignant ameloblastoma is rare, and the term defines ameloblastoma that has metastasised, while maintaining the benign histologic features of the primary lesion.[15] Malignant ameloblastoma occur at a younger age than their benign counterpart and are usually seen in the mandible. Metastasis is typically to the lung (this is thought to be by aspiration) and occasionally to local lymph nodes.[1] Only few cases of malignant ameloblastoma have been reported; therefore, these cases should be properly followed up and investigated.[17]

In recent times, various molecular alterations responsible for development and progression of odontogenic tumours have been identified. [18,19] Extracellular matrix components such as collagens, fibronectin, tenascin and laminin have been detected in various odontogenic tumours. [9,20]

Matrix metalloproteinases (MMPs) are thought to play an important role in ameloblastoma invasion, because of their function in the breakdown of extracellular matrix (ECM). [18] Matrix metalloproteinases (MMPs) are a family of zinc and calcium dependent proteolytic enzymes that play a central role in the regulation of extracellular matrix during embryonic development and tissue remodelling. However, they also participate in tumour invasion and metastasis in which case, their pathological activity is detected in tumour cells and surrounding stroma. [10,21]

MMP-2 (Gelatinase A) is described as one of the most important proteolytic enzymes that degrade basement membrane components (such as type IV collagen and laminin) and extracellular

matrix during tumour invasion and metastasis. [22] In contrast to most MMPs, progelatinase A is produced constitutively in high concentrations by many types of cells and is not induced by cytokines that regulate other MMPs. The final activation of this proenzyme (an important step in controlling gelatinase A activity) involves a cell surface activation mechanism which partly requires the participation of another MMP, MT1-MMP.

Tissue inhibitors of matrix metallo-proteinases (TIMPs) function to inhibit active and latent forms of MMPs by forming non-covalent bimolecular complexes with MMPs. That is, TIMPs inhibits the activity of fully activated MMPs. TIMPs appear to block or retard the activation of MMP precursors. [23] Binding of TIMPs to MMPs is thought to be a 1:1 stoichiometry. [24] The role played by TIMPs in regulating matrix degradation is important in physiological tissue remodelling such as wound healing and trophoblastic implantation as well as in the pathologic process of tumour invasion. [10, 25]

TIMP-2 is unique because it may function both as a MMP inhibitor and activator. The conversion of MMP proenzymes to the activated forms is controlled by the stoichiometric binding of TIMPs which are synthesized by cells, such as fibroblasts, endothelial cells, and tumour cells. The amount of TIMP2 determines the balance between the level of activator (free MT1-MMP) and receptor (MT1-MMP/TIMP2 complex), which regulates the degree of activation of progelatinase A. Thus TIMP2 may have a dichotomous mode of action. On one hand, it can act as a specific inhibitor of MMP-2 (Gelatinase A). On the other hand, it is implicated in the mechanism of progelatinase activation [26] Apart from regulating MMP-2 activity, TIMP-2 has also been found to also regulate cellular responses to growth factors in the tumour microenvironment. [26, 27]

MMP-2 has been closely associated with ameloblastoma invasion and high MMP2 expression and activity is related to a more aggressive infiltrative behaviour in ameloblastoma.[28, 29]

Previous studies done have shown that ameloblastomas generally, with no implication of specific clinical types, have an elevated expression of MMP-2 and MMP-9, but are devoid of or have an abnormal expression of TIMP-2. [28, 30]

An established association between the clinical types of ameloblastoma and the expression of these proteins is sparse in literature, though a study found a significantly higher expression of TIMP-2 and MT1-MMP (MMP-14) mRNA in recurrent and solid multicystic ameloblastoma compared to primary and unicystic types.[31] The reason for this is not clear.

Identification and correlation of the relative expression of these proteins may thus provide useful molecular markers for early prediction of recurrence and appropriate treatment planning for cases of ameloblastoma. Thus, this study aims to determine and compare the relative Immunohistochemical expression of MMP-2 to TIMP-2 in the clinical types of ameloblastoma.

MATERIALS AND METHODS

The study was carried out at the Department of Oral Pathology/Oral Medicine, University College Hospital (UCH), Ibadan. Immunohistochemistry was done at the Breast Cancer Research Laboratory of the Institute for Advanced Medical Research and Training (IAMRAT) located within the same institution, using 50 formalin-fixed paraffin embedded (FFPE) tissues of ameloblastoma histologically diagnosed between January 2000 and December 2011. New slides were reviewed by the investigators for re-confirmation of initial histological diagnosis. Clinical data was retrieved from clinic/laboratory registers and cases were classified according to the 2017 WHO classification of odontogenic tumours, grouping them into Conventional and Unicystic clinical types, as no cases of Peripheral and Malignant Ameloblastoma were found in our series.

Selected paraffin blocks of the ameloblastoma were re-mounted and representative sections of each cut serially on a manual microtome at 4 micron thickness, sections were floated on a

warm water bath, placed on adhesive coated slides and then placed on a warmer set at 60ºC. Two slide sections were prepared from each block, in preparation for immune-histochemical staining with antibodies for MMP-2 and TIMP-2 respectively.

The sections were deparaffinised by passing them through changes of xylene twice for 5 minutes each, then rehydrated in decreasing grades of alcohol as follows: 100% ethanol twice for 3 minutes each, followed by 95% and 70% ethanol for 1 minute each. This was followed by rinsing in phosphate buffered saline (PBS).

Following this, the slides were immersed in heat induced epitope retrieval citrate buffer (10 mM sodium citrate buffer at pH 6.0) and pre-treated in a microwave oven set at 90^0C for 1 hour. They were removed and placed in fresh citrate to cool for 20 minutes. This was followed by a 10 - minute rinse in phosphate buffered saline (PBS).

The slides were placed in a humidity chamber, then, the tissue periphery marked with a hydrophobic pen. Blocking of endogenous peroxidase was done with 3% hydrogen peroxide for 10 minutes at room temperature after which the slides were rinsed in PBS.

The two slide sections prepared from each paraffin block were incubated for an hour with recommended amount of appropriately characterized and diluted primary antibodies (40 – 130 µl, depending on the surface area of the tissue): anti-TIMP-2 (1:200 mouse monoclonal antibody, by abcam) and anti-MMP-2 (1:200 mouse monoclonal antibody, by abcam) respectively.

This was followed by incubation of the slide sections with the appropriately labelled polymer horse radish peroxidase (HRP) conjugated anti-mouse secondary antibody for 30 minutes, after which they were rinsed in PBS. 1ml of diaminobenzidene (DAB) chromogen was added to cover the specimen. After visualization of the reaction with the diaminobenzidine chromogen, incubation in humidity chamber for 15 minutes was done. The slides were immersed in a bath of aqueous haematoxylin about 10 times for 1 minute each and then rinsed in distilled water for 3 minutes after 10 immersions for counterstaining. The slides were then passed by dipping through a series of graded alcohol to dehydrate the sections (70%, 95% then 100%) and then also rinsed with xylene. A mounting fluid (glycerine gel) was applied and a cover slip placed.

Positive and negative staining controls for each antibody were included to validate the procedure. Breast adenocarcinoma was used as a positive control for MMP-2, human placental tissue was used for TIMP-2. [32, 33] Laboratory negative staining controls were other slide sections produced from each of the two laboratory control paraffin blocks, but incubated with PBS only, instead of the indicated primary antibody. Slide sections were evaluated by the investigator and two experienced consultant Oral Pathologists. A consensus opinion of staining patterns observed visually using a binocular light microscope (Zeiss Axioscope, Switzerland) was recorded for each case. Both stromal and tumour cells were assessed for staining in cases of ameloblastoma. Cytoplasmic staining was used for positive staining in each of the antibodies.

Immunohistochemical staining of stromal and tumour cells was assessed using both stain intensity and proportion of cells stained. Stain intensity was graded using a semi quantitative 4-point scale: 0 = no staining; 1= mild staining; 2= moderate staining; and 3= intense staining. The proportion of stromal and tumour cells stained was assessed at X100 magnification using a semi quantitative 4-point scale: 0 = no cell staining in any microscopic fields; 1= <25% staining; 2= 25-50% staining; and 3= >50% staining.

The combined score (proportion plus intensity) was determined. The final combined score in the was determined by finding the average of combined score in the stromal and tumour epithelial cells. The combined score was categorised as follows: <2, negative staining or low staining (-); 2 and 3, moderate staining (+); and ≥4, strong staining (++). A combined score equalling or exceeding (+) was defined as positive for either MMP-2 or TIMP-2. Data was analysed with SPSS version 20.0

Qualitative data are expressed as proportions/percentages. Mean scores were compared within clinical types, using the Independent sample t-test. Correlation tests were done to compare the scores of MMP-2 with TIMP-2 among the clinical types. The level of significance was set at $p < 0.05$.

Ethical Approval- Ethical clearance was obtained from the Joint University of Ibadan/ University College Hospital Ethical Review Committee (Number: UI/EC/12/0046)

RESULTS

A total of one hundred and seventy-nine (179) jaw neoplasms were histologically diagnosed as ameloblastoma within the period under review. The blocks of 126 of these cases were retrievable, out of which 79 were re-confirmed to have satisfied the histological criteria for diagnosis of ameloblastoma. The conventional ameloblastoma was the most common clinical type, accounting for 45 (57.0%) followed by unicystic type with a total of 34 (43.0%)[Fig. 1]. Neither peripheral nor malignant ameloblastoma was seen in this series.

Immunohistochemical Findings

There was a statistically significant difference in the mean expression of TIMP2 between the biological types ($F = 6.32$, $df = 48$, $p= 0.02$). There was also a significant difference in the ratio of MMP2:TIMP2 between the clinical types ($F =8.47$, $df =48$, $p = 0.01$)[Table 1].

Table 1 - Semi-quantitative expression of proteins within the Clinical types of ameloblastoma

CLINICAL TYPES	MMP2	TIMP2	MMP2:TIMP2
Conventional (n = 30)	4.60 (SD±1.22)	3.50 (SD±1.66)	2.0:1 (SD± 1.84)
Unicystic (n = 20)	5.00 (SD±1.30)	4.40 (SD±1.24)	1.2:1 (SD±1.08)
p value	0.78	0.02*	0.01*

***** Significant p value

In conventional ameloblastoma, there was a very weak positive correlation between MMP-2 [Fig. 2] and TIMP-2 expression which was not statistically significant (Pearson's correlation coefficient=0.20, p=0.30) [Figs 3 and 4]. Similarly, in unicystic ameloblastoma, there was a very weak positive correlation between MMP-2 and TIMP-2 expression, which was not statistically significant (Pearson's correlation coefficient = 0.382, p= 0.10)[Figure 5].

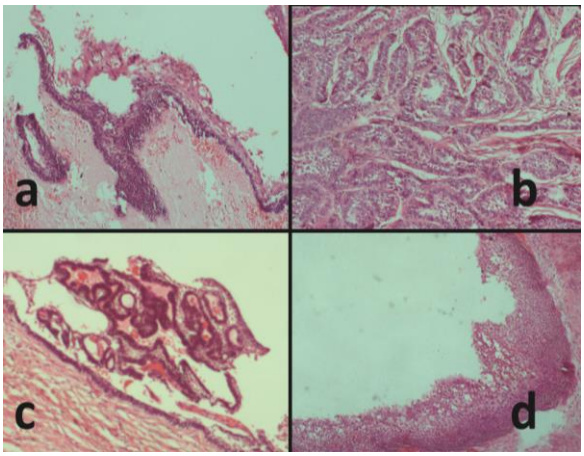

Figure 1a-d: Photomicrographs showing representative examples of ameloblastoma a-Cystic mural (H&E, X100); b- Follicular (H&E, X10); c- Cystic intraluminal (H&E, X10); d- Cystic luminal (H&E, X100)

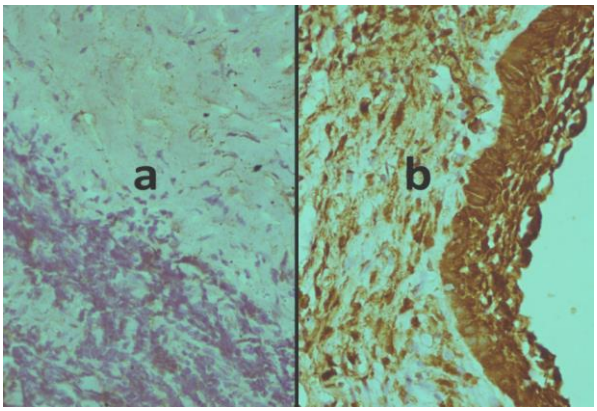

Figure 2: a. Photomicrograph showing weakly positive staining for MMP2 in a case of ameloblastoma (X400); b- Photomicrograph showing strongly positive staining for MMP2 in a case of ameloblastoma (X400)

DISCUSSION

The present study followed the 2017 WHO classification of odontogenic tumours, which grouped benign ameloblastoma into four main clinical types, namely: conventional, unicystic, peripheral and malignant/metastastazing.[5]

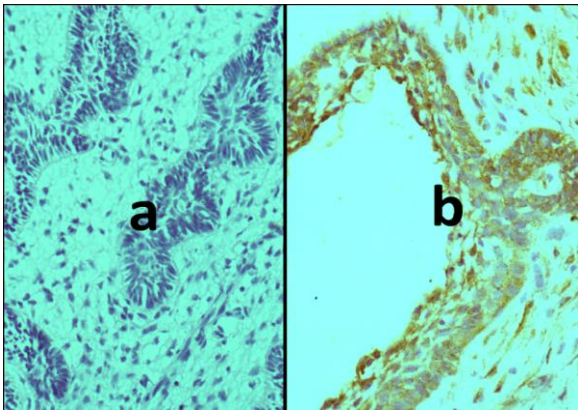

Figure 3: a. Photomicrograph showing negative staining for TIMP2 in a case of ameloblastoma (X400); b- Photomicrograph showing strongly positive staining for TIMP2 in a case of ameloblastoma (X400)

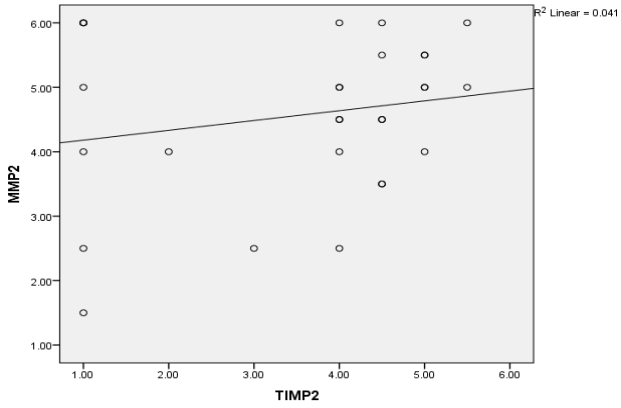

Figure 4- Scatter plot showing the correlation of MMP2 expression with TIMP2 expression in conventional ameloblastoma.

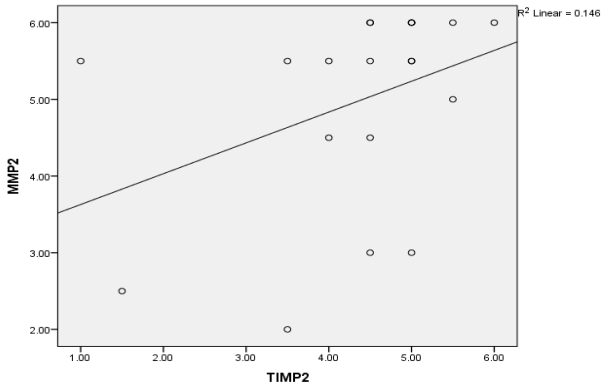

Figure 5- Scatter plot showing the correlation of MMP2 expression with TIMP2 expression in unicystic ameloblastoma

Ameloblastoma is typified by the conventional type.[8] It is the commonest of all types as found in this study, followed by the unicystic type. The relative rarity of the malignant and peripheral types in literature [15,17] is confirmed in this study with no case found in our series.

In all cases of ameloblastoma, the expression of MMP-2 was greater than TIMP-2. This imbalance may thus be contributory to the invasive characteristic of these neoplasms. There was however no significant difference in the expression of MMP-2 among the clinical types. The unicystic type in this study, had a slightly higher mean expression of MMP2 compared to the conventional type, though not statistically significant. Rosenstein *et al* [34] had also found a higher proliferative index in unicystic type than in the solid type but this does not translate to higher aggressiveness in the unicystic type.

A similar study we conducted showed that MMP-2 may also be expressed to a similar degree in certain non-invasive inflammatory lesions. Thus, MMP-2 may not be singularly useful in predicting aggressiveness/invasion in ameloblastoma and its clinical types. There was a statistically significant difference in the mean expression of TIMP2 between the clinical types, in which the conventional ameloblastoma had less expression than the unicystic ameloblastoma. This seems to indicate increasing inhibition of MMP2 by TIMP2 in the solid and unicystic clinical types respectively, supporting that unicystic type is the least invasive.

Previous studies have indicated that TIMP2 may not be independently useful in predicting invasive property in ameloblastoma. [35,36] However compared to MMP-2 expression, the pattern of mean TIMP-2 expression shown in this study is in keeping with the biologic behaviours of the clinical types and may thus be useful in predicting their aggression/ invasiveness. Consequently, the mean MMP2:TIMP2 was higher (approaching 2:1) in conventional type than the unicystic type which was close to a 1:1 ratio with a slight excess of TIMP-2 in unicystic ameloblastoma, indicating slight inhibition to invasiveness. In both clinical

types, the ratio of MMP2:TIMP2 were in favour of local invasion. [36] This is also consistent with the flipside role of TIMP2, in which, a stoichiometric ratio of MMP2:TIMP2 equal to 1:1 makes TIMP2 contribute to the further activation of MMP2, observed in invasive lesions as established in reviews and models given by Polette et al [35] and Lu et al. [36] They explain that the role of TIMP2 in inhibiting MMP2 has to be taken in consideration with other factors like MT-MMP (MMP14) in stoichiometric proportions and its actions are thus more complex.

In this study, the weak positive (direct) correlations between MMP2 and TIMP2 expressions observed in both unicystic and conventional types of ameloblastoma largely follows the pattern of the diagrammatic model depicted by Lu *et al*. [36] He presented the relationship between the MMP2/MT1-MMP and TIMP2 expression as studied in glioblastoma, a highly invasive tumour. He explained that MMP2 activation initially increases as TIMP-2 increases until TIMP-2 reaches the optimum for maximal MMP2 activation. Thereafter, increases in TIMP2 are inhibitory to activation of pro MMP2, and at higher levels, inhibitory to MMP-2 activity.

CONCLUSION:

We conclude that MMP-2: TIMP-2 is a useful parameter which shows that TIMP-2 contributes to the activation of MMP2 in the clinical types of ameloblastoma at the expression levels observed. Also, the direct correlation between TIMP-2 and MMP-2 in both conventional and unicystic ameloblastoma depicted by the scatter plots in our study, appear to approach a plateau of saturated activation of MMP-2 by TIMP-2, at which point, we suggest that exogenous inhibitors of matrix metallo-proteinases may be explored as adjuvant therapy to provide necessary inhibitory activity. Futhermore, TIMP-2 expression is a better singular indicator of biologic aggression in the clinical types of ameloblastoma than MMP-2 expression.

Acknowledgement- The authors appreciate the technical support of the laboratory staff of the Breast Cancer Research Laboratory of the Institute of Advanced Medical Research and Training, University of Ibadan, Nigeria.

Conflict of Interest-The authors declare no conflict of interest in the conduct of this research.

Funding- This research received no specific grant from any funding agency in the public, commercial or not- for- profit sectors.
Guarantor- Not applicable.
Contributor - Not applicable.

REFERENCES

1. Regezi JA Scuibba J, Jordan C. (Eds.). Odontogenic Tumors. Oral Pathology:Clinico pathologic correlations. 5th edition, Saunders Elsevier; 2003: 265-270.
2. Gruica B, Stauffer E, Buser D, Bornstein M. Ameloblastoma of the follicular, plexiform, and acanthomatous type in the maxillary sinus: a case report. Quintessence Int. 2003;34(4):311-314.
3. Junquera L, Ascani G, Vicente JC, Garcia-Consuegra L, Roig P. Ameloblastoma revisited. The Annals of Otology, Rhinology, and Laryngology. 2003;112(12):1034-1039.
4. Fletcher DM. (Ed.). Odontogenic Tumors. Diagnostic Histopathology of Tumors. 2nd edition, Churchill livingstone; 2000: 217-220.
5. Soluk-Tekkesin M, Wright JM. The World Health Organization Classification of Odontogenic Lesions: A Summary of the Changes of the 2017 (4th) Edition. Turkish Journal of Pathology. 2017; 34(1):1-18
6. Li TJ, Kitano M, Arimura K, Sugihara K. Recurrence of unicystic ameloblastoma: a case report and review of the literature. Archives of Pathology & Laboratory Medicine. 1998;122(4):371-374.
7. Ackermann GL, Altini M, Shear M. The unicystic ameloblastoma: a clinicopathological study of 57 cases. Journal of Oral Pathology. 1988;17(9-10):541-546.
8. Barnes L EJ, Reichart P, Sidransky D. (Eds.). World Health Organization Classification of Tumors.Pathology and Genetics of Head and Neck Tumors. Lyon: IARC Press; 2005.
9. Salo T, Kainulainen T, Parikka M, Heikinheimo K. Expression of laminin-5 in ameloblastomas and human fetal teeth. Journal of Oral Pathology & Medicine. 1999;28(8):337-342.
10. Mitchell R N KV, Abass A.K, Fausto N. (Eds.).Pocket companion to Robbins and Cotran Pathologic Basis of Disease. 7th edition ed.

Pennsylvania,United States of America: Saunders Elsevier; 2006.
11. Philipsen HP, Reichart PA, Takata T. Desmoplastic ameloblastoma (including "hybrid" lesion of ameloblastoma). Biological profile based on 100 cases from the literature and own files. Oral Oncology. 2001;37(5):455-460.
12. Shashikanth MC, Neetha MC, Ali IM, Shambulingappa P. Desmoplastic ameloblastoma in the maxilla: a case report and review of literature. Indian Journal of Dental Research. 2007;18(4):214-217.
13. Eversole LR, Leider AS, Hansen LS. Ameloblastomas with pronounced desmoplasia. Journal of Oral and Maxillofacial Surgery. 1984;42(11):735-740.
14. Olaitan AA, Adekeye EO. Unicystic ameloblastoma of the mandible: a long-term follow-up. Journal of Oral and Maxillofacial Surgery. 1997;55(4):345-348; discussion 349-350.
15. Curi MM, Dib LL, Pinto DS. Management of solid ameloblastoma of the jaws with liquid nitrogen spray cryosurgery. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics. 1997;84(4):339-344.
16. Philipsen HP, Reichart PA, Nikai H, Takata T, Kudo Y. Peripheral ameloblastoma: biological profile based on 160 cases from the literature. Oral Oncology. 2001;37(1):17-27.
17. Ikemura K, Tashiro H, Fujino H, Ohbu D, Nakajima K. Ameloblastoma of the mandible with metastasis to the lungs and lymph nodes. Cancer. 1972;29(4):930-940.
18. Kumamoto H. Molecular pathology of odontogenic tumors. Journal of Oral Pathology & Medicine. 2006;35(2):65-74.
19. Heikinheimo K, Jee KJ, Niini T, Aalto Y, Happonen RP, Leivo I, et al. Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. Journal of Dental Research. 2002;81(8):525-530.
20. Heikinheimo K, Morgan PR, Happonen RP, Stenman G, Virtanen I. Distribution of extracellular matrix proteins in odontogenic tumours and developing teeth. Virchows Archiv B, Cell pathology including Molecular Pathology. 1991;61(2):101-109.
21. Coussens LM, Werb Z. Matrix metalloproteinases and the development of cancer. Chemistry & Biology. 1996;3(11):895-904.
22. Wang Y, Xu HT, Ueda Y, Shimasaki M, Wang EH. Activation ratio of MMP-2 and expression of MT1-MMP are correlated in thymic epithelial tumours. Pathology. 2007;39(5):486-490.
23. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, et al. Matrix metalloproteinases: a review. Critical reviews in Oral Biology and Medicine. 1993;4(2):197-250.
24. Wang A, Zhang B, Huang H, Zhang L, Zeng D, Tao Q, et al. Suppression of local invasion of ameloblastoma by inhibition of matrix metalloproteinase-2 in vitro. BMC Cancer. 2008;8:182.
25. Bast BT, Pogrel MA, Regezi JA. The expression of apoptotic proteins and matrix metalloproteinases in odontogenic myxomas. Journal of Oral and Maxillofacial Surgery. 2003;61(12):1463-1466.
26. Stetler-Stevenson WG. The tumour microenvironment: regulation by MMP-independent effects of tissue inhibitor of metalloproteinases-2. Cancer Metastasis Reviews. 2008;27(1):57-66.
27. Siqueira AS, Carvalho MR, Monteiro AC, Freitas VM, Jaeger RG, Pinheiro JJ. Matrix metalloproteinases, TIMPs and growth factors regulating ameloblastoma behaviour. Histopathology. 2010;57(1):128-137.
28. Zhang B, Zhang J, Xu ZY, Xie HL. Expression of RECK and matrix metalloproteinase-2 in ameloblastoma. BMC Cancer. 2009;9:427.
29. Fregnani ER, Sobral LM, Alves FA, Soares FA, Kowalski LP, Coletta RD. Presence of myofibroblasts and expression of matrix metalloproteinase-2 (MMP-2) in ameloblastomas correlate with rupture of the osseous cortical. Pathology Oncology Research. 2009;15(2):231-240.
30. Zhong M, Han YP, Wang J, Li ZJ, Bao G, Yue YL. [Expression of matrix metalloproteinases and tissue inhibitor of metalloproteinase in ameloblastoma]. Shanghai Journal of Stomatology. 2003;12(6):427-431.
31. Zhang B, Zhang J, Huang HZ, Xu ZY, Xie HL. Expression and role of metalloproteinase-2 and endogenous tissue regulator in ameloblastoma. Journal of Oral Pathology & Medicine. 2010;39(3):219-222.
32. Abcam plc. Anti-MMP-2 antibody [6E3F8](ab86607). www.abcam.com.Downloaded on 20th February 2013.
33. Abcam plc. Anti-TIMP2 antibody [3A4] (ab1828).www.abcam.com.Downloaded on 20th February 2013.

34. Rosenstein T, Pogrel MA, Smith RA, Regezi JA. Cystic ameloblastoma--behavior and treatment of 21 cases. Journal of Oral and Maxillofacial Surgery. 2001;59(11):1311-1316; discussion 1316-1318.
35. Polette M, Birembaut P. Membrane-type metalloproteinases in tumour invasion. The International Journal of Biochemistry & Cell Biology. 1998;30(11):1195-1202.
36. Lu KV, Jong KA, Rajasekaran AK, Cloughesy TF, Mischel PS. Upregulation of tissue inhibitor of metalloproteinases (TIMP)-2 promotes matrix metalloproteinase (MMP)-2 activation and cell invasion in a human glioblastoma cell line. Laboratory investigation; a Journal of Technical Methods and Pathology. 2004;84(1):8-20.