

Original Article**A Comparative Study of Cathepsin D Expression in Peripheral and Central Giant Cell Granuloma of the Jaws by Immunohistochemistry Technique**Massoumeh Zargaran¹, Abbas Moghimbeigi², Noushin Afsharmoghadam³, Mohsen Nasr Isfahani⁴, Atefeh Hashemi⁵¹ Dental Research Center, Dept. of Oral and Maxillofacial Pathology, Dental School, Hamadan University of Medical Sciences, Hamadan, Iran.² Modeling of Noncommunicable Disease Research Center, Dept. of Biostatistics and Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran.³ Dept. of Pathology, AL Zahra Medical Center, Isfahan University of Medical Sciences, Isfahan, Iran.⁴ Pathology Technologist, Dept. of Pathology, AL Zahra Medical Center, Isfahan University of Medical Sciences, Isfahan, Iran.⁵ Dept. of Oral and Maxillofacial Pathology, Dental School, Arak University of Medical Sciences, Arak, Iran.**KEY WORDS**Giant Cell Granuloma;
Cathepsin D;
Immunohistochemistry;
Jaw**ABSTRACT****Statement of the Problem:** Peripheral and central giant cell granuloma are two common benign lesions of the oral cavity. In spite of histopathological similarities, they have different clinical behaviors. Cathepsin D is a lysosomal enzyme which has different functions on the basis of protein and applied peptide cleavage.**Purpose:** This research aimed to evaluate and compare the expression level of Cathepsin D in these two lesions to find the reasons for the differences in clinical and biologic characteristics.**Materials and Method:** The expression of Cathepsin D was investigated by using the immunohistochemistry method in 20 samples of peripheral giant cell granuloma and 20 samples of central giant cell granuloma. The percentage of stained giant cells (labeling index), the intensity of staining of giant cells, and staining-intensity-distribution in both groups were calculated and compared.**Results:** The labeling indices of Cathepsin D in peripheral giant cell granuloma and central giant cell granuloma were 95.9 ± 4.03 and 95.6 ± 2.34 , respectively. There was no significant difference in the percentages of stained giant cells between the two groups ($p = 0.586$). The intensity of staining of giant cells in central giant cell granuloma was stronger than that of peripheral giant cell granuloma ($p < 0.001$). Staining-intensity-distribution of giant cells in central giant cell granuloma was significantly greater than that of the peripheral type of lesion ($p = 0.001$).**Conclusion:** The higher expression level of Cathepsin D in central giant cell granuloma compared to peripheral type of lesion can explain more aggressive behavior of central giant cell granuloma.**Corresponding Author:** Hashemi A., Dept. of Oral and Maxillofacial Pathology, Dental School, Arak University of Medical Sciences, Arak, Iran. Tel: +98-8632724522 Fax: +98-8632724524
Email: dr.hashemi91@yahoo.comReceived June 2015;
Received in Revised form October 2015;
Accepted December 2015;**Cite this article as:** Zargaran M., Moghimbeigi A., Afsharmoghadam N., Nasr Isfahani M., Hashemi A. A Comparative Study of Cathepsin D Expression in Peripheral and Central Giant Cell Granuloma of the Jaws by Immunohistochemistry Technique. *J Dent Shiraz Univ Med Sci.*, 2016 June; 17(2): 98-104.**Introduction**

Peripheral giant cell granuloma (PGCG) occurs as a red or purple nodule exclusively on the gingiva and alveolar ridge. These lesions originate from the periodontal ligament or mucoperiosteum of the alveolar ridge as a result of local irritation or trauma. [1] PGCG can develop

at any age, especially during the fifth and sixth decades of life with a slight female predilection. [1-2]

In some cases, PGCG affects the underlying bone and may cause cupping resorption. [2] Central giant cell granuloma (CGCG) occurs within the jaw bones and appears as radiolucent defects in radiographs which may

be unilocular or multilocular. [3] The majority of these lesions are noted in young adults with a predilection for females. [4-5] Even some case of this lesion have been associated with significant asymmetries, [6-7] repeated recurrences, [8] multifocal incidences, [8-9] invasion and extensive destruction of jaw bones. [10] Both CGCG and PGCG exhibit similar histopathological features, and are characterized by the presence of abundant mononuclear stromal cells, admixed with a large number of multinucleated giant cells and a rich vascularized stroma with extravasated erythrocytes, hemosiderin deposition, and blood. Meanwhile, these lesions may have different clinical behaviors. [11-12]

Although various parameters have been compared between these two lesions in different studies, the reasons behind the differences in the biologic behaviors of these lesions are still to be elucidated. [3, 13-14] Cathepsin D is a soluble lysosomal aspartic endopeptidase which is released from the rough endoplasmic reticulum as procathepsin D and after elimination of the signal peptide, procathepsin D is carried into the intracellular vesicular structures. The various physiologic functions of Cathepsin D depend on its capacity to cleave functional and structural proteins and peptides. [15] Research has shown the presence of Cathepsin D in the vacuoles and vesicles of alveolar bone osteoclasts in mice, indicating that they are necessary for bone resorption and remodeling. [16-17] In addition, it has been demonstrated that giant cells in the giant cell tumor of long bones contain Cathepsin D. This enzyme has a positive relationship with the local invasion of this tumor, which might be attributed to its role in bone resorption. [18] Given the presence of Cathepsin D in osteoclasts and its effect on resorption of bone, the present study was undertaken to evaluate and compare its incidence through an immunohistochemical technique in PGCG and CGCG in an attempt to explain the reasons for different biological behaviors of these two tumors.

Materials and Method

The present descriptive analytical study was carried out by using a cross-sectional design. A total of 40 samples were selected from the archives of the Department of Oral Pathology, Faculty of Dentistry of Hamadan University of Medical Sciences and the archives of the de-

partment of pathology, AL Zahra Medical Center, Isfahan University of medical sciences in two groups. Group A consisted of 20 samples of PGCG and group B consisted of 20 samples of CGCG. The CGCG samples were selected based on the occurrence of central lesions in the jaws as shown on radiographic views (if available) or according to the report made by the radiologist in the patient's medical file (if radiograph was not available). Cases without sufficient documents were excluded from the study. An indirect immunohistochemistry technique was used to evaluate and compare the samples.

Immunohistochemical Technique

The indirect immunohistochemical technique to stain for Cathepsin D consisted of the following steps:

1. Deparaffinization step: the slides with the specific tissue were placed in an oven for 60 minutes and heated to 60° C. They were then immersed in xylol and descending concentrations of alcohol (90%, 80% and 70%, respectively) for the tissue water absorption in preparation for the test.
2. Hydrogen peroxide step: The prepared slides were placed in 3% hydrogen peroxide, and were rinsed in distilled water.
3. The Cathepsin D primary antibody step: Cathepsin D antibody (Rabbit Polyclonal Antibody, PU 205-UP, BioGenex, USA) was poured on the slides and rinsed with PBS buffer solution after one hour of incubation at room temperature.
4. Envision step: the secondary Envision antibody was applied on the slides for 30 minutes and then the slides were incubated at room temperature.
5. Chromogen step: The chromogen used was DAD (Diaminobenzidine), which was applied on the slides for 5-10 minutes, and the slides were rinsed with distilled water.
6. Background staining step: Hematoxylin was used to stain the background by applying it on the slides for 5 minutes. The slides were rinsed with distilled water. Finally the slides were mounted with entellan glue which is suitable for visualization under a light microscope. Based on the manufacturer's instructions, the breast carcinoma cells were used as the positive controls, in which the cancerous cells exhibit cytoplasmic staining for the Cathepsin D marker.

Immunohistochemical evaluation

The stained giant cell counts were determined by count-

Table 1: Frequency distribution of the two study groups regarding gender and age

Group	No.	Gender		Mean	SD	Age	
		Male	Female			Maximum	Minimum
A	20	(45%)9	(55%)11	31.6	20.34	65	7
B	20	(40%)8	(60%)12	32.75	16.48	66	8
Total	40	(42.5%)17	(57.5%)23				

ing these cells in 8 fields on each slide under a light microscope (Olympus B 40X; Japan) at 400 X magnification. Then the labeling index (LI) for Cathepsin D was calculated for each sample based on the number of stained cells. Counting procedures were carried out twice to avoid errors. The Cathepsin D LI (the percentage of stained giant cells) was analyzed and compared between group A (PGCG) and group B (CGCG).

In addition, the Cathepsin D immunoreactivity was evaluated semi-quantitatively and scored based on the percentage of stained cells ; score 0 when <1% of cells were stained, and score 1 to 4 when 1-25%, 26-50%, 51-75%, and >75% of cells were stained respectively.

Staining intensity was evaluated and scored as 0 (no cell staining), 1 (weak staining), 2 (moderate staining), and 3 (intense staining) based on the staining strength of the cells. [19]

The scores were determined in each field. In order to achieve more accurate results and more reliable determination of staining intensity in the whole slide, 8 fields were scored separately and their means were calculated.

Staining intensity distribution (SID) was calculated separately for each field by multiplying the percentage of giant cells stained in that field by the intensity of staining in that field; the mean SID was calculated for each sample.

Statistical Analyses

Statistical analyses were carried out by using SPSS software, version 16. Kolmogorov-Smirnov was used to determine the distribution of the three variables of stained cells, staining intensity, and SID.

T test was used to evaluate Cathepsin D LI and SID between the two groups. Fisher's exact test was used to

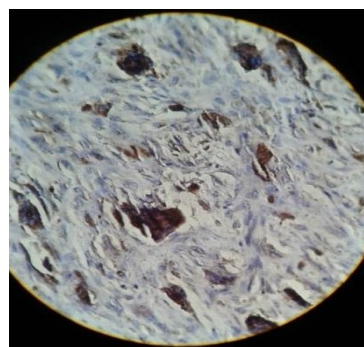
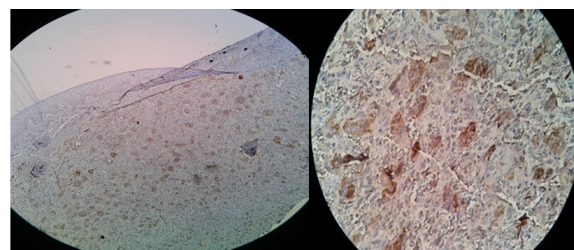
evaluate and compare the staining intensity of giant cells between the two groups.

Results

By assessment of study group documents, the patients' information such as age, sex, and site of the lesions were extracted. (Tables 1 and 2)

Immunohistochemical findings

Positive immunostaining giant cells had cytoplasmic brown staining in the two groups (Figures 1 and 2).

**Figure 1:** Staining of giant cells for Cathepsin D in CGCG**Figure 2:** Staining of giant cells for Cathepsin D in PGCG

Kolmogorov-Smirnov test showed normal distribution of the three variables of stained giant cells, staining intensity, and SID. The mean percentage of stained giant cells was 95.09 ± 4.03 and 95.6 ± 2.34 in groups A and B respectively, with no statistically significant differences between the two groups ($p = 0.586$).

Table 2: Frequency distribution of the two study groups regarding the site of lesions

Group	N	Site of the samples					Total
		Anterior jaw (unilateral)	Anterior jaw (bilateral)	Posterior jaw	Anterior and Posterior jaw	Unknown	
A	20	10 (50%)	1 (5%)	5 (25%)	4 (20%)	0 (0%)	20 (100%)
B	20	8 (40%)	1 (5%)	6 (30%)	4 (20%)	1 (5%)	20 (100%)

Table 3: The staining intensities for Cathepsin D in the two study groups

Group	Staining Intensity			
	Score 0 (no staining)	Score 1 (weak staining)	Score 2 (moderate staining)	Score 3 (severe staining)
A	0 (0%)	13 (65%)	7 (35%)	0 (0%)
B	0 (0%)	4 (20%)	14 (70%)	2 (10%)
Total	0 (0%)	17 (45%)	21 (52.5%)	2 (5%)

Table 3 presents the results of staining intensity of giant cells in the two study groups. The results of statistical analyses showed significant differences in staining intensity between the two groups. The mean SID in groups A and B was 7.140 ± 1.40 and 9.21 ± 1.90 , respectively, indicating statistically significant differences between the two groups.

Discussion

In the present study, expression of Cathepsin D was detected in the giant cells of both CGCG and PGCG lesions. Different studies evaluated the giant cells in these two lesions and all showed the osteoclastic nature of these cells. [19-20]

On the other hand, Goto *et al.* evaluated the growth plates of the femur and detected large amounts Cathepsin D suggesting that this enzyme is necessary for osteoclastic resorption. [16] In another study, Goto *et al.* showed that Cathepsin D has an active indirect role in osteoclastic resorption. [17] Demertzis *et al.* carried out a study on giant cell tumor and reported that all the giant cells in all samples expressed this enzyme. They reported a significant relationship between the expression of Cathepsin D and local invasion and expansion of the tumor. [18] Therefore, the expression of Cathepsin D in giant cells, which is considered a factor involved in bone destruction and one of the enzymes found in osteoclasts, might confirm the osteoclastic nature of giant cells in both PGCG and CGCG.

Osteoclasts contain several enzymes for resorption of bone, including the Cathepsin group (A, B, Z, etc.). Czupalla *et al.* reported that Cathepsin D is a type of Cathepsin synthesized in osteoclasts. [21] As discussed above, various studies showed the osteoclastic nature of giant cells in PGCG and CGCG lesions. Thus, the presence and expression of this enzyme in these cells can be expected. However, the question is what the reason is behind the differences in the expression intensity of this enzyme in giant cells in these two lesions. Among the most important factors involved in the osteoclastogene-

sis and bone resorption are proinflammatory mediators. For example it was reported that TNF- α and IL-1 play an important role in the formation and activation of osteoclasts. [22] TNF- α exerts its osteoclastogenic effect in association with other cytokines such as IL-1 β . In this context, mice that had no IL-1 β , but received TNF- α , exhibited no bone destruction despite persistent inflammation. [23]

A study by Papanicolaou *et al.* found that TNF- α and IL-1 β proinflammatory factors were expressed in both PGCG and CGCG lesions. However, IL-1 β was expressed in CGCG giant cells at a significantly higher rate than that in PGCG giant cells. [24] As previously mentioned, TNF- α plays a role in osteolysis only in the presence of IL-1 β . Since IL-1 β is expressed in CGCG giant cells at a higher rate, TNF- α can be more effective in osteoclastogenesis of these cells, and consequently in production of its products such as Cathepsin D. [25] During osteoclastogenesis, the enzymes required for the activity of these cells (including Cathepsin D) are synthesized. Due to the apparent osteoclastic nature of giant cells in PGCG and CGCG, the more intense expression of Cathepsin D in CGCG might be attributed to more significant and noticeable presence of IL-1 β cytokine in these cells.

Cathepsin D plays a role in various physiologic and pathologic processes including bone resorption. [16-18] A higher rate of its expression by CGCG giant cells compared to PGCG might help explaining the processes of bone invasion and more bone destruction in CGCG since Cathepsin D can play a role in the destruction of extracellular matrix (ECM) by affecting the proteins of ECM, proteoglycans, and collagen. [26-27] Therefore, a higher rate of its expression in CGCG lesions compared to PGCG might explain greater destruction in these lesions.

Likewise, Cathepsin D in osteoclasts plays an indirect role in the destruction of bone matrix through activation of Cathepsin B and L. [17] Therefore, a higher concentration of Cathepsin D in CGCG giant cells

might be considered as a factor to produce more Cathepsin B and L that are active, and consequently more bone destruction. Cathepsin B and L that are active will prevent further osteolytic activity in the lesion.

Moreover, Moles *et al.* showed that under *in vitro* conditions, an increase in the expression of Cathepsin D resulted in an increase in the synthesis of TGF- β in hepatic cells. [28] Therefore, a higher rate of expression of Cathepsin D in CGCG compared to PGCG might lead to higher expression of TGF- β . The expression of TGF- β in CGCG lesions was higher than that in PGCG lesions. It was consistent with the results of a study reported by de Matos *et al.* [29] On the other hand; Quan *et al.* reported that TGF- β had an effective role in the bone resorption process which caused higher survival rate of osteoclasts and increased the capacity of MMP-9 in bone resorption. [30]

Zhang *et al.* showed that Src factor was activated by TGF- β in lung cancerous cells [31] and as mentioned above, de Matos *et al.* showed that TGF- β was expressed at a lower rate in PGCG compared to CGCG. [29] Khiavi *et al.* noticed that Src factor was expressed in both lesions, but at a higher rate in CGCG compared to PGCG. [19] Based on the study by Moles *et al.*, the increase in the expression of TGF- β was affected by Cathepsin D. [28] Therefore, its higher expression of Cathepsin D and TGF- β might explain a higher expression of Src in CGCG giant cells. Apart from its role in osteoclastogenesis, Src plays a significant role in the polarity of osteoclasts, formation of a brush border, and bone resorption. [32]

Hu *et al.* reported that Cathepsin D affected the activity of MMP-9 in cells; therefore, use of Pepstatin A, the inhibitor of Cathepsin D, *in vitro* (similar to the specific inhibitor of MMP-9) resulted in inhibition of this enzyme. [33] MMP-9 has a role in absorption of bone through proteolysis of the organic matrix of bone. [34] Matos *et al.* carried out a study on PGCG and CGCG and observed that MMP-9 was expressed in CGCG giant cells at higher rates compared to the PGCG giant cell. [35] Based on the results of the present study, a higher rate of expression of Cathepsin D in CGCG giant cells might be associated with higher expression of MMP-9 in these cells, resulting in greater bone destruction in these lesions. Rundhaug has also shown that a higher rate of expression of MMP-9 results in an in-

crease in VEGF levels. [36] VEGF has a role in osteoclastogenesis as a recruiting agent for osteoclast progenitors [37] and a mediator for their differentiation, directly affect the differentiated osteoclasts, in addition to its role in angiogenesis. [34] Likewise, in the study by Matos, VEGF was expressed in CGCG giant cells at a higher rate compared to PGCG giant cells. [35] Therefore, Cathepsin D can also indirectly have a role in the resorption of bone through these mechanisms.

In an *in vitro* study, Diment *et al.* showed that Cathepsin D could convert parathyroid hormone (PTH) to its active form, PTHrP. [1-34, 38] Constant exposure to PTH or PTHrP activates resorption of bone by osteoclasts. In addition, their effect halts the osteoblast maturation process. [39] Houpis *et al.* evaluated PGCG and CGCG samples and reported that PTHrP and its receptor (PTHR) were expressed in all samples at a higher rate in CGCG giant cells compared to PGCG giant cells. [40] Therefore, it might be suggested that a higher rate of expression of Cathepsin D in CGCG giant cells results in the synthesis of more PTHrP (the active form of PTH). Accordingly, Cathepsin in the presence of a higher concentration of PTHrP in CGCG giant cells might explain a higher rate of bone resorption in this lesion.

Based on the available data and a literature review, this study appears to be the first in which Cathepsin D was evaluated in PGCG and CGCG quantitatively and semi-quantitatively. Hence, the mechanisms suggested in the present study to explain a higher rate of expression of Cathepsin D in CGCG compared to PGCG should be further evaluated and confirmed by further studies. In addition, given the complexities and the extent of involved cellular-molecular mechanisms, the effect of these currently-unknown factors cannot be ruled out.

Conclusion

The number of stained giant cells was similar in both groups (CGCG and PGCG) in the present study. The giant cells in CGCG stained more intensely than in PGCG. SID in the CGCG was significantly different from that in the PGCG group; it was higher in the former group. There was no relationship between the expression of Cathepsin D in the PGCG and CGCG giant cells and age and sex.

Acknowledgment

This manuscript is based on the thesis prepared by Dr. Atefeh Hashemi in Dental School, Hamadan University of Medical Sciences, Hamadan, Iran (Grant number 930126377). The authors do not have any conflict of interest.

Conflict of Interest

The authors of this manuscript certify that they have no conflict of interest.

References

- [1] Motamedi MH, Eshghyar N, Jafari SM, Lassemi E, Navi F, Abbas FM, et al. Peripheral and central giant cell granulomas of the jaws: a demographic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007; 103: e39-e43.
- [2] Shadman N, Ebrahimi SF, Jafari S, Eslami M. Peripheral giant cell granuloma: a review of 123 cases. *Dent Res J (Isfahan).* 2009; 6: 47-50.
- [3] Torabinia N, Razavi SM, Shokrolahi Z. A comparative immunohistochemical evaluation of CD68 and TRAP protein expression in central and peripheral giant cell granulomas of the jaws. *J Oral Pathol Med.* 2011; 40: 334-337.
- [4] Amirchaghmaghi M, Falaki F, Mohtasham N, Imanimoghaddam M. Central Giant Cell Granuloma of the Jaws: A Clinical and Radiographic Study in Khorasan. *J Appl Scien.* 2010; 10: 777-780.
- [5] Noleto JW, Marchiori E, Sampaio RK, Iron KL, Collers FB. Radiological and epidemiological aspects of central giant cell granuloma. *Radiol Bras.* 2007; 40: 167-171.
- [6] Jadu FM, Pharoah MJ, Lee L, Baker GI, Allidina A. Central giant cell granuloma of the mandibular condyle: a case report and review of the literature. *Dentomaxillofac Radiol.* 2011; 40: 60-64.
- [7] Ebrahimi H, Yazdani J, Pourshahidi S, Esmaeli F, Zenouz AT, Mehdipour M. Central giant cell granuloma of the posterior maxilla: a case report. *J Dent Res Dent Clin Dent Prospects.* 2008; 2: 71-75.
- [8] Bilodeau E, Chowdhury K, Collins B. A case of recurrent multifocal central giant cell granulomas. *Head Neck Pathol.* 2009; 3: 174-178.
- [9] Curtis NJ, Walker DM. A case of aggressive multiple metachronous central giant cell granulomas of the jaws: differential diagnosis and management options. *Int J Oral Maxillofac Surg.* 2005; 34: 806-808.
- [10] De Corso E, Politi M, Marchese MR, Pirroni T, Ricci R, Paludetti G. Advanced giant cell reparative granuloma of the mandible: radiological features and surgical treatment. *Acta Otorhinolaryngol Ital.* 2006; 26: 168-172.
- [11] Flórez-Moreno GA, Henao-Ruiz M, Santa-Sáenz DM, Castañeda-Peláez DA, Tobón-Arroyave SI. Cytomorphometric and immunohistochemical comparison between central and peripheral giant cell lesions of the jaws. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2008; 105: 625-632.
- [12] Souza PE, Mesquita RA, Gomez RS. Evaluation of p53, PCNA, Ki-67, MDM2 and AgNOR in oral peripheral and central giant cell lesions. *Oral Dis.* 2000; 6: 35-39.
- [13] Duarte AP, Gomes CC, Gomez RS, Amaral FR. Increased expression of NFATc1 in giant cell lesions of the jaws, cherubism and brown tumor of hyperparathyroidism. *On-col Lett.* 2011; 2: 571-573.
- [14] Liu B, Yu SF, Li TJ. Multinucleated giant cells in various forms of giant cell containing lesions of the jaw express features of osteoclasts. *J Oral Pathol Med.* 2003; 32: 367-375.
- [15] Benes P, Vetvicka V, Fusek M. Cathepsin D--many functions of one aspartic protease. *Crit Rev Oncol Hematol.* 2008; 68: 12-28.
- [16] Goto T, Tsukuba T, Ayasaka N, Yamamoto K, Tanaka T. Immunocytochemical localization of cathepsin D in the rat osteoclast. *Histochemistry.* 1992; 97: 13-18.
- [17] Goto T, Tsukuba T, Kiyoshima T, Nishimura Y, Kato K, Yamamoto K, et al. Immunohistochemical localization of cathepsins B, D and L in the rat osteoclast. *Histochemistry.* 1993; 99: 411-414.
- [18] Demertzis N, Kotsiandri F, Giotis I, Apostolikas N. Giant-cell tumors of bone and progesterone receptors. *Orthopedics.* 2003; 26: 1209-1212.
- [19] Khiavi MM, Aghbali AA, Halimi M, Kouhsoltani M, Hamishehkar H. Immunohistochemical expression of Src protein in peripheral and central giant cell granulomas of the jaws. *J Oral Maxillofac Pathol.* 2013; 17: 358-362.
- [20] Tiffée JC, Aufdemorte TB. Markers for macrophage and osteoclast lineages in giant cell lesions of the oral cavity. *J Oral Maxillofac Surg.* 1997; 55: 1108-1112.
- [21] Czupalla C, Mansukoski H, Riedl T, Thiel D, Krause E, Hoflack B. Proteomic analysis of lysosomal acid hydrolases secreted by osteoclasts: implications for lytic enzyme transport and bone metabolism. *Mol Cell Proteomics.* 2006; 5: 134-143.

- [22] Jung SM, Kim KW, Yang CW, Park SH, Ju JH. Cytokine-mediated bone destruction in rheumatoid arthritis. *J Immunol Res.* 2014; 2014: 263625.
- [23] Polzer K, Joosten L, Gasser J, Distler JH, Ruiz G, Baum W, et al. Interleukin-1 is essential for systemic inflammatory bone loss. *Ann Rheum Dis.* 2010; 69: 284-290.
- [24] Papanicolaou P, Chrysomali E, Stylogianni E, Donta C, Vlachodimitropoulos D. Increased TNF- α , IL-6 and decreased IL-1 β immunohistochemical expression by the stromal spindle-shaped cells in the central giant cell granuloma of the jaws. *Med Oral Patol Oral Cir Bucal.* 2012; 17: e56-e62.
- [25] Sakai E, Miyamoto H, Okamoto K, Kato Y, Yamamoto K, Sakai H. Characterization of phagosomal subpopulations along endocytic routes in osteoclasts and macrophages. *J Biochem.* 2001; 130: 823-831.
- [26] Chodynicky S, Chyczewski L, Olszewska E. Immunohistochemical investigations of cathepsin D activity in the structures of cholesteatoma. *Med Sci Monit.* 2002; 8: BR184-BR186.
- [27] Skrzydlewska E, Sulkowska M, Koda M, Sulkowski S. Proteolytic/antiproteolytic balance and its regulation in carcinogenesis. *World J Gastroenterol.* 2005; 11: 1251-166.
- [28] Moles A, Tarrats N, Fernández-Checa JC, Marí M. Cathepsins B and D drive hepatic stellate cell proliferation and promote their fibrogenic potential. *Hepatology.* 2009; 49: 1297-1307.
- [29] de Matos FR, de Moraes M, Nonaka CF, de Souza LB, de Almeida Freitas R. Immunoexpression of TNF- α and TGF- β in central and peripheral giant cell lesions of the jaws. *J Oral Pathol Med.* 2012; 41: 194-199.
- [30] Quan J, Elhousiny M, Johnson NW, Gao J. Transforming growth factor- β 1 treatment of oral cancer induces epithelial-mesenchymal transition and promotes bone invasion via enhanced activity of osteoclasts. *Clin Exp Metastasis.* 2013; 30: 659-670.
- [31] Zhang H, Davies KJ, Forman HJ. TGF β 1 rapidly activates Src through a non-canonical redox signaling mechanism. *Arch Biochem Biophys.* 2015; 568: 1-7.
- [32] Boyce B, Xing L. Src inhibitors in the treatment of metastatic bone disease: rationale and clinical data. *Clin Investig (Lond).* 2011; 1: 1695-1706.
- [33] Hu L, Roth JM, Brooks P, Luty J, Karpatkin S. Thrombin up-regulates cathepsin D which enhances angiogenesis, growth, and metastasis. *Cancer Res.* 2008; 68: 4666-4673.
- [34] Engsig MT, Chen QJ, Vu TH, Pedersen AC, Therkidsen B, Lund LR, et al. Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones. *J Cell Biol.* 2000; 151: 879-889.
- [35] Matos FR, Nonaka CF, Miguel MC, Galvão HC, de Souza LB, Freitas Rde A. Immunoexpression of MMP-9, VEGF, and vWF in central and peripheral giant cell lesions of the jaws. *J Oral Pathol Med.* 2011; 40: 338-344.
- [36] Rundhaug JE. Matrix metalloproteinases and angiogenesis. *J Cell Mol Med.* 2005; 9: 267-285.
- [37] Niida S, Kaku M, Amano H, Yoshida H, Kataoka H, Nishikawa S, et al. Vascular endothelial growth factor can substitute for macrophage colony-stimulating factor in the support of osteoclastic bone resorption. *J Exp Med.* 1999; 190: 293-298.
- [38] Diment S, Martin KJ, Stahl PD. Cleavage of parathyroid hormone in macrophage endosomes illustrates a novel pathway for intracellular processing of proteins. *J Biol Chem.* 1989; 264: 13403-13406.
- [39] Horwitz MJ, Tedesco MB, Sereika SM, Prebehala L, Gundberg CM, Hollis BW, et al. A 7-day continuous infusion of PTH or PTHrP suppresses bone formation and uncouples bone turnover. *J Bone Miner Res.* 2011; 26: 2287-2297.
- [40] Houpis CH, Tosios KI, Papavasileiou D, Christopoulos PG, Koutlas IG, Sklavounou A, et al. Parathyroid hormone-related peptide (PTHrP), parathyroid hormone/parathyroid hormone-related peptide receptor 1 (PTHrP1), and MSX1 protein are expressed in central and peripheral giant cell granulomas of the jaws. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010; 109: 415-424.