

Expression of Matrix Metalloproteinases 2 and 19 in Odontogenic Keratocysts and Dentigerous Cysts

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Abstract

Background and Aim: Odontogenic keratocyst (OKC) and dentigerous cyst (DC) are two common developmental cysts involving the jaws. The role of matrix metalloproteinases 2 (MMP2) and 19 (MMP19) in progression and invasion of some cysts and tumors has been documented. This study sought to assess the expression of MMP2 and MMP19 in OKCs and DCs.

Materials and Methods: In this descriptive analytical study, 58 paraffin blocks including 20 DCs, 20 OKCs and 18 dental follicles (DF) were chosen from the archives of the Oral and Maxillofacial Pathology Department, Shahid Beheshti University of Medical Sciences. Immunohistochemistry (IHC) staining was performed to detect the expression of MMP2 and MMP19 using the EnVision technique. Data were analyzed using the Chi square, Fisher's exact, Kruskal Wallis, Mann Whitney and Spearman's correlation tests.

Results: Both markers were expressed in OKCs and DCs. Expression of MMP19 was higher in OKCs compared to DCs and DFs. Significant differences existed among the groups in the intensity of staining of the epithelium ($P<0.05$) and the connective tissue ($P<0.001$) for MMP19. Expression of MMP2 in DCs was higher than in OKCs and DFs. Significant differences existed among the groups in the intensity and percentage of staining of the epithelium ($P<0.001$) and the connective tissue ($P<0.05$) for MMP2.

Conclusion: Our findings indicated the potential role of MMP19 in the invasive behavior of OKC. Greater expression of MMP2 in DCs may indicate its role in multi-dimensional growth of this cyst. Nevertheless, further studies are required to make an evidence-based decision.

Key Words: Odontogenic Cysts, Dentigerous Cyst, Matrix Metalloproteinases

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Introduction

Odontogenic cysts are highly prevalent oral lesions among which, OKC and DC are very common [1]. Odontogenic keratocyst (OKC), recently known as keratocystic odontogenic tumor, is among the important odontogenic developmental cysts

requiring special attention due to its unique clinical and histological characteristics. A case of mandibular OKC invading the base of skull with an aggressive behavior mimicking that of low grade squamous cell carcinoma (SCC) has been reported in the literature, which shows the complex

behavior of this cyst and the resultant complications [2]. Thus, it is important to find a method for early detection of this tumor. Immunohistochemical markers that can reliably confirm the presence of OKC can greatly help in this respect [3].

Dentigerous cyst (DC) is the most common odontogenic developmental cyst accounting for 25% of the cysts of the jaws [4]. Small cysts are often asymptomatic and are occasionally found on radiographs. However, they have the potential of extensive growth and can dislocate the adjacent teeth or the mandibular canal [5]. Also, DCs may cause bone resorption probably due to the release of some cytokines from the lesion [6]. Pathogenesis of DC has yet to be clearly understood. Although most DCs are considered developmental, some cases seem to have an inflammatory origin [5]. Its histopathological pattern is non-specific, resembling that of dental follicle (DF). The diagnosis is made based on clinical presentation of an unerupted tooth and attachment of the cyst to the cemento-enamel junction of the impacted tooth on radiographs and during surgery. Differential diagnosis of a DC from a normal or enlarged DF is difficult if relied solely on histopathological findings. Rarely, primary neoplastic changes may occur in the lining of the cyst such as SCC, ameloblastoma or mucoepidermoid carcinoma [7].

DF is composed of a fibro-vascular layer surrounding the dental papilla and enamel organ and contains a heterogeneous population of cells showing various phenotypes. Cystic changes in DF are seen in 46% of the cases even on a conventional radiograph [8].

Matrix metalloproteinases (MMPs) are a large family of zinc-endopeptidases that degrade the extracellular matrix (ECM) in developmental, proliferative, inflammatory or neoplastic processes. MMP2 degrades type IV collagen, which is the main constituent of the basement membrane and plays a role in stromal invasion by neoplastic cells [9]. MMP-19 is found in cutaneous fibroblasts, keratinocytes and inflammatory cells such as monocytes and macrophages, indicating its role in development of inflammatory processes and cutaneous tumors. MMP19 seems to play a role in some tissue regeneration processes related to

adipogenesis and tumor progression [10]. Down-regulation of MMP19 gene has been documented in nasopharyngeal carcinoma and catalytic activity of MMP19 appears to be necessary for its tumor suppressive and anti-angiogenic behavior in this carcinoma [11].

MMP19 is highly expressed in proliferating astrocytoma/glioma cells and can enhance the invasion of tumoral cells to the ECM [12].

Only a few previous studies investigated the expression of these markers in odontogenic lesions [13,14]

Still, the relationship between their expression and biological behavior of these odontogenic lesions has not been clearly identified. Based on the potential roles of MMPs such as promoting the invasive behavior or expansive processes [15], we hypothesized their possible role in OKC (as a bone invading odontogenic tumor) [16] and dentigerous cyst (DC) as a highly expansive odontogenic cyst [17].

Considering the existing controversies regarding the expression of these markers in odontogenic cysts and gap of information about their pattern of expression, this study sought to assess the expression of MMP2 and MMP19 in OKCs, DCs and DFs with inflamed subgroups.

Materials and Methods

This descriptive cross-sectional study was conducted on paraffin blocks of patients with OKC and DC presenting to the Oral and Maxillofacial Pathology Department of Shahid Beheshti University, Dental School between 2008-2012. A total of 58 cases were entered in the study including 20 DCs, 20 OKCs and 18 DFs (as controls). Age, sex, site of involvement and radiographs of patients were evaluated.

Sections (4µm in thickness) of formalin-fixed paraffin-embedded blocks were mounted on silane-coated slides (silanized s 3003; Dako, Copenhagen, Denmark), dewaxed in xylene, rehydrated in graded ethanol and then treated with 3% hydrogen peroxide. Antigen retrieval was carried out in 10 mM citrate solution (pH of 6.0) in a 750W microwave oven for 10 minutes.

The tissue specimen on the slides was then outlined by a pen and incubated for one hour in a screw-top container with the respective primary

antibody at a specific concentration, anti-MMP2 antibody (NCL: MMP2-507:1:40; Novocastra, London, England) and anti-MMP19 antibody (Prediluted Ab15477; Abcam, London, England). After one hour, the slides were immersed in rinse buffer with a pH of 7.5 for five minutes (the rinse buffer composed of one unit of salt, Tris, distilled water, hydrochloric acid and 4 units of distilled water). The EnVision solution was then added to the slides in the same screw-top container and the slides were then rinsed. Afterwards, one or two drops of DAB chromogen (Novocastra, London, England) at a ratio of 1 chromogen to 20 substrates were added to each slide to stain the above-mentioned complex. After two to three minutes, specimens were evaluated under a microscope and after ensuring their staining (cell cytoplasm became brown), they were rinsed with tap water for five minutes. The specimens were then immersed in hematoxylin and lithium carbonate for staining of the tissue matrix and rinsed with tap water after five minutes. They were then dehydrated in graded alcohol and cleared in xylol twice each time for five minutes. Specimens were then mounted. After drying, the slides were evaluated under a light microscope (Nikon, Tokyo, Japan) to determine the number of positively stained cells and the intensity of staining.

Gingival tissue served as the positive control and the same tissue without the primary antibody was considered as the negative control. Control specimens were stained to eliminate errors. In case of occurrence of error, paraffin block was sliced again and new slides were prepared and stained.

Two experienced and calibrated examiners separately and blindly performed immunohistochemical analysis. Distribution of staining in the basal and para-basal layers was evaluated. Distribution of staining in the connective tissue was divided into two groups of focal and diffuse [18]. All semi-quantitatively obtained data were evaluated according to the criteria by Wahlgren et al. [19] in 2003. The proportion score (percentage of stained cells for MMP2 and MMP19) in the connective tissue was classified as follows: (0): 0-10%, (1): 11-50% and (2): 51-100%. The intensity of staining of cells in the epithelium and connective tissue in OKCs, DCs and DFs was classified into three groups of mild,

moderate and severe. The percentage of staining of cells in the epithelium was determined as 0%, $\leq 50\%$ and $>50\%$.

Data were analyzed using descriptive and analytical statistics. The Kruskal Wallis test was used to compare the intensity of expression of MMP2 and MMP19. In case of significant differences, Mann Whitney test was used for pairwise comparisons. Chi square test was used to compare qualitative variables among different groups such as incidence rate, location of lesion and status; otherwise, Fisher's exact test was applied. To assess the correlation of variables such as the intensity of expression of MMP2 and MMP19, the Spearman's rho test was used. Type 1 error was considered as 0.05 and $P < 0.05$ was considered statistically significant.

Results

Frequency distribution of lesions: A total of 58 paraffin blocks were evaluated including 20 OKCs, 20 DCs and 18 DFs belonging to patients presenting to the Pathology Department of Shahid Beheshti University from 2008 to 2012. Patients had a mean age of 28.33 ± 14.07 years (range 8 to 67 years). There were 35 males (60%) and 23 females (40%).

Considering the location of the lesions, posterior mandible had the highest and anterior maxilla had the lowest frequency of involvement. Table 1 shows the frequency distribution of lesions based on age, sex, site of involvement and presence of inflammation. Of all, eight OKCs, 10 DCs and five DFs showed signs of inflammation. No significant difference existed in these lesions in terms of presence or absence of inflammation ($P > 0.05$).

Expression of MMP2:

Epithelium: The most common site of expression of MMP2 marker in the epithelium was in the para-basal layer of OKCs and DCs; lack of expression had the highest frequency in DF (Table 2). In cases with inflammation, the para-basal layer in the DCs showed the highest expression of MMP2. In DFs and OKCs, expression of this marker was equal in the para-basal and basal plus para-basal layers (Table 2).

The Kruskal Wallis test found a significant difference in the intensity of expression of MMP2 among the three groups ($P = 0.001$). Pairwise

Table 1. Frequency distribution of OKCs, DCs and DFs based on sex, age and site of involvement

Characteristics	Type of lesion		
	OKC Number(%)	Dentigerous cyst Number(%)	Dental follicle Number(%)
Mean age	36(±14.85)	27.2(±15.75)	21.06(±4.43)
Males	8(40)	18(90)	9(50)
Females	12(60)	2(10)	9(50)
Anterior mandible	2(10)	1(5)	0(0)
Posterior mandible	15(75)	16(80)	11(61)
Anterior maxilla	0(0)	1(5)	1(6)
Posterior maxilla	3(15)	2(10)	6(33)
Inflammation	8(40)	10(50)	5(28)

Table 2. Frequency distribution of site of expression of MMP2 in the epithelium in the three groups of lesions with and without inflammation

Type of lesion	Site of expression of MMP2 in the epithelium with/without inflammation							
	With/without inflammation	No expression		Basal layer		Para-basal layer		Basal and para-basal layer
		Number	(%)	Number	Percentage	Number	Percentage	Number Percentage
OKC	Without inflammation	2	(10)	0	0	14	70	4 20%
	With inflammation	0	0	0	0	4	50	4 50
DC	Without inflammation	0	0	1	5	14	70	5 25
	With inflammation	0	0	0	0	6	60	4 50
DF	Without inflammation	14	77.8	0	0	2	11.1	2 11.1
	With inflammation	1	20	0	0	2	40	2 40

comparison of groups with the Mann Whitney test showed a significant difference between OKCs and DCs ($P=0.02$) and the intensity of staining was higher in DCs. The difference between OKC and DF groups was not significant ($P=0.19$). DCs and DFs had a significant difference in this regard ($P<0.001$) and the intensity of staining in DCs was higher. Kruskal Wallis test found no significant difference in the intensity of MMP2 expression in the epithelium among the three groups with inflammation ($P=0.173$).

Assessment of the percentage of stained cells for MMP2 marker using the Kruskal Wallis test showed a significant difference among the groups ($P<0.001$). Pairwise comparison of the groups

using Mann Whitney U test showed that OKC and DC had a significant difference in this regard and the percentage of stained cells was significantly higher in the DC group ($P=0.01$). The difference in this regard between OKC and DF was not significant ($P=0.08$). But, the difference between DC and DF was significant, and DC showed higher percentage of stained cells ($P<0.001$). The percentage of stained cells for MMP2 in the epithelium using the Kruskal Wallis test showed no significant difference among the three groups with inflammation ($P=0.109$).

Connective tissue: Fisher's exact test showed a significant difference in expression of MMP2 in the connective tissue among the three groups

($P < 0.01$). Pattern of expression of MMP2 in the connective tissue was diffuse in OKC and DF and mostly diffuse (75%) in DC (25% diffuse + focal). Fisher's exact test found no significant difference in expression of MMP2 among inflammatory lesions in the three groups ($P = 0.051$; 100% diffuse in both OKC and DF and 50% diffuse and 50% diffuse + focal in DC; Table 3).

Fisher's exact test found no significant difference among the groups in the intensity of staining of the connective tissue for MMP2 ($P = 0.132$). Fisher's exact test found no significant difference in this regard among the inflammatory lesions either ($P = 0.394$; Figure 1).

Expression of MMP19:

Epithelium: Expression of MMP19 in all specimens was in the basal and para-basal layers. Using the Kruskal Wallis test, a significant difference was found in the expression of MMP19 among the three groups. Pairwise comparison by Mann Whitney U test showed that OKC and DC groups were not significantly different in this regard ($P = 0.718$), while OKC and DF and also DC and DF had significant differences (both P s < 0.05 ; Table 4). Kruskal Wallis test found no significant difference in expression of MMP19 among the groups with inflammation ($P = 0.345$; Table 4). Percentage of stained cells in all specimens was

over 50%.

Connective tissue: Fisher's exact test showed a significant difference in expression pattern of MMP19 in the connective tissue among the three groups ($P < 0.05$). Expression was mainly diffuse in all groups (55% in OKCs, 70% in DCs and 94.4% in DFs; the remaining were diffuse + focal). Also, significant differences were found in the expression of MMP19 in the connective tissue among the three groups with inflammation ($P < 0.001$).

Fisher's exact test showed a significant difference in the expression score of MMP19 in the connective tissue among the three groups ($P < 0.001$). Score 2 was the most common in OKCs and DCs and score 1 was the most common in DFs. A significant difference was also found in this regard among the three groups with inflammation ($P < 0.05$); score 2 was the most common in OKCs and DCs and score 1 was the most common in DFs.

Assessment of the intensity of staining of connective tissue for MMP19 by the Fisher's exact test revealed no significant difference (95% severe in OKCs and DCs and 88.9% severe in DFs, the remaining were moderate; $P = 0.678$). The intensity of staining in all three groups with inflammation was severe (Figure 2).

Table 3. Frequency distribution of expression of MMP2 marker in the connective tissue in the three groups of lesions with and without inflammation

	With/without inflammation	Expression of MMP2 in connective tissue with/without inflammation			
		Score 0 (0-10%)		Score 1 (11-50%)	
		Number	Percentage	Number	Percentage
OKC	Without inflammation	14	70	6	30
	With inflammation	7	87.5	1	12.5
DC	Without inflammation	7	35	13	65
	With inflammation	3	30	7	70
DF	Without inflammation	13	72.2	5	27.8
	With inflammation	3	60	2	40

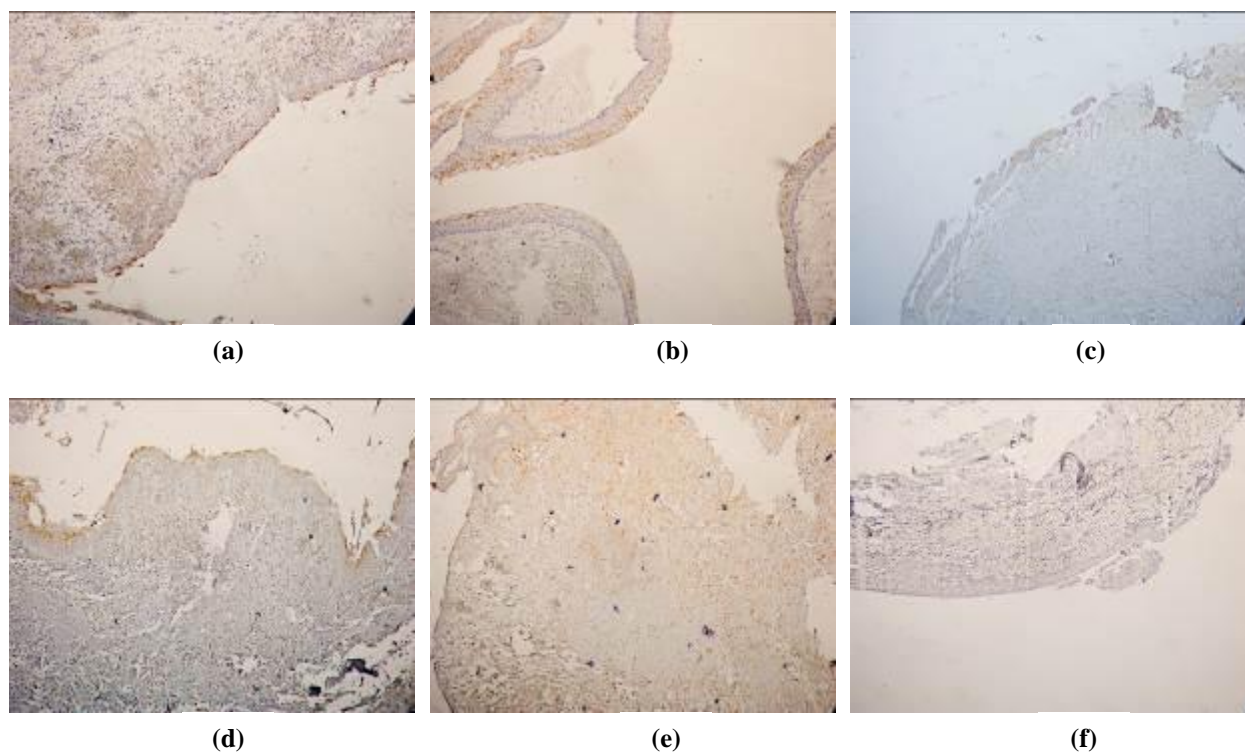


Figure 1. IHC staining of MMP2 at $\times 100$ magnification (a) non-inflammatory DC (b) non-inflammatory OKC (c) non-inflammatory DF (d) inflammatory DC (e) inflammatory OKC (f) inflammatory DF

Table 4. Frequency distribution of the expression of MMP19 in the epithelium in the three groups with and without inflammation

Type of lesion	With/without inflammation	Expression of MMP19 in the epithelium with/without inflammation							
		+		++		+++		++++	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
OKC	Without inflammation	0	0	3	15	5	25	12	60
	With inflammation	0	0	2	25	1	12.5	5	62.5
DC	Without inflammation	1	5	5	25	2	10	12	60
	With inflammation	1	10	1	10	0	0	8	80
DF	Without inflammation	0	0	0	0	0	0	18	100
	With inflammation	0	0	0	0	0	0	5	100

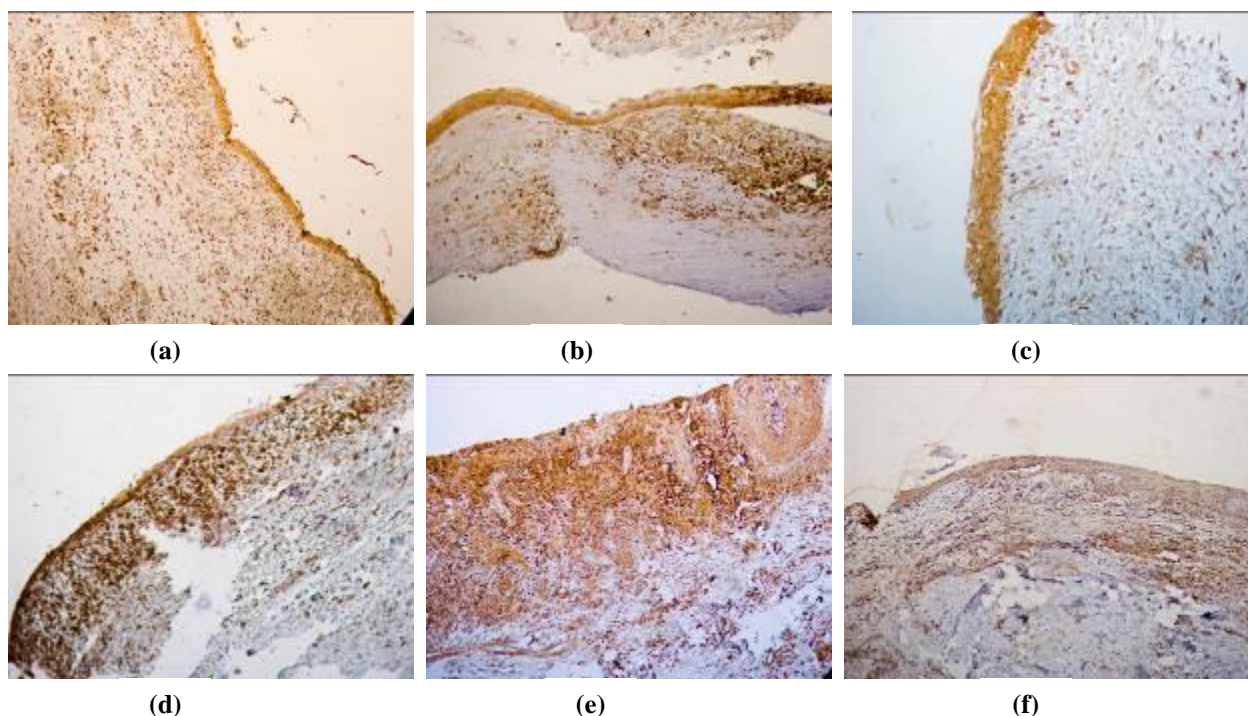


Figure 2. IHC staining of MMP19 at $\times 100$ magnification (a) non-inflammatory DC (b) non-inflammatory OKC (c) non-inflammatory DF (d) inflammatory DC (e) inflammatory OKC (f) inflammatory DF

Discussion

DC with a prevalence of 36-11% [20] and OKC with a prevalence of 2-11% are among the most common cysts of the jaws [21]. DC often occurs in the age range of 10-30 years. This range is 10-40 years for OKC [1]. OKC has an aggressive behavior and high rate of recurrence. The nature of OKC is a matter of debate and it has been stated that some features of OKC are similar to those of a neoplasm [22]. Numerous markers have been investigated for assessment of the biological behavior of these lesions, which are mainly related to angiogenesis, tissue regeneration and transcription factors [23]. Techniques for detection and assessment of expression of these antigens are variable and include Western blot, polymerase chain reaction, RT-PCT, IHC, etc. P53, TIMPs, MMPs and nuclear factors are among these antigens.

In the current study, expression of MMP2 and MMP19 was assessed using IHC. This technique allows for assessment of expression of markers and their pattern of distribution in tissues. Moreover,

this technique does not require fresh tissue and can be performed using formalin-fixed paraffin-embedded blocks. It is easy and reproducible as well.

Expression of MMPs plays a role in prognosis and invasion of tumors. These markers degrade the basement membrane, which is mainly composed of type IV collagen [9]. The pivotal role of MMP2 in cancer cells has been confirmed, and increased expression of MMP2 has been clearly linked to tumor invasion [24]. Role of MMP2 in odontogenic tumors such as ameloblastoma, odontogenic myxoma and calcifying cyst odontogenic tumor and epithelial tumors such as SCC has been evaluated. A previous study showed that this marker played no role in growth or invasion of odontogenic myxoma and calcifying cyst odontogenic tumor [25]; whereas, MMP2 may play a role in the aggressive behavior of OKC and ameloblastoma [19]. No study has assessed the role of MMP19 in oral lesions; but its role in nasopharyngeal carcinoma, melanoma and astrocytoma has been investigated. Expression of

MMP19 is necessary to suppress nasopharyngeal carcinoma [11]; on the other hand, its increased expression has been reported in astrocytoma and melanoma. It is believed that expression of MMP19 and MMP2 is related to melanoma [12,26].

In the current study, patients with OKC and DC had a mean age of 31.6 years (range 8-67 years) with a male to female ratio of 13:7. Also, the most common site of involvement was the posterior mandible, which is in accordance with the findings of a previous study [1]. MMP2 was expressed in all specimens in our study, which is similar to previous studies; also, both focal and diffuse patterns of MMP2 expression were seen [25].

Wahlgren et al. [19] showed that percentage of stained cells and score of expression of MMP2 in the epithelium of OKCs were greater than in DFs. Also, the intensity and score of staining in OKCs were more than in DFs. Moreover, higher number of OKC specimens compared to DFs showed expression of this marker in the basal layer [19]; these findings are in line with our observations.

In our study similar to a previous investigation [19], expression of MMP2 in areas with inflammation was higher than in areas without inflammation. In our study, expression of MMP2 in the epithelium of the three groups was significantly different, and higher expression was noted in DCs, but no significant difference was found between OKCs and DFs in this regard. Therefore, MMP2 may play a role in growth of DC, which is different from that of OKC. Our findings revealed that MMP2 does not play a role in the occurrence of inflammatory types of OKCs either. Another reason for decreased expression of MMP2 in inflammatory OKCs may be the spontaneous drainage of OKCs. Expression of MMP2 in the connective tissue of three groups of specimens was significantly different in our study. Since the OKC originates from the dental lamina or its remnants, connective tissue of OKC and DF may act similarly. Thus, the expression score of MMP2 in both inflammatory and non-inflammatory types of OKCs and DFs was zero. This value was one in DCs.

Results of studies regarding the expression of MMP19 are highly controversial. Expression of MMP19 increases in astrocytoma, melanoma and

congenital dysplasia and it has been reported to enhance cell division, migration and adhesion of keratinocytes to type I collagen [25]. However, its expression decreases in some other types of cancers such as the skin cancer, breast cancer, colon cancer and nasopharyngeal carcinoma [11, 27]. Anti-tumoral effect of MMP19 may be due to its anti-angiogenic property because tumors can exceed 2mm in size only if angiogenesis is performed and cells are supplied with oxygen [11]. However, another study reported angiogenesis due to the expression of MMP19 [10]. Expression of MMP19 can be detected in hyper proliferative keratinocytes in basal cell carcinoma and early stages of SCC. It is expressed in areas with high mitotic rate and malignant parts of chronic wounds but it is not seen in malignant nests of SCC [19]. However, MMP19 expression increases in systemic or lymphatic metastasis of oropharyngeal SCC. It is not expressed in early stages of melanoma, but its expression in advanced stages has been detected [26]. Staining of MMP19 in our study was mainly detected in the cytoplasm of epithelial cells and less commonly in the connective tissue; which confirms the findings of Chan et al [11].

Expression of MMP19 in areas with high mitotic rate has been documented, which explains its high expression in our study; MMP19 is also found in normal tissues. It plays a role in degradation of basement membrane and ECM in physiological conditions, which explains its expression in DFs [27].

Our study showed diffuse pattern of expression of MMP19 in the connective tissue, which has also been shown by Lettau et al, [12] in astroglial tumors. Müller et al. [26] showed that the expression of MMP19 in normal skin tissue was limited to undifferentiated keratinocytes present in the basal layer of epithelium and it was not expressed in differentiated keratinocytes present in the supra-basal layer and melanocytes. As the melanoma advanced, increased expression of this marker in supra-basal keratinocytes and dermal fibroblasts was noted. However, in the current study, expression of this marker was noted in all dermal fibroblasts of all lesions. Sadowski et al. [28] showed the expression of this marker in undifferentiated keratinocytes of basal layer. In our

study, MMP19 was expressed in the basal layer, similar to a previous report [26]. Irregular expression of MMP19 occurs in some skin lesions with abnormal mitosis such as eczema and psoriasis, and expression of this marker is noted in supra-basal layer and spinous layer of epidermis [28].

Kolb et al. [29] reported the expression of MMP19 in the capillary endothelial cells to be related to acute inflammation, which indicates the role of this marker in angiogenesis. In our study, inflammation increased the expression of MMP19, which indicates its role in tissue destruction.

Significant differences were noted in our study in the intensity of MMP19 expression in the epithelium (almost similar in OKC and DC but different in DF), which may indicate high rate of epithelial proliferation in these lesions compared to normal tissue with no such epithelial growth. Expression of MMP19 in the connective tissue of specimens was also different in the three groups in our study and indicated the role of inflammation in greater expression of this marker.

Controversial results have been reported in the literature regarding the relationship of MMP19 and MMP2 expression. Some studies [27,30] have demonstrated increased expression of both markers in advanced tumors; in mammary gland cancer, the expression of MMP19 decreases while that of MMP2 increases [30]. Decreased expression of MMP19 in advanced mammary gland cancer may be due to the superior role of MMP2 over that of MMP19. Such an inverse relationship has been noted not only in tumoral cells, but also in smooth muscle and endothelial cells around the lesions, indicative of inhibition of malignant transformation [30]. In colon cancer, expression of MMP19 decreases while that of MMP2 increases as the lesion advances [27].

Considering the obtained results, similar studies are required on other odontogenic and epithelial tumors. Moreover, assessment of the correlation of these markers with the clinical behavior and recurrence rate of tumors is an interesting topic for future studies.

Conclusion

Within the limitations of this study, the results showed higher expression of MMP19 in OKCs

compared to DCs and DFs; whereas, expression of MMP2 in DCs was higher than in OKCs and DFs. MMP19 seems to play a role in the aggressive behavior of OKCs. Higher expression of MMP2 in DCs may indicate its potential role in multi-dimensional growth of DCs. However, further investigations with diverse methods are needed in addition to these semi-quantitative findings, to enhance making an evidence-based decision.

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