

## ORIGINAL PAPER

**A POSITIVE CORRELATION BETWEEN IMMUNOHISTOCHEMICAL EXPRESSION OF CD31 AND MAST CELL TRYPTASE IN ODONTOGENIC TUMORS**MARYAM KOUHSOLTANI<sup>1</sup>, MONIREH HALIMI<sup>2</sup>, SANA DIBAZAR<sup>3</sup><sup>1</sup>Dental and Periodontal Research Center and Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran<sup>2</sup>Hematology Oncology Research Center and Department of Pathology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran<sup>3</sup>Drug Applied Research Center and Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran

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In this study, we compared mast cell tryptase and CD31 expression between odontogenic tumors with the aim of predicting the clinical behavior of these lesions at the time of initial biopsy. We also evaluated the correlation between mast cell tryptase and CD31 expression to clarify the role of mast cells (MCs) in the growth of odontogenic tumors. Immunohistochemical staining with anti-MC tryptase and anti-CD31 antibodies was performed on 48 cases of odontogenic tumors including solid ameloblastoma (SAM), unicystic ameloblastoma (UAM), odontogenic myxoma (OM), cystic calcifying odontogenic tumor (CCOT) and adenomatoid odontogenic tumor (AOT). Ten high power fields were analyzed for each sample. Total MC count was significantly increased in SAM compared to other odontogenic tumors ( $p < 0.05$ ). Microvessel density was statistically higher in SAM and AOT compared to remaining odontogenic tumors ( $p < 0.05$ ). A significant correlation was observed between MCs and microvessels in odontogenic tumors ( $p = 0.018$ ,  $r = 0.34$ ). Our findings suggest a role for MCs in aggressive clinical behavior of odontogenic tumors. The significant correlation found between MC count and microvessel density in odontogenic tumors is in agreement with the theory of participation of MCs in tumor progression. Targeting MC activity may represent an important nonsurgical therapeutic approach, especially for aggressive odontogenic tumors.

**Key words:** angiogenesis, ameloblastoma, myxoma.

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**Introduction**

Odontogenic tumors originate from the dental organ and related structures. These lesions are an important aspect of oral and maxillofacial pathology due to the considerable bone destruction that may occur [1, 2]. The development of odontogenic tumors is controlled by the intrinsic proliferative potential of the tumor cells and the environmental circumstances, e.g. local inflammation at the tumor site [3]. Mast

cells (MC) are immune cells characterized by small nuclei and granular cytoplasm [4]. On activation, these cells secrete biologically active substances that either reside in the cytoplasmic granules (e.g. histamine, MC tryptase, heparin) or are produced on cell activation (e.g. growth factors, chemokines, and cytokines) [5]. Mast cells are known for participation in anaphylaxis and bone resorption and interaction with other immune cells [4-6].

There has been much interest in the possible roles of MCs in tumor biology, e.g. the studies performed by Jaafari-Ashkavandi *et al.* and Dyduch *et al.* [6, 7]. *In vitro* studies have shown that these cells influence many aspects of tumor biology, e.g. tumor growth, tumor-induced angiogenesis, and tissue remodeling. However, the definitive *in vivo* contribution of MCs to tumor biology remains controversial [5, 8, 9]. It is yet not clear whether MCs are for or against tumor growth [10]. Mast cells have been shown to play an important role in angiogenesis by producing several factors, e.g. tryptase, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), tumor necrosis factor (TNF), interleukin 8 (IL-8), histamine and heparin [5, 11, 12]. Angiogenesis, based on endothelial cell proliferation and migration, has a critical role in many pathologic conditions such as tumor development and metastasis [13]. On the other hand, MCs may restrict the growth of the lesions by producing several factors including IL-1, IL-4, IL-6 and TNF [11, 14].

In the present study, we compared MC tryptase and CD31 expression by immunohistochemistry between various odontogenic tumors to clarify the role of MCs and angiogenesis in the growth and development of odontogenic tumors. In the case of involvement of MCs in angiogenesis and tumor development, treatment strategies including pharmacologic agents can be used to inhibit MC activity via angiogenic pathways as an aid to reduce mutilating surgery regarding odontogenic tumors. To our knowledge, a study evaluating the relation between MCs and angiogenesis and their roles in the development of odontogenic tumors is not available.

## Material and methods

Forty-eight samples were collected from the archives of three laboratories of Tabriz University of Medical Science, Tabriz, Iran. The specimens included 15 cases of solid ameloblastoma (SAM: 10 females and 5 males, ranging in age from 23 to 49 years), 15 cases of unicystic ameloblastoma (UAM: 9 males and 6 females, ranging in age from 23 to 55 years), 7 cases of odontogenic myxoma (OM: 4 males and 3 females, ranging in age from 1 to 33 years), 6 cases of cystic calcifying odontogenic tumor (CCOT: 5 males and 1 female, ranging in age from 14 to 41 years), and 5 cases of adenomatoid odontogenic tumor (AOT: 3 females and 2 males, ranging in age from 13 to 24 years). These findings indicated that most cases of odontogenic tumors occurred between the ages of 15 and 55. 90% of the cases of SAM, 95% of the cases of UAM, 65% of the cases of OM, 50% of the cases of CCOT, and 50% of the cases of AOT occurred in the mandible. These results revealed that

most cases of odontogenic tumors were located in the mandible.

Each formalin-fixed and paraffin-embedded tissue block was used to acquire two sections of 4  $\mu$ m thickness. The sections were stained through standard immunohistochemical staining methods according to the manufacturer's instructions (DAKO, Glostrup, Denmark). The sections were mounted on glass slides and deparaffinized in xylene and then rehydrated through graded alcohol. To block endogenous peroxidase activity, the sections were incubated in 1% hydrogen peroxide. For antigen retrieval, the sections were microwaved in citrate buffer solution (0.01 M, PH 6.0) for 20 min, and rinsed with distilled water. Subsequently, sections were incubated with 1 : 20 and 1 : 100 diluted monoclonal mouse anti-human primary antibodies for CD31 and MC tryptase detection, respectively, and then rinsed with phosphate buffered saline (PBS) and incubated with secondary antibody. Reaction products were visualized using 0.3% diaminobenzidine (DakoCytomation). As a final point, the sections were counterstained in Harris hematoxylin.

For immunohistochemical counting, the sections were scanned at 100 $\times$  magnification. Hot spot fields (the fields most populated by microvessels and MCs) were identified and ten representative high power fields (five intratumoral and five peritumoral) were analyzed in each section. We counted the number of positively stained MCs and microvessels in each field. The mean numbers of MCs and microvessels in five intratumoral fields and five peritumoral fields were calculated. Moreover, the means of ten fields (including five intratumoral and five peritumoral fields) were considered as the total counts for each sample. Evaluations were performed by two independent observers, with an agreement level of 92%.

Data analysis was performed using Statistical Package for Social Sciences (SPSS) 20.0 (SPSS, Chicago, IL). Interobserver reproducibility was verified through six double evaluations. Results were stated as the mean  $\pm$  standard error of mean (SEM). One-way ANOVA followed by Tukey's HSD test was used to compare MC tryptase and CD31 expression between various odontogenic tumors. Intratumoral and peritumoral MC tryptase and CD31 expression in each lesion were compared using the paired t-test. To assess the correlation between MC tryptase and CD31 expression, Pearson's correlation coefficient test was used. A p-value of < 0.05 was considered as statistically significant.

## Results

Mast cells were present in all odontogenic tumors, except one case of myxoma (equivalent to 97% of samples). These were mostly round-shaped, rough

cells with brownish immunostaining of cytoplasmic granules (Fig. 1). MCs were found in intratumoral and peritumoral areas without a specific tendency for any area (paired t-test). Total MC count was statistically higher in SAM compared to CCOT, OM, AOT (one-way ANOVA, Tukey's HSD test, Fig. 2, Table I).

Angiogenesis, assessed by CD31 expression, was increased significantly in SAM and AOT compared to other odontogenic tumors (one-way ANOVA, Tukey's HSD test, Fig. 3, Table I). CD31 expression did not differ significantly between intratumoral and peritumoral areas.

The correlation between MC count and angiogenesis was statistically significant in odontogenic tumors:  $p$ -value = 0.018,  $r = 0.34$  (Pearson's correlation coefficient test, Fig. 4).

## Discussion

In the present study, we found that MCs were more numerous in SAM compared to UAM, COT and AOT, OM ( $p < 0.05$ ). Solid ameloblastoma is a benign tumor with aggressive biological behavior and a high degree of infiltration, bone destruction

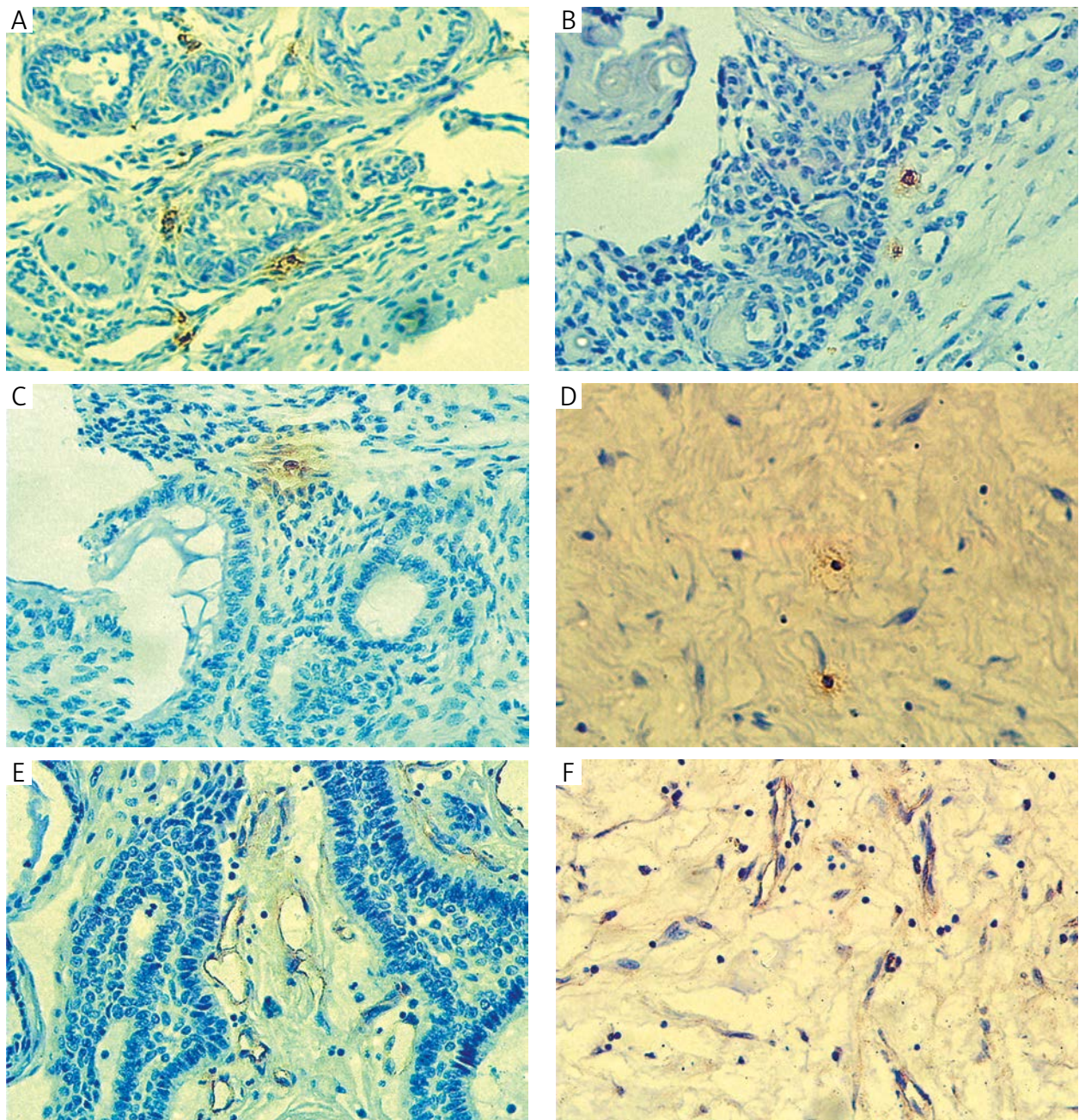
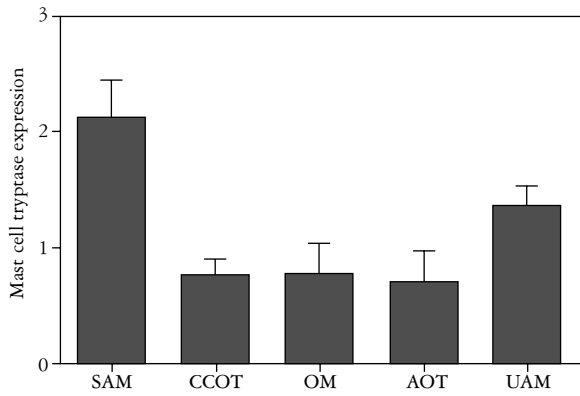
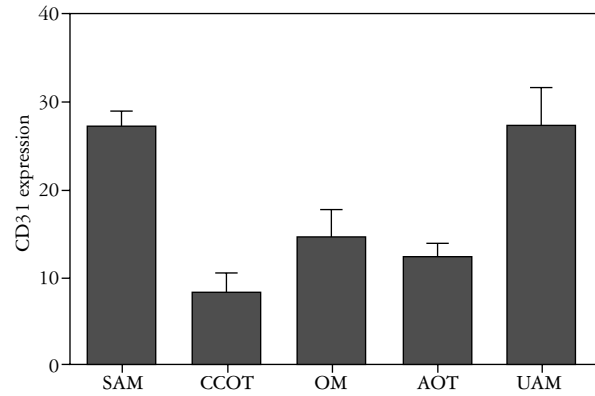


Fig. 1. Mast cells in: A) ameloblastoma, B) cystic calcifying odontogenic tumor, C) adenomatoid odontogenic tumor, D) myxoma; and microvessels in E) ameloblastoma, F) myxoma (IHC stain, magnification 400×)



**Fig. 2.** Histogram of mast cell tryptase expression in solid ameloblastoma, cystic calcifying odontogenic tumor, odontogenic myxoma, adenomatoid odontogenic tumor, unicystic ameloblastoma

SAM – solid ameloblastoma; CCOT – cystic calcifying odontogenic tumor; OM – odontogenic myxoma; AOT – adenomatoid odontogenic tumor; UAM – unicystic ameloblastoma



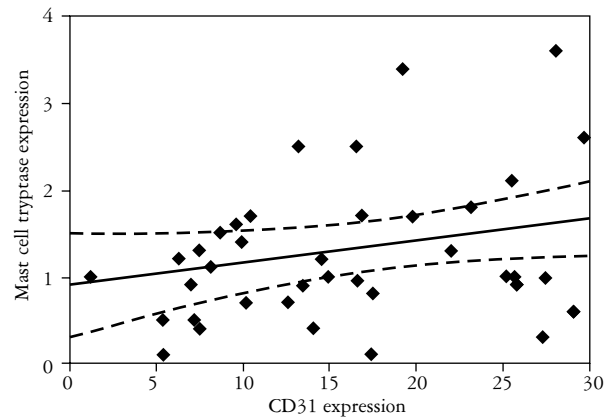
**Fig. 3.** Histogram of CD31 expression in solid ameloblastoma, cystic calcifying odontogenic tumor, odontogenic myxoma, adenomatoid odontogenic tumor

SAM – solid ameloblastoma; CCOT – cystic calcifying odontogenic tumor; OM – odontogenic myxoma; AOT – adenomatoid odontogenic tumor; UAM – unicystic ameloblastoma

and recurrence. However, UAM is a cystic tumor with much less invasive clinical behavior. The tumoral nature of this lesion is often not distinguished on the basis of the clinical and radiographic features, unless a histopathological examination is carried out. AOT is generally accepted as a benign tumor with non-invasive clinical behavior, and many researchers even consider this lesion as a hamartoma rather than a tumor. Likewise, CCOT has favorable clinical behavior and a low recurrence rate [1, 2]. Considering the clinical characteristics of the studied tumors, our findings are in agreement with the theory of participation of MCs in the aggressive clinical behavior of the lesions. In the majority of previous studies, MCs were clearly associated with tumor development and poor prognosis, e.g. in carcinoma of the stomach, oral cavity and melanoma [3, 8, 11]. Pereira *et al.* [8] evaluated the role of MCs in odontogenic tumors, but did not observe a significant difference in MC density between odontogenic tumors with different clinical behaviors. The sample size for each group in the present study was greater than that in the study of Pereira *et al.* [8], especially for SAM and UAM.

OM is an exception in our findings, possibly because of the mesenchymal origin of this tumor. All other studied odontogenic tumors have epithelial origins. OM is a benign and aggressive tumor, with clinical behavior similar to ameloblastoma. Our finding in the case of OM is in agreement with the findings of Pereira *et al.* [8] and Martínez-Mata *et al.* [15], who did not report high MC densities in this lesion. Further studies on odontogenic tumors with mesenchymal and mixed origins can make the concept more clear.

According to our findings, MC tryptase expression was increased in intratumoral areas compared



**Fig. 4.** Correlation between mast cells and microvessels in odontogenic tumors

**Table I.** Mast cell tryptase and CD31 expression in odontogenic tumors

CD31 EXPRESSION	MAST CELL TRYPTASE	STUDIED GROUPS
MEAN ± SEM	MEAN ± SEM	
27.30 ± 1.65	2.12 ± 0.32	SAM
11.05 ± 1.64	1.36 ± 0.17	UAM
7.03 ± 1.45	0.72 ± 0.25	AOT
27.30 ± 1.62	0.77 ± 0.27	OM
8.25 ± 2.17	0.77 ± 0.13	CCOT

SAM – solid ameloblastoma; UAM – unicystic ameloblastoma; AOT – adenomatoid odontogenic tumor; OM – odontogenic myxoma; CCOT – cystic calcifying odontogenic tumor

to peritumoral regions (although not statistically significant), which is in agreement with most studies evaluating MCs in odontogenic cysts, including odontogenic keratocyst, which has a controversial na-



ture. In the mentioned studies, higher MC densities were observed in the subepithelial regions compared to the depths of connective tissues or progressive fronts of odontogenic cysts. However, Pereira *et al.* [8] found higher densities of MCs in peritumoral areas compared to intratumoral regions.

When a tumor becomes larger than 2 mm, angiogenesis must occur for further growth. This is because nutritional substances and oxygen need to be provided by new blood vessels. Unlike physiologic conditions, angiogenesis is not a favorable survival indicator in many cancers, e.g. breast, prostate and oral cavity [11, 16]. We found that microvessel density was increased significantly in SAM compared to UAM and CCOT. Our findings are in agreement with the studies performed by Seifi *et al.* [17] and Kumamoto *et al.* [13]. Seifi *et al.* [17] demonstrated an increase of angiogenesis, assessed by CD34 expression, in SAM compared to odontogenic keratocyst and dentigerous cyst and concluded that angiogenesis has a role in aggressive behavior of SAM. Kumamoto *et al.* [13] assessed vascular endothelial growth factor (an angiogenic mediator mostly secreted by MCs) in ameloblastoma and its malignant counterpart and suggested that upregulation of this protein might be associated with neoplastic or malignant changes of odontogenic epithelium.

Our immunohistochemical studies revealed a significant positive correlation between MC tryptase and CD31 expression. Recent clinical studies evaluating the role of MCs in tumorigenesis have also suggested a strong association between these cells and angiogenesis, and thus tumor development [18]. Furthermore, *in vitro* studies have confirmed aggregation of MC with microvessels [12]. This phenomenon may be related to the secretory enzymes of MCs, e.g. MC tryptase, that directly stimulate proliferation and migration of endothelial cells or indirectly degrade the connective tissue scaffold [19]. In two studies on oral squamous cell carcinoma, Lamaroon *et al.* and Pyziak *et al.* reported a significant relation between the number of MCs and angiogenesis, in agreement with the mentioned theory [12, 20]. This positive correlation has also been observed in various tumors, e.g. lung cancer [21]. On the other hand, in some studies, e.g. by Niczyporuk *et al.* [19] on non-small cell lung cancer or Jaafari *et al.* [6] on oral squamous cell carcinoma, no significant positive correlation between MCs and angiogenesis was observed.

## Conclusions

The significant correlation found between mast cell count and microvessel density in odontogenic tumors is in agreement with the theory of participation of mast cells in tumor progression via promotion of angiogenesis. Furthermore, the findings of the

present study suggest a role for MCs in aggressive clinical behavior of odontogenic tumors. However, further investigations to confirm the potential prognostic value of these cells in aggressive behavior of odontogenic tumors, particularly ameloblastoma, are encouraged. Based on this study, we suggest that targeting mast cell activities via blocking angiogenesis may be an important nonsurgical treatment strategy, especially for aggressive odontogenic tumors.

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