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EXPRESSION OF COLLAGEN IV AND MATRIX METALLOPROTEINASE-9 IN AMELOBLASTOMA AND DENTAL GERM

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ABSTRACT

Introduction: collagen IV is a component of the basement membrane (BM) in all stages of tooth development. Destruction of the basement membrane collagen IV occurs in most odontogenic neoplasms. Matrix metalloproteinase-9 (MMP9) is an important enzyme that plays a pivotal role in the destruction of collagen IV. This study was aimed to compare the expression of collagen IV and MMP9 in different histopathological types of ameloblastoma as well as their various biological behaviors with dental germ.

Materials and methods: among 31 ameloblastoma and 10 dental germ samples, expression of MMP9 in stromal and parenchymal regions of ameloblastoma and dental germ was investigated. Also, expression of COIIV in the basement membrane of ameloblastoma neoplastic epithelium and the basement membrane around inner enamel epithelium (IEE) and outer enamel epithelium (OEE) of dental germ was analyzed by immunohistochemical technique.

Results: collagen IV in the basement membrane of ameloblastoma epithelial islands in most of the samples showed $<50\%$ staining as thin and discontinuous. Expression of collagen was lower in the samples with higher expression of MMP9. There was only a significant correlation between MMP9 expression at epithelial surface and MMP9 expression in the stroma around epithelium with $<50\%$ and $>50\%$ staining rates for collagen IV, respectively ($p<0.05$). Expression of MMP9 at stromal region was higher than that of the parenchymal neoplastic ameloblastoma in most of the samples, but ameloblastoma did not indicate a significant difference in different histopathological types.

MMP9 expression in the epithelial and stromal regions far from neoplastic ameloblastoma did not show a direct correlation with presence of inflammation ($p<0.05$). Further, MMP9 did not show any expression in the dental germ. Collagen IV in the basement membrane of OEE and IEE, except in small areas, was expressed as continuous and thick, which was higher than ameloblastoma. However, only a significant correlation was obtained for the higher consistency of collagen IV in the basement membrane of OEE than in ameloblastoma ($p<0.05$).

Conclusion: higher expression of matrix metalloproteinase-9 (MMP9) and inconsistency of collagen IV in ameloblastoma compared with dental germ may be due to different biological behaviors than dental germ. Moreover, lack of difference in the expression of MMP9 and collagen IV in various histopathological types of ameloblastoma and lack of correlation between expression of MMP9 and collagen IV in all groups may be indicative of lack of correlation between these two variables and biological behavior of different histopathological patterns of ameloblastoma.

Keywords: ameloblastoma, tooth germ, MMP9, collagen IV, immunohistochemical

INTRODUCTION

Ameloblastoma is a tumor of epithelial origin with slow growth that comprises approximately 1% of oral mucosa tumors and 18% of odontogenic tumors [1]. Ameloblastoma is one of the most common odontogenic tumors with odontogenic epithelial origin [1]. Odontogenic tumors originate from tooth-constituting tissues. Ameloblastoma is a slow-growing and locally-aggressive tumor which in most cases has a benign course [1]. It can also exhibit a high level of recurrence and metastasis, which is considered a unique behavior. That is why ameloblastoma, its biologic behavior and the factors affecting it have dramatically attracted many researchers [2, 3]. The role of various biomarkers in different stages of diagnosis, development and prognosis of this tumor has been investigated.

Collagen IV plays an important role in cell attachment, migration control, cell differentiation and cell growth. This collagen can be damaged during physiologic and pathologic processes. These processes include tissue remodeling, angiogenesis and tumor development [4, 5]. Collagen IV is a component of basement membrane (BM) that is involved in all tooth development processes. Further, changes and remodeling of basement membrane collagen IV occur in most odontogenic neoplasms [6].

Basement membrane is a disk-shaped structure of extracellular matrix that provides a protective structure on endothelial and epithelial cells. Most of the studies have shown the significance of protecting the basement membrane and its destruction during different stages of aggressive carcinoma [7, 8]. Evidence shows that basement membrane play a pivotal role in tumors by releasing the mediators that participate in oncogenesis, and collagen IV α chain 1 plays a key role in cytodiffrentiation and tumor development in ameloblastoma [9].

Matrix metalloproteinases (MMPs) are a large family of calcium-dependent zinc-containing endopeptidases that are able to reduce the extracellular matrix (ECM) and basal layer, and are involved in physiologic and pathologic processes. If produced in low physiologic levels, matrix metalloproteinases (MMPs) can play a role in such processes as structural and functional modification of extracellular matrix and biomineralization of dentine and enamel. However, overexpression of matrix metalloproteinases (MMPs) results in imbalance of their activators and inhibitors, which is followed by pathologic processes. On the other hand, overexpression of matrix metalloproteinases (MMPs) in pathologic conditions destroys extracellular matrix and basement membrane and accelerates tumor growth, invasion and metastasis [10]. Matrix metalloproteinases (MMPs) are produced by both epithelial and mesenchymal cells [11]. Among MMPs, MMP9 is the most important proteinase associated with bone loss [12].

Researches carried out about epigenetic regulation of matrix metalloproteinases (MMPs) in ameloblastoma have shown that ameloblastoma local invasion is associated with MMP2 and MMP9 and overexpression of MMP9 in ameloblastoma with lack of methylation of the related gene may be associated with the invasive behavior of ameloblastoma and its frequent recursions [13].

Although ameloblastoma has an unknown etiology, it is believed that it is made of the epithelium covering dental follicle [14]. The epithelial components of ameloblastoma are similar to the components of developing dental germ [15]. Histologically, the epithelium of ameloblastoma is similar to the enamel of developing dental organ. The cylindrical cells around the follicular tumor are arranged the same as those of ameloblastoma. The central cells of follicular tumor are also similar to the star-shaped reticulum of dental germ [16].

Cell differentiation of epithelial tumor cells in ameloblastoma has been determined by different biomarkers with development and identification of molecular mechanisms in the cells. Therefore, pathological comparison of ameloblastoma and dental germ by the abovementioned biomarkers can be quite helpful in their diagnosis, development and prognosis.

Since no study has evaluated the correlation of MMP9 and COLIV expression in ameloblastoma and dental germ so far, the present study was aimed to compare immunohistochemical expression of MMP9 and COLIV in different histopathological types of ameloblastoma and dental germ and to analyze the association of MMP9 with destruction of basement membrane and biological behavior of these lesions.

MATERIALS AND METHODS

In this retrospective descriptive-analytical study, the ameloblastoma and dental germ samples were studied. This study was carried out over a year from 2013 to 2014. The research proposal was reviewed and approved by the ethical committee and deputy of research at Islamic Azad University, Khorasegan branch. The ameloblastoma samples were obtained from the archive of pathology department of Shariati hospital, Tehran, and the dental germ samples were prepared from the autopsy embryos referred to Dr. Kariminegad and Jajmabadi laboratory, Tehran.

The clinical information, including age, gender and lesion site were analyzed. A 5μ section was prepared for haematoxylin-eosin staining. Having observed haematoxylin-eosin staining slides, the slides with sufficient tissue and appropriate fixation were selected. The histological samples with improper fixation, lesions with extensive inflammation, extensive bleeding and high inflammation were excluded from the study. Diagnosis of ameloblastoma tumor was performed according to the World Health Organization classification in 2005 as well as other studies [17, 18, 19].

MMP9 expression in stromal and parenchymal regions of ameloblastoma and dental germ were analyzed. Also, collagen IV expression in the basement membrane of ameloblastoma neoplastic epithelium and the basement membrane around the enamel organ (outer enamel epithelium and inner enamel epithelium) of dental germ was analyzed by immunohistochemical staining method. Expression levels of the mentioned biomarkers were compared in ameloblastoma and dental germ.

Immunohistochemical staining techniques

Immunohistochemical staining was performed by Envision method using MMP9 monoclonal antibody (Biogenex co, U.S.) and collagen IV monoclonal antibody (Dako co, U.S.). 3μ sections were prepared from the blocks and placed on the glass slides. During deparaffinization and dehydration process, the slides were placed in the thermal oven (60 °C) for 30 minutes and then put in two xylol containers for 5 minutes and three descending ethanol containers (100%, 80% and 65%, respectively) for 2 minutes for the tissues to dehydrate. The slides were then placed in 3% hydrogen peroxide and washed with distilled water after 5 minutes.

In antigen retrieval stage for matrix metalloproteinase-9 (MMP9), the slides were put in citrate buffer (pH=6) in microwave for 25 minutes at 95 °C and MMP9 primary antibody was added and incubated at ambient temperature for 30 minutes. For collagen IV marker, the slides were placed in k proteinase enzyme for 40 minutes and collagen IV primary antibody was poured on the tissue and incubated at ambient temperature for 60 minutes. The slides for collagen IV and

MMP9 antibodies were washed with Tris-buffered saline (TBS) and each group of slides was incubated for 1 hour at room temperature and then washed with Tris-buffered saline (TBS). Next, diaminobenzoic acid (DAB) chromogen was poured on the slides and after 5 minutes incubation at room temperature, the slides were washed with distilled water, and hematoxylin-eosin staining was performed for background staining. During Envision stage, the secondary antibody or Envision was poured on the slides and kept at room temperature for 30 minutes and the slides were then washed with Tris-buffered saline (TBS). Finally, the slides were mounted with Entellan glue and observed by optic microscope. Omission of primary antibody was employed as negative control, while tissue of long and skin were used as positive control for MMP9 and collagen IV.

Having stained the slides, they were evaluated by two pathologists using an optic microscope. According to the techniques presented by Gomes Henrix, collagen IV staining in basement membrane between epithelium and mesenchyme was analyzed. The percentage of collagen IV staining along the basement membrane was analyzed semi-quantitatively and presented as >50% or ≤50%. Also, the collagen IV staining pattern was determined in terms of continuity (continuous, non-continuous) and thickness (thin, thick, mixed) [20].

The expression of matrix metalloproteinase-9 (MMP9) was separately investigated in epithelium and mesenchyme. In mesenchyme, the staining of endothelial cells, fibroblasts and inflammatory cells was observed. The percentage of MMP9 staining was determined semi-quantitatively by the method presented by Gong [21]. In this technique, the staining percentage is determined by three scores; 0 (<10% staining), 1 (10-50% staining) and 2 (>50% staining). Brown cytoplasm was considered as positive staining. The severity of inflammation was also determined as + (low inflammation), ++ (moderate inflammation) and +++ (high inflammation).

Statistical analysis

The obtained data were analyzed by SPSS (version 20) software using chi-square, Mann-Whitney, Fisher's exact test and Tukey test.

RESULTS

In this study, 31 ameloblastoma samples and 10 dental germ samples were analyzed. The ameloblastoma samples were taken from the patients with the age range of 12-72 and mean age of 71±12.872. The dental germ samples were related to the late bell stage and the embryos were aged 14-24 weeks.

From the 31 paraffin blocks of ameloblastoma, 27 samples belonged to posterior mandible, 2 samples belonged to maxilla, 1 sample belonged to temporal and 1 sample belonged to soft palate. Of 10 dental germ samples, 50% were from mandible and 50% were from maxilla. Histopathologically, all ameloblastoma samples included different unicystic ameloblastoma patterns (15 samples), multicystic or solid (16 samples). The solid type comprised of 8 samples with follicular pattern and 8 samples with plexiform pattern. Among the samples, 5 of them were recurrent.

The expression level of matrix metalloproteinase-9 (MMP9) in dental germ was reported to be 0%. The expression frequency of matrix metalloproteinase-9 (MMP9) in epithelium and adjacent and far stroma is presented in Table 1. The detailed expression of matrix metalloproteinase-9 (MMP9) according to different characteristics of ameloblastoma is provided in Table 2.

The expression levels of collagen IV in pathologic samples of ameloblastoma and dental germ were reported to be 100% (10.10) and 58.1% (31.18), respectively (p=0.01). The expression levels of collagen IV at the basement membrane in terms of continuity (continuous or discontinuous) and thickness (thin, thick, mixed) in pathologic samples of ameloblastoma and dental germ are presented in Table 2.

Table1. Frequency [n (%)] of immunohistochemical expression of matrix metalloproteinase-9 (MMP9) in epithelium and adjacent and far stroma of ameloblastoma

Immunohistochemical expression of MMP9	Score 0	Score 1	Score 2	P value
Epithelial	20	8	3	
Adjacent stroma	13	9	9	0.2
Far stroma	13	12	6	

Table2. Frequency [n (%)] of immunohistochemical expression of matrix metalloproteinase-9 (MMP9) according to different characteristics of ameloblastoma

Immunohistochemical expression of MMP9	Score 1	Score 2	Score 3	P value
Sex				
-Male	15	12	6	0.725
-Female	28	17	12	
-Total	43	29	18	
Location of tumor				0.158
-Mandible	41	25	15	

-Maxilla	1	2	3	
-Soft palate	3	0	0	
-outside jaw	1	2	0	
Inflammation(epithelium)				
-0	11	5	1	.023
-+	2	0	0	
-++	0	1	2	
-+++				
Inflammation(adjacent stroma)				
-0	7	3	4	.061
-+	0	3	3	
-++	0	1	2	
-+++				
Inflammation(far stroma)				
-0	6	5	1	.049
-+	1	2	3	
-++	0	3	2	
-+++				

Table2. Expression [n (%)] of collagen IV and its patterns (continues or discontinues) and thickness at the basement membrane of ameloblastoma and dental germ [outer enamel epithelium (OEE) and inner enamel epithelium (IEE)]

	Ameloblastoma n=31	Dental germ (OEE) n=10	Dental germ (IEE) n=10	P value
Expression				
-< 50% staining	8	0	0	0.02
->50% staining	10	10	10	
Staining pattern				
- Continuous	3	10	9	*1.000 **.024
- Discontinuous	12	0	1	
- Mixed (continuous & discontinuous)	3	0	0	
Thickness				
-Thin	13	9	7	1.000 .148
-Thick	1	1	3	
- Mixed (thin and thick)	4	0	0	

*** Ameloblastoma & IEE, ** Ameloblastoma & OEE**

DISCUSSION

In this study, collagen IV and matrix metalloproteinase-9 (MMP9) expression in different histopathological types of ameloblastoma and dental germ was analyzed and compared. The findings of the study showed a higher expression of matrix metalloproteinase-9 (MMP9) and more discontinuity of collagen IV in ameloblastoma than in dental germ. MMP9 expression in different histopathological types of ameloblastoma was not reported to be significantly different. Collagen IV expression was significantly higher in dental germ than in ameloblastoma. The staining level of collagen IV in dental germ was not significantly different than ameloblastoma (higher staining in dental germ).

The findings of previous studies show that collagen IV αchain is destroyed in the early invasion stages of many cancers. On the other hand, evidence is indicative of the production of cancer cells in the early stages of invasion and metastasis through destruction of basement membrane, which is caused by the effect of matrix metalloproteinases that are produced from cancer cells as well as reduction of inhibitors of matrix metalloproteinases.

Expression of matrix metalloproteinases (MMPs) is regulated by inflammatory cytokines, growth factors, and cell-cell and cell-matrix interaction. Also, endogenous tissue inhibitors of MMP (TIMP subtypes) inhibit their production. In fact, the balance between metalloproteinases (MMPs) and their tissue inhibitors is a vital factor for maintaining the normal and remodeling conditions of the tissue [22, 23, 24].

In addition, various studies, analyzing the role of collagens, have shown that except the mentioned mechanisms, hypermethylation of collagen chains in COL4A5 and COL4A6 regions can be responsible for the lost expression of α5(IV)/α6(IV) chains and remodeling of basement membrane along the invasion of cancer cells [25]. As for the developing neoplastic odontogenic lesions, modification of basement membrane collagen IV has also been observed [26]. Since reduced expression of collagen α5(IV)/α6(IV) chains has been seen in many cancers, it seems necessary to evaluate the role of these biomarkers in odontogenic lesions [27].

Since the exact molecular mechanism of ameloblastoma invasion has not been determined so far, and given the possible role of matrix metalloproteinases in this regard and their destructive effect on collagen IV, one of the main constituents of basement membrane, the current study was conducted to evaluate the role of the above factors in the invasive activity of ameloblastoma and to anticipate their biological behavior.

Various studies have assessed the role of collagen IV in this regard. Nagai et al carried out a study on collagen α chains (IV) in different stages of mice dental development and showed that the location and distribution of collagen IV α chains during the development of molar tooth germ was essential for morphogenesis and cell differentiation [28].

Some studies analyzed the distribution of collagen IV α chains in ameloblastoma and dental germ and reported that collagen alpha1 (IV), alpha 2 (IV), alpha 5 (IV) alpha 6 (IV) chains were distinctively expressed around neoplastic epithelial islands without discontinuity. They were also reported to be well-expressed in dental germ at IEE of alpha1 (IV), alpha 2 (IV), and alpha 4 (IV) in the basement membrane, and in OEE of alpha1 (IV), alpha 2 (IV), alpha 5 (IV), and alpha 6 (IV) chains at basement membrane. The authors suggested that distribution of collagen IV in the basement membrane of ameloblastoma played an important role in cell differentiation and development.

In another study, Kou et al investigated the distribution of collagen IV in ameloblastoma and tooth germ by indirect immunofluorescence and suggested that collagen IV can play a pivotal role in cell differentiation and development [30].

In their analysis of odontogenic tumors by histochemical and immunohistochemical staining techniques, Hengelbruke et al [31] analyzed 19 samples of ameloblastic odontogenic tumors, ameloblastic fibroma, ameloblastic fibro-odontoma, odontogenic myxoma and odontogenic fibroma by H&E, Picrosirius, Reticulin and Masson Trichrome and immunoperoxidase staining techniques. They assessed the distribution of collagen I-IV in each lesion and reported that collagen IV was not expressed in odontogenic tumors except around the blood vessels.

In the current study, collagen IV expression in dental germ was significantly higher than that of ameloblastoma. Collagen IV staining in tooth germ was significantly different than that of ameloblastoma (higher staining in dental germ). Although staining continuity and thickness of collagen IV in ameloblastoma was more discontinuous and thinner than in dental germ, no statistically significant difference was found between them. This seems to be due to a small sample size in this study.

Gomes Henriques et al [32] carried out a comparative analysis of collagen IV and MMP9 expression in odontogenic cysts and tumors by immunohistochemical staining technique. Collagen IV staining in most radicular and dentigerous cysts was reported to be continuous in epithelial basement membrane, whereas KOTs and ameloblastoma displayed a discontinuous and mixed (thick-thin) staining pattern. There was a significant difference in the collagen IV expression among different lesions, and the minimum collagen immunostaining (≤50%) was reported for ameloblastoma. Also, in the present study from the 18 collagen IV samples, 10 samples were reported to have

>50% staining with discontinuous and thin pattern in most cases. In the analysis of histopathological types of ameloblastoma, no significant difference was observed; however, a significant difference was reported for dental germ where collagen IV expression with a thick and continuous pattern was seen in all samples.

The results of the analysis of matrix metalloproteinase-9 (MMP9) expression revealed a higher expression of MMP9 in mesenchymal keratocystic odontogenic tumor than in dentigerous and radicular cysts. This can be a reason for the more invasive behavior and higher development potential of keratocystic odontogenic tumor than other odontogenic tumors. Moreover, the findings of this study indicated no significant difference for MMP9 expression in epithelial keratocystic odontogenic tumor, dentigerous cyst, radicular cyst and ameloblastoma, but MMP9 expression in mesenchymal keratocystic odontogenic tumor and ameloblastoma was significantly higher than that of dentigerous and radicular cysts. Further, for MMP9 expression in ameloblastoma, staining was more observed in peripheral cells and neoplastic epithelial islands. In the present study, MMP9 expression in ameloblastoma was higher than in dental germ, and MMP9 expression in far and adjacent stroma was higher than that of epithelium, which showed no significant difference between different histopathological types of ameloblastoma. In the epithelial region, the peripheral cells and star-shaped reticulum of neoplastic epithelial islands around these cells were reported to have a higher level of staining. Moreover, the results of analyses in the current research showed no significant correlation between MMP9 and COLIV expression in all histopathological groups of ameloblastoma.

Furthermore, [31] showed that MMP9 expression in mesenchymal and epithelial components in 100% of lesions was reported to be dispersed, and there was a little difference in the staining pattern of the stained cells. Also, MMP9 staining in the mesenchymal region for fibroblasts, inflammatory cells and endothelial cells, the maximum level of staining was reported for ameloblastoma in >50% of cases. Unlike the results of the present study, there was a significant correlation between the inflammatory cells in the epithelium and stroma far from epithelial islands and MMP9 expression in the same region (epithelial and stroma far from epithelium), and no significant association was seen between inflammatory cells in the stroma adjacent to epithelial islands and MMP9 expression in this region.

Kumamoto et al [33] examined the immunohistochemical expression of matrix metalloproteinase-1, -2 and -9, TIMP-1 and TIMP-2 in ameloblastoma and dental germ. The frozen histological sections of tooth germ and ameloblastoma samples were evaluated by immunohistochemical staining technique. The results showed that matrix metalloproteinases-1, -2 and -3, TIMP-1 and TIMP-2 were highly expressed in the mesenchymal components (dental papilla and dental follicle) of dental germ and ameloblastoma; indicating a higher expression for matrix metalloproteinase-9 (MMP9) in ameloblastoma stromal cells than in dental papilla and dental follicle mesenchymal cells. Dental lamina indicated a poor expression of matrix metalloproteinase-2 and matrix metalloproteinase-9. Parenchymal or epithelial area of ameloblastoma showed a poor expression of matrix metalloproteinase-2, matrix metalloproteinase-9 and TIMP-1 in all ameloblastoma samples. In line with this study, in our study the samples with MMP9 expression obtained a higher score than those of epithelial region, which showed no significant difference among different histopathological types of ameloblastoma, with minimum MMP9 expression in epithelium. In contrast to the results of Kumamoto et al, however, no expression of MMP9 was observed in both dental germ in mesenchymal stroma (dental papilla and dental follicle) and parenchymal region (dentallamina).

Matrix metalloproteinase-9 expression in dentigerous cyst, keratocystic odontogenic tumor and ameloblastoma was evaluated by Gheisari et al [34] through immunohistochemical staining technique using Envision method. Parenchymal (epithelial) MMP9 expression in solid ameloblastoma, unicystic ameloblastoma and keratocystic odontogenic ameloblastoma was higher than that of parenchymal (epithelial) dentigerous cyst, and statistical analyses indicated only a significant difference between dentigerous cyst and different types of ameloblastoma. Further, MMP9 expression in the stroma of most solid ameloblastoma and keratocystic odontogenic samples was higher than that of unicystic ameloblastoma and dentigerous cysts. The results also showed a statistically significant difference between dentigerous cyst and other lesions under study. MMP9 expression in the stroma and parenchyma of all dental follicular samples was less than those of the other samples. Moreover, the findings revealed that higher expression of MMP9 can be one of the reasons for more aggressive behavior of ameloblastoma and higher recurrence of keratocystic odontogenic tumors. However, in our study no significant difference was reported for MMP9 expression among various histopathological types, unicystic, solid, recurrent and non-recurrent, and follicular and plexiform subtypes. MMP9 expression in stromal region was also higher than parenchymal region, which showed no significant difference in all ameloblastoma groups and was in contrast with the findings of our study.

However, compared with dental follicle, no MMP9 expression was found in dental germ in our study. Further, in previous studies no comparison has been made between MMP9 and COLIV expression in ameloblastoma and tooth germ, but in the present study MMP9 expression in ameloblastoma was higher than that of dental germ and most of the dental germ samples displayed a higher level of collagen expression, especially in OEE than in ameloblastoma. Thus, it seems that MMP9 expression is directly correlated with lower expression of collagen IV in ameloblastoma and dental germ.

CONCLUSION

Matrix metalloproteinase-9 (MMP9) expression and collagen IV inconsistency in ameloblastoma may be one of the reasons for different biological behaviors of ameloblastoma than dental germ. Lack of difference in MMP9 and collagen IV expression in different pathological types of ameloblastoma and lack of correlation between MMP9 and collagen IV expression in all groups may be indicative of lack of association between these two variables and biological behavior of various histopathological patterns of ameloblastoma.

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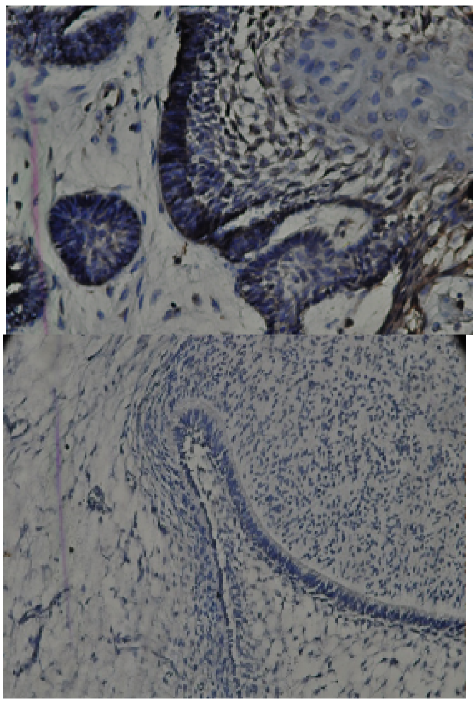

Figure 1. A. MMP9 expression of follicular ameloblastoma with acanthomatous changes- In central regions, MMP9 expression at satellited reticulum except acanthomatous regions of the center of islands and at basal layer around epithelial islands indicated score 2 and the stromal region adjacent to epithelial islands showed score 1. B. Dental germ of cervical loop (IHC staining, ×400)

Non-expression of MMP9 at dental papilla (upper right hand), non-expression of MMP9 at enamel organ of satellited reticulum, inter enamel epithelial and outer enamel epithelial (center of image) and non-expression of MMP9 at dental follicle (left side) (IHC staining, ×200)

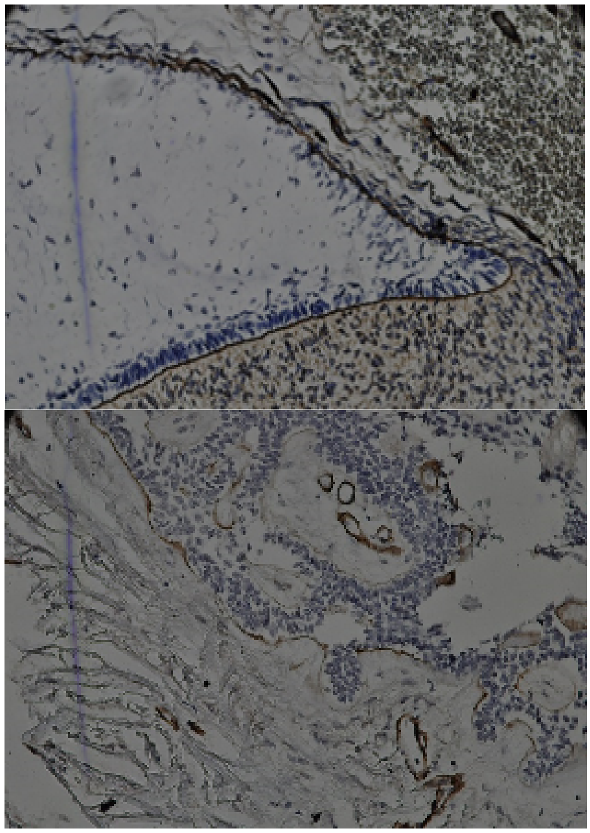

Figure 2. Expression of collagen IV: A. Dental germ- positive staining of collagen IV: Basement membrane of outer enamel epithelial adjacent to satellited reticulum dental follicle and basement membrane of inter enamel epithelial adjacent to starred reticulum and dental papilla, stained continuously and thickly. B. Follicular ameloblastoma- Staining pattern of collagen at basement membrane, shown as non-continuous and thin.