Immunohistochemical Assessment of Angiogenesis and Cell Proliferation in Aggressive versus Non-Aggressive Central Giant Cell Granuloma

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Abstract:

Objective: The current study investigated the expression of MCM3 and CD31 in aggressive versus non-aggressive central giant cell granuloma (CGCG). **Materials and Methods:** Thirty-three paraffin blocks for CGCG cases were collected from Oral Pathology Departments, Faculty of Dentistry, Mansoura, and Alexandria Universities. The Immunohistochemical staining for CD31 and MCM 3 was performed, assessed, and scored. **Results:** Mean age of the studied cases was 35.6±9.1 years. The lesions that occurred in the mandible represented the majority of cases [27 cases (81.8%)]. The study revealed an association between aggressiveness and the presence of large swelling, pain, cortical perforation, root resorption, and recurrence where they were significantly higher in group 1 (aggressive cases) than in group 2 (non-aggressive cases). Group 1 revealed histologically numerous MNGCs, more cellular connective tissue Stroma, numerous vascularity, and areas of Hemorrhage. Our study showed a highly statistically significant association between MCM3 expression and size and recurrence of the lesion. Statistically, a significant correlation existed between aggressive cases and MCM3 expression. All aggressive cases were associated with a high reaction of MCM3. Comparisons of size and recurrence of the lesions with CD31 expression revealed a highly significant association with high marker reaction in large-size lesions and recurrent cases. The Association between CD31 expression and the presence of aggressiveness was significant. All aggressive cases were associated with a high reaction of CD31 and showed a significant increase in MVD. MCM3 showed a highly significant positive correlation with CD31. **Conclusions:** Highly significant correlation existed between cell proliferation represented by MCM3 expression and angiogenesis represented by CD31 expression in CGCGs.

Introduction:

The CGCG is an MNGC-rich lesion with a poorly understood etiology that is

Found predominantly in the maxillae of young individuals. The CGCG was previously thought to be a reparative granuloma of jaw bones due to its less aggressive behavior from giant cell tumors. Generally, CGCGs are histologically indistinguishable from peripheral CGCG variants which are considered inflammatory lesions and commonly occur on the gingiva. CGCG can be divided into aggressive and non-aggressive according to clinical and radiographic data.[1] The mini-chromosome maintenance protein complex (MCM) is a DNA helicase essential for genomic DNA replication the human MCM3 gene is located on the minus strand and spans 20868 bps of a genomic region. This gene has 7 transcripts (splice variants). It has 853 amino acids and its size is about 91 kDa. It contains a nuclear localization signal and possesses an ATPase/helicase region at the center. It has a total of 17 exons and extends a bit more than 20 KB of genomic DNA.[2] Ha et al.[3] evaluated the association of giant cell tumors recurrence with MCM3 and suggested that MCM3.

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Immunohistochemical marker can be a useful prognostic factor.CD31 or platelet endothelial cell adhesion molecule (PECAM-1) is a Transmembrane glycoprotein, a member of the immunoglobulin superfamily, expressed on early and mature vascular endothelial cells.[4] Razavi and Yahyaabadi[5] investigated the association between clinical behavior and Histopathological features using an Immunohistochemical vascular CD31 marker. The evaluation of the expression rate for the vascular CD31 marker showed that there might be a positive relationship between the clinical features and histopathology of CGCG. Furthermore, clinical behavior may be predicted based on features such as the number of blood vessels and the proliferation of fibro-endothelial cells.

Aim of the work: The present study was carried out to investigate the expression of CD31 in aggressive versus non-aggressive CGCG, assess the expression of MCM3 in aggressive versus non-aggressive CGCG and determine any correlation between CD 31 and MCM3 expression in studied CGCG cases.

Materials and methods:

Thirty-three paraffin blocks for central giant cell granuloma (CGCG) cases were collected from Oral Pathology Departments, Faculty of Dentistry, Mansoura, and Alexandria Universities.

Clinical data: The clinical and radiographic data are collected from patients` records with emphasis on a gender, site of lesions, size of lesions as aggressive

lesions usually larger than 5 cm, presence of root resorption, and the patient complaint of pain. Based on clinic-radiographic collected data, cases were classified into two groups: aggressive (group1) and non-aggressive (group 2) CGCG. The other types of giant cell lesions like (PGCG) were excluded from the study, and the remaining and the remaining samples were included in the analysis.[6]

Histopathological examination: All the selected slides were analyzed in terms of histological parameters such as the presence of fibro-endothelial tissue and multinucleated giant cells in the background of the lesion and were recorded in a specific table. The retrieved paraffin blocks were employed to prepare 4-micron sections to conduct the following techniques: a) Haematoxylin and eosin stain (H&E) it was performed to confirm the diagnosis by two oral pathologists to ensure the diagnosis. b) Immunohistochemical staining for CD31 and MCM 3The Immunohistochemical staining for CD31 and MCM 3 was performed according to the manufacturer`s instructions. This staining was assessed and scored. For Immunohistochemical staining by the avid in-biotin technique, antigen amplification, antigen retrieval, deparaffinization, and rehydration and processing were performed, respectively. After irrigating the samples with phosphate buffer solution (PBS), drops of monoclonal antibody were applied with 1:100 dilutions for MCM3 marker and without dilution for CD31 marker as it was ready to use. The slides were incubated at room temperature overnight then sections were washed - for five minutes in PBS- three times. Finally, the samples were placed in ethanol with various concentrations for dehydration and then in xylene for clearing the slides, and were mounted by a P.V-mounting system. Negative control was established by replacing the primary antibody with PBS. Pyogenic granuloma and colon cancer were used as the positive control for CD31 and MCM3 markers respectively. c) Evaluation and scoring for MCM3: Immunohistochemical results were evaluated under a light microscope, the stained cells were randomly counted in 5 microscopic fields at x400 magnification (HPF) and scored as follows; 0: no detectable staining (<5%), 1: weak but detectable staining (5< and <25%), 2: moderate staining (25%< and <75%) and 3: abundant staining (>75%).[7]

Evaluation and scoring for CD31: The stained vessels were randomly counted in 5 microscopic fields at x400 magnification (HPF) and the proportion of stained cells and overall staining were assessed for each field. The proportion of Stained cells in each field was assessed as 0, no stained cells; 1, 25% stained cells; 2, 25-50% stained cells; and 3, more than50% stained cells.[8]

Micro-vessel density (MVD): To determine MVD, all stained sections were screened at 40× by two observers using a double-headed microscope and Vascular hotspots (areas containing the highest amount of Vascularization) were identified. Of these, five were included all brown- selected for counting micro-vessels At 400×, which stained cells situated individually or in small clusters and separate from other connective tissue elements in addition to identifiable micro-vessels of any size and shape, with or without red blood cells. Large vessels containing muscular walls were not included in the MVD count. MVD was expressed as the mean number of counted micro-vessels per high power field.[9]

Statistical analysis: Data were tabulated, coded then analysed using the computer program SPSS (Statistical package for social science) version 23.0. Descriptive statistics were calculated in the form of mean ± standard deviation (SD), minimum and maximum, and frequency (number and per cent).In the statistical comparison between the different groups, the significance of the difference was tested using one of the following tests: One-way ANOVA (analysis of variance) was used to compare more than two different groups of numerical (parametric) data. Inter-group comparison of categorical data was performed by using Monte-Carlo (>2 x 2 table).

Spearman's correlation coefficient test was used to correlate different parameters, P value <0.05 was considered statistically significant in all analyses. The test used is the student's t-test (unpaired), p-value <0.05 was considered statistically significant.

Results:

As shown in Table 1, the association between the presence of aggressiveness & gender samples were included in the analysis[6] was non-significant (p=0.08) and the frequency of females was higher in group 1 (69.2%) while the male frequency was higher in group 2 (65%). The association between the presence of aggressiveness & site of lesion was non-significant (p=1.00) and frequency in the mandible of aggressive and non-aggressive cases was 84.6% and 80% respectively, while the frequency of maxilla in cases with aggressiveness was 15.4% and cases without was 20%.

The association between the presence of aggressiveness & large swelling, pain, perforation, and root resorption were Significant (p=<0.001*, <0.001*,0.017*,<0.001* respectively). The presence of large swelling was higher in group 1 (92.3%) while it was only 10% in group 2 cases. The presence of pain was higher in group 1 (100%) while it was only (15%) in group2 cases. The presence of perforation was higher in group 1 (84.6%). The presence of root resorption was higher in group 1 (92.3%). The Association between the presence of aggressiveness & recurrence was significant (p=<0.001*). The presence of recurrence was higher in group 1 (84.6%, Table 1). Most of the cases showed numerous MNGCs, they were highly distinct and mostly evenly distributed in 21 cases (63.6%) and focally distributed only in 12 cases (36.3%).All samples were positive for MCM3 staining, it was restricted to the nuclei of either mononuclear cell in the stroma and MNGCs. The reaction was detected as a homogenous brown stain. In the studied cases of CGCGs, the cases were evaluated and scored according to the percentage

Table 1: Association between aggressiveness and other parameters (gender, site, swelling, pain, perforation, root resorptionand recurrence)

		Aggressiveness				P
		No		Yes		
		No	%	No	%	
Gender	Male	13	65.0%	4	30.8%	0.08
	Female	7	35.0%	9	69.2%	
Site	Mandible	16	80.0%	11	84.6%	1.00
	Maxilla	4	20.0%	2	15.4%	
Swelling size	small	18	90.0%	1	7.7%	<0.001*
	large	2	10.0%	12	92.3%	
Pain	No	17	85.0%	0	0.0%	<0.001*
	Yes	3	15.0%	13	100.0%	
Perforation	No	20	100.0%	9	69.2%	0.017*
	Yes	0	0.0%	4	30.8%	
Root resorption	No	16	80.0%	1	7.7%	<0.001*
	Yes	4	20.0%	12	92.3%	
Recurrence	No	20	100.0%	2	15.4%	<0.001*
	Yes	0	0.0%	11	84.6%	

Data expressed as frequency (Number-percent) p:probability *significance<0.05Test used monte-carlo for data expressed as frequancy

of stained cells (incidence, Figure 1: A and B).

As shown in Table 2, the association between aggressive cases and MCM 3 expression was significant (p=<0.001*). All aggressive cases (100%) were associated with a high reaction of MCM3.CD31 is an endothelial marker that was known to stain both old- and newly-formed vessels. The reaction was membranous and/or cytoplasmic. The reaction appeared as a homogenous brown stain. No reaction in MNGCs or other stromal cells (Fig. 1: C and D). The association between CD31 expression and the presence of aggressiveness was significant (p=<0.001*). All aggressive cases (13 cases) (100%) were associated with a high reaction of CD31, Table 3.As shown in Table 4, aggressive cases (19.52±.89) showed a significant increase in MVD compared to non- Aggressive ones

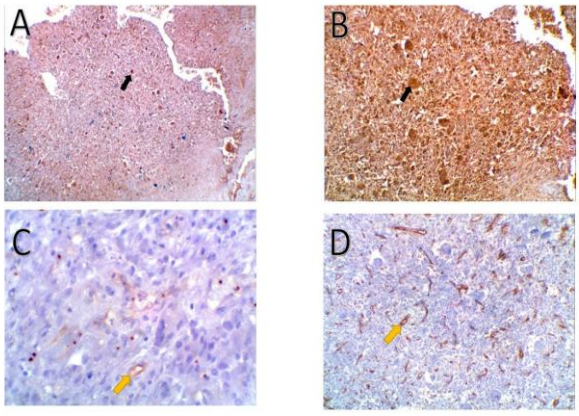

Figure: (A) Photomicrograph of CGCG with low expression of MCM3 marker in both MNGCs (black arrow) & stromal cells (ABC-DAB x100)**, (B)** Photomicrograph of CGCG with high expression of MCM3 marker in both MNGCs (black arrow) & stromal cells (ABC-DAB x200)**, (C)** Photomicrograph of CGCG with low reaction of CD31 marker in endothelial cells (yellow arrow) (ABC-DAB x400), **(D)** Photomicrograph of CGCG with high reaction of CD31 marker in endothelial cells (yellow arrow) of newly formed capillaries (ABC-DAB x100).

(8.90±4.91) (p=<0.001). As shown in Table 5, MCM3 showed a highly significant positive correlation with CD31 (p=<0.001*).

Discussion:

Central giant cell granuloma (CGCG) is a non-neoplastic bone lesion with unknown etiology.[10] Most of the lesions are non-aggressive. They are relatively small and have mild symptoms, or are asymptomatic. Their growth is slow, and they do not cause cortical bone perforation or root resorption in the adjacent teeth. They are usually detected during a routine radiographic examination or based on the painless swelling of bone. Aggressive lesions are characterized by symptoms such as pain, rapid growth, cortical Perforation, root resorption, tooth displacement, paraesthesia, and certain recurrence potential. Spread to the soft tissue and mucosal ulceration may also occur in some cases. Radiographically, CGCG is a multi-ocular radiolucent lesion with defined outlines.[11] Cell proliferation plays a basic role in cell growth and the maintenance of tissue homeostasis, and also in several biological and pathological events, such as tumor development. Identification of cell proliferation markers could be a useful diagnostic or prognostic tool to understand or predict the clinical and biological behavior of many pathologic lesions.[12] Due to contradictions regarding the relationship between clinical behavior and histological features in aggressive and non-aggressive CGCGs reported by studies until now, and the few studies were done on the role of angiogenic activity and cell proliferation in CGCG with a big controversy in their results, the current

study was conducted to investigate the expression of CD31 and MCM3 in aggressive versus non-aggressive CGCG and to determine any correlation between CD31 andMCM3 expression in studied CGCG cases. In the current study, the mean age of the studied cases was 35.6±9.1 years, ranging from 19 to 58 years; this was in accordance with Atarbashi Moghadam and Ghorbanpor[11] as they observed that clinically, CGCG had occurred during a wide age range (2-80 years), approximately in 60% of cases before the age of 30. Also, Sadri et al.[9] and Mourad et al.[13] reported the same results. Regarding gender distribution among the studied cases in the present study, 17 cases were males (51.5%) and 16 cases were females (48.5%) contradicting the results of Mourad et al.[13] as they found that lesions tend to occur mostly in women. A situation of the female predominance of GCG was attributed to the role of particular ovarian sex hormones that could be involved in its formation process.[14] This contradiction might be due to variations in race and sample sizes between the current study and other studies. In this study, the lesions occurred in the mandible representing the majority of cases [27 cases (81.8%)], while the maxilla represents only 6 cases (18.2%). This was in acceptance by Sadri et al. [9] and Mourad et al.[13], who reported the frequent location of the CGCGs anterior to the mandibular first molar and often cross the midline The present study revealed an association between aggressiveness and different clinical parameters as the presence of large swelling, pain, cortical perforation, root resorption, and recurrence where they were significantly higher in group 1 (aggressive cases) than in group 2 (non-aggressive cases), this was in acceptance with Atarbashi Moghadam and Ghorbanpour[11] as they found that aggressive lesions are characterized by the previous symptoms in addition to tooth displacement, paraesthesia, and certain recurrence potential. In the current study, the aggressive cases (group 1) revealed histologically numerous MNGCs, more cellular connective tissue stroma, numerous vascularity, and areas of hemorrhage, this was in line with Neville et al.[10] who reported that the relationship between the histopathological features and clinical behavior of CGCG lesion has remained debatable. It is said that more aggressive lesions may be associated with a greater number of giant cells, more surface occupation by the giant cells, and a higher mitotic index. They also suggested that increased vascular concentration and incidence of markers related to angiogenesis can be attributed to the aggressive clinical behavior of this lesion.

Table 2**:** Association between Aggressiveness of CGCG cases& MCM 3 immunoreactivity

		Aggressiveness				P
		No		Yes		
		No	%	No	%	
MCM 3	low reaction	3	15.0%	0	0%	<0.001*
	Intermediate reaction	9	45.0%	0	0%	
	High reaction	8	40.0%	13	100%	

Data expressed as frequency (Number-percent), P: Probability*: significance <0.05
Test used: Monte-Carlo for data expressed as Frequency

Table3: Association between Aggressiveness& CD31expression in CGCG cases

		Aggressiveness				P
		No		Yes		
		No	%	No	%	
CD 31	low reaction	8	40.0%	0	0%	<0.001*
	intermediate reaction	12	60.0%	0	0%	
	high reaction	0	0%	13	100%	

P: Probability*: significance <0.05
Test used: Monte-Carlo for data expressed as frequency

Table 4: Comparison of MVD regarding to

	Aggressiveness		P
	No	Yes	
MVD	8.90±4.91	19.52±.89	<0.001*

Data expressed as mean ±SD (SD: standard deviation)
P: Probability *: significance <0.05
Test used: Student's t-test (Unpaired)

Table 5: Correlation between MCM3 and CD31

	CD 31	
	R	P
MCM 3	0.762	<0.001*

R: Spearman's rho
P: Probability *: significance <0.05

Peacock et al.[15] reported a higher level of angiogenesis in aggressive giant cell lesions than in non-aggressive ones. However, Peacock et al.[16] concluded that, using the histopathological criteria including, the number of or giant cell nuclei in 41 CGCG samples to between the aggressive and non-aggressive CGCGs alone was not considered sufficient. To the best of our knowledge in English literature, this study was the first one that used MCM3 in CGCLs. MCM3 was used as a proliferation marker in the current study as it was expressed in the early G1 phase and throughout the cell cycle. In contrast with Ki-67, the immunodepression was expressed from the late G1 phase to the mitotic (M) phase, whereas it is important to note that MCM family proteins are involved in the early stages of genome replication of eukaryotic cells. MCM proteins are important components of cell replication machinery and ensure that DNA replicates only once per mitotic cycle. This difference suggests that MCM3 is a more sensitive proliferation marker than Ki-67.[12,17] All present samples were positive for MCM3 staining, it was restricted to the nuclei of either mononuclear cell in the stroma and MNGCs, this is in line with Rezvani et al.[7] who observed that MCM3 expression was restricted to the nuclei of basal cell layer and a few cells in the immediate suprabasal layers in premalignant lesions and squamous cell carcinoma. The reaction was detected as a homogenous brown stain. The current study showed a highly statistically significant association between MCM3 expression related to the size and recurrence of the lesion, this observation was consistent with Carreon-Burciaga et al.[12] who reported that assessment of MCM2 and MCM3 expression would be useful to predict tumor behavior. Tumors with higher expression indexes might have been associated with greater growth, tumoral invasion and recurrence.The present study revealed that a statistically significant correlation existed between aggressive cases and MCM3 expression. All aggressive cases (100%) were associated with a high reaction of MCM3 and these results are in parallel to Razavi and Yahyaabadi[5] who investigated the association between clinical behavior and histopathological features using Ki67 as a proliferative marker on CGCGs, the higher expression was found in aggressive cases. However, Al Sheddi et al.[18] stated that the expression of Ki67 showed no significant difference between the two groups. Hence, they concluded that it is not possible to predict the clinical behavior of CGCG by histological analyses and proliferation parameters; therefore, this index could not be used for predicting the clinical behavior of CGCG. In the present study, CD31 marker which is an endothelial marker that was known to stain both old- and newly-formed vessels, the reaction was membranous and/or cytoplasmic and it appeared as a homogenous brown stain, this was in accordance with Razavi and Yahyaabadi.[5] Comparisons of both the size of the lesions and recurrence with CD31 expression revealed a highly significant expression with high reaction observed in large size lesions and recurrent cases in the current study. Various markers have assessed the vascularity in GCGs and suggested the angiogenic activity of GCLs and it was proposed as a determinant of the aggressive nature of GCLs. In the current study association between CD31 expression and the presence of aggressiveness was significant. All aggressive cases (13 cases) (100%) were associated with a high reaction of CD31 moreover, aggressive cases showed a significant increase in MVD Compared to non-aggressive ones and this was in agreement with Razavi and Yahyaabadi[5] who showed that the mean expression of CD31 was higher in aggressive CGCGs. Also, Mourad et al.[13] observed increased levels of VEGF in the MNGCs and the surrounding stroma of aggressive lesions as compared with non-aggressive lesions. Their results were corroborating the results of Peacock et al.[15] and substantiate the hypothesis that a proliferative vascular component within GCLs may be accountable for their clinical aggressiveness. Furthermore, their results advocated that the major role of VEGF in CGCL is related to the osteoclast genesis process and, consequently, to the stimulation of bone resorption.Similar to the findings of the current study, Nair et al.[19] compared the angiogenesis between aggressive and non-aggressive forms of CGCG and reported a significantly higher level of vascularity in aggressive versus non-aggressive lesions. Hallikeri et al.[20] also observed a significantly higher MVD in CGCGs and this can be explained that the increased vascular proliferation in aggressive CGCGs may be responsible for the aggressive behavior of this lesion. In line with this theory that vascular proliferation in aggressive CGCGs may be responsible for the aggressive behavior of this lesion, it can be argued that increased angiogenesis can differentiate the blood vessels into osteoclasts, which in turn induces the rapid growth, osteolysis, and enlargement of this lesion. Razavi and Yahyaabadi[5] also evaluated the expression of VEGF, CD31, and CD34 markers in CGCGs. They found that the angiogenesis level was higher in the aggressive samples than in non-aggressive samples. Therefore, the incidence rate of these markers can be a basis for predicting the clinical behavior of CGCG. In this study, MCM3 showed a highly significant positive correlation with CD31, this was accepted by Razavi and Yahyaabadi[5] who observed a significant difference between the mean expression of CD31 and KI67 markers in non-aggressive and aggressive CGCGs and they showed that there might be a positive relationship between the clinical features and histopathology of CGCG. Furthermore, they concluded that the clinical behavior may be predicted based on features such as the number of blood vessels and proliferation of fibro-endothelial cells. Whereas El-Attar and Wahba[8] found in their study on Ki67 and CD31 markers in 10 CGCG non-aggressive samples and 8 aggressive CGCG lesions, the expression of CD31 marker did not show a significant difference between the two

groups, but the number of Ki67 marker was higher in aggressive lesions. Accordingly, it was concluded that the expression of Ki67 marker helped determine the clinical behavior of CGCGs. The positive point of the present study is that along with the use of CD31 marker, as the most well-known immunohistochemical vascular marker, we decided to use MCM3 marker, which is a sensitive cellular proliferation marker. An important characteristic of this marker is that it is present in all active stages of mitosis, so it can be well associated with cell proliferation. A point worth to be mentioned is that in the current study the number of studied samples was by far more than those of the aforementioned studies. Accordingly, it can be argued that the results of the present research are more reliable. Thus, considering the obtained results in the current study, it can be argued that the incidence of MCM3 marker can also be used as a reliable histopathological parameter to anticipate the clinical behavior of CGCG.

Conclusions:

The clinical behavior of CGCG might be partly predicted by using cell proliferation (MCM3) and angiogenic (CD31) markers and analyzing their expression. The histological criteria should be considered as a proper index for early diagnosis of aggressive CGCG and consequently a guideline for clinicians and surgeons for making a treatment plan.The combined evaluation of old- and newly-formed vessels by endothelial markers CD31 showed differences between aggressive and non-aggressive lesions, supporting the possible vascular-proliferative nature of aggressive lesions.Histologically MVD is one of the important prognostic tools that can be used to differentiate between aggressive versus non-aggressive CGCGs. A highly significant correlation existed between cell proliferation represented by MCM3 expression and angiogenesis represented by CD31 expression in CGCGs.

CGCLs require further investigations using large scales of cases and long-term follow-up. Using auxiliary ways in the treatment plan of CGCLs as anti-proliferative and anti-angiogenic agents is recommended especially in aggressive one.

Reference:

1. Samson M, Corbera-Bellalta M, Audia S, Planas-Rigol E, Martin L, Cid MC, et al. Recent advances in our understanding of giant cell arteritis pathogenesis. Autoimmun Rev. 2017;16(8):833–844.
2. Zhai Y, Cheng E, Wu H, Li N, Yung PYK, Gao N, et al. Open-ringed structure of the Cdt1–Mcm2–7 complex as a precursor of the MCM double hexamer. Nat Struct Mol Biol. 2017;24(3):300–308.
3. Ha J-K, Jeong H, Kim Y-J, Lee K-H, Choi K-E. Association with recurrence of giant cell tumor of bone between immunohistochemical marker (MCM3, Ki-67 and HH3) expression rate. J Korean Bone Jt Tumor Soc. 2007;13(2):67–74.
4. Müller AM, Hermanns MI, Skrzynski C, Nesslinger M, Müller K-M, Kirkpatrick CJ. Expression of the endothelial markers PECAM-1, vWf, and CD34 in vivo and in vitro. Exp Mol Pathol. 2002;72(3):221–229.
5. Razavi SM, Yahyaabadi R. Comparative study of correlation between angiogenesis markers (CD31) and Ki67 marker with behavior of aggressive and nonaggressive central giant cell granuloma with immunohistochemistry technique. Asian Pac J Cancer Prev. 2018;19(8):2279–2283.
6. Vasconcelos RG, Vasconcelos MG, Queiroz LMG. Peripheral and central giant cell lesions: etiology, origin of giant cells, diagnosis and treatment. J Bras Patol Med Lab. 2013;49(6):446–452.
7. Rezvani G, Andisheh-Tadbir A, Ashraf MJ, Amanpour S, Kamali F, Fardisi S. Evaluation of minichromosome maintenance-3 (MCM3) in oral squamous cell carcinoma. J Dent (Shiraz). 2015;16(2):87–92.
8. HM El Attar R, Wahba OM. Expression of Ki67, CD31, CD68 and P53 in peripheral and central giant cell granuloma of the jaws. Arch Cancer Res. 2016;4(2):1–8.
9. Sadri D, Shahsavari F, Hezarkhani M, Shafizadeh M. Expression of CD34 and CD31 in central and peripheral giant cell granulomas. J Dent (Shiraz). 2019;20(1):10–15.
10. Neville BW, Damm DD, Allen CM, Bouquot J. Oral and Maxillofacial Pathology. 4th ed. Saunders; 2015.
11. Atarbashi Moghadam S, Ghorbanpour M. Central giant cell granuloma of the jaws: Correlation between vascularity and biologic behavior. J Dental Materials and Techniques. 2017;6(1):35–39.
12. Carreón-Burciaga RG, González-González R, Molina-Frechero N, Bologna-Molina R. Immunoexpression of Ki-67, MCM2, and MCM3 in ameloblastoma and ameloblastic carcinoma and their correlations with clinical and histopathological patterns. Dis Markers. 2015; 2015:683087.
13. Mourad S, Awad S, Shamaa A. Vascularity and angiogenesis level correlations with the clinical behavior of the central and peripheral giant cell granulomas of the oral cavity. Egypt Dent J. 2019;65(3):2209–2218.
14. Peñarrocha-Diago MA, Cervera-Ballester J, Maestre-Ferrín L, Peñarrocha-Oltra D. Peripheral giant cell granuloma associated with dental implants: clinical case and literature review. J Oral Implantol. 2012;38 Spec No(S1):527–532.
15. Peacock ZS, Jordan RCK, Schmidt BL. Giant cell lesions of the jaws: does the level of vascularity and angiogenesis correlate with behavior? J Oral Maxillofac Surg. 2012;70(8):1860–1866.

16. Peacock ZS, Resnick CM, Susarla SM, Faquin WC, Rosenberg AE, Nielsen GP, et al. Do histologic criteria predict biologic behavior of giant cell lesions? J Oral Maxillofac Surg. 2012;70(11):2573–2580.
17. Valverde L de F, de Freitas RD, Pereira T de A, de Resende MF, Agra IMG, Dos Santos JN, et al. MCM3: A novel proliferation marker in oral squamous cell carcinoma. Appl Immunohistochem Mol Morphol. 2018;26(2):120–125.
18. Al Sheddi M, Mosadomi H, Al Dayel F. Central giant cell granuloma of the jaws and giant cell tumor of long bones. A clinicopathologic, cytometric, and immunohistochemical comparative study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;98(2):195–196.
19. Nair S, Nayak R, Bhat K, Kotrashetti VS, Babji D. Immunohistochemical expression of CD105 and TGF-β1 in oral squamous cell carcinoma and adjacent apparently normal oral mucosa and its correlation with clinicopathologic features. Appl Immunohistochem Mol Morphol. 2016;24(1):35–41.
20. Hallikeri K, Acharya S, Koneru A, Trivedi DJ. Evaluation of microvessel density in central and peripheral giant cell granulomas. J Adv Clin Res Insights.2015;2:20–25.