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ORIGINAL PAPER

Prognostic Significance of Mast Cell Count and Angiogenesis in Central Giant Cell Granulomas of the Jaws

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ABSTRACT

Objectives: *Mast cells (MCs) secrete different angiogenic factors stimulating angiogenesis. The purpose of this study was to assess MC count and microvessel density (MVD) in aggressive and non-aggressive central giant cell granulomas (CGCGs) of the jaws.*

Materials and methods: *Sixteen aggressive and 14 non-aggressive paraffin-embedded samples of CGCGs were prepared for immunohistochemical analysis using anti-MC tryptase and anti-CD31 antibodies.*

Results: *Mean values±SEM (standard error of mean) for MC count and MVD in aggressive and non-aggressive CGCGs were: 7.48±0.84, 3.85±0.51, 5.55±0.89, 3.19±0.61, respectively. All CGCG cases demonstrated MCs. Differences for both MC count and MVD were statistically significant between aggressive and non-aggressive CGCGs (p=0.002 and p=0.045, respectively),* i.e., *MC count and MVD were significantly higher in aggressive lesions. Moreover, there was a significant positive correlation between MC count and MVD in CGCG (r=0.570, p=0.001).*

Conclusion: *Mast cell count and MVD might be used as prognostic markers of aggressive behavior of CGCGs at the time of initial biopsy. Our findings might postulate that therapeutic strategies against MC mediators and angiogenesis may benefit in aggressive CGCG as a non-surgical modality of treatment.*

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With further studies, their prognostic significance and therapeutic application can be evaluated in patients suffering from aggressive CGCG.

Keywords: angiogenesis, central giant cell granuloma, CGCG, immunohistochemistry, mast cell, microvessel.

INTRODUCTION

Central giant cell granuloma (CGCG) is a non-odontogenic benign lesion of the jaw bones. This lesion accounts for about 10% of all benign lesions of the jaws and mostly occurs in people younger than 30 years of age. Peripheral variant of giant cell granuloma is a reactive lesion which occurs almost exclusively in the gingiva. On the contrary, different origins of reactive, neoplastic, infective or mesenchymal proliferation have been described for CGCG (1, 2).

Based on its clinical and radiographic behavior, CGCG is classified into two non-aggressive and aggressive categories. Aggressiveness is described as rapid growth, cortical perforation, recurrence and root resorption (1, 3, 4). Histopathologically, the hallmark of both types of CGCG is multinucleated giant cells within a fibrovascular stroma. Therefore, the custom histopathological evaluation cannot distinguish these two forms of CGCGs (3-5) and recognizing molecular and cellular biomarkers is very beneficial to predict the clinical behavior and prognosis of CGCG. Identifying the characteristic parameters of tumor progression and aggressiveness can help to plan targeted therapeutic strategies. Molecular targeting can reduce serious complications such as facial distortions, aesthetic and functional damages, tooth loss and recurrences, particularly in aggressive forms of these lesions (1, 6-8).

Mast cells (MCs) are granular immune cells scattered throughout the body, particularly in association with the blood vessels in tumor microenvironment. These cells play principal roles in the formation of the granulation tissue, wound healing, tissue remodeling and growth of various lesions (9-12). Mast cells have important roles in the pathogenesis of different lesions, e.g., renal cell carcinoma, gastric and lung cancers. Therefore, MC count has been appeared to be a significant prognostic biomarker in many lesions (13-16). It has been shown that MCs contain several angiogenic factors such as vascular endothelial growth factor, tryptase, FGF, tumor necrosis factor, histamine, heparin and IL-8. Consequently, angiogenesis which has an essential role in the progression of various lesions is influenced by MCs (17, 18). On the other hand, the hypothesis that CGCG belongs to the jaw vascular lesions has been proposed. However, characterization of the vascular component has not been completely investigated. Interestingly, anti-angiogenic therapy with alpha and beta interferons has been introduced as a promising strategy for the treatment of aggressive CGCGs (19, 20). Considering the possible role of MCs and microvessels in clinical behavior of these lesions, we aimed to assess MC count and MVD in aggressive and non-aggressive CGCGs. ❑

MATERIALS AND METHODS

Tissue samples

The research protocol was approved by ethics committee of Tabriz University of Medical Sciences (No. 432). In this descriptive analytical study, the paraffin-embedded cases with the diagnosis of CGCG were retrieved from the archives of three pathology laboratories. The patient records were reviewed to obtain the clinical and radiographic information and the microscopic slides were assessed to confirm the diagnosis. Fourteen samples characterized by slow growth rate, no or negligible symptoms and lack of cortical perforation or root resorption were considered as non-aggressive CGCGs. Sixteen samples characterized by pain, high growth rate, cortical perforation or root resorption and recurrence were considered as aggressive CGCGs. The inclusion criteria were: (i) giant cell granulomas located centrally within the bone; (ii) patients submitted to surgery as the initial treatment; (iii) the presence of adequate microscopic fields for immunohistochemical analysis; and (iv) the presence of appropriate paraffin blocks to prepare new slides. Samples with (i) histopathologic similarity to central giant cell granuloma, but clinically different (e.g., brown tumor

of hyperparathyroidism, cherubism and aneurysmal bone cyst); (ii) patients to whom treatment other than surgery was performed; (iii); insufficient microscopic fields to evaluate the samples; and (v) inappropriate paraffin blocks to prepare microscopic sections were excluded (2).

Immunohistochemical staining

Two sections of 4 μm thickness were prepared from each formalin-fixed paraffin-embedded specimen. A standard immunohistochemical procedure was carried out following the guidelines (DAKO, Denmark). Deparaffinization of the samples in xylene and then rehydration through alcohol with different concentrations were carried out. Peroxidase activity was inhibited in 1% hydrogen peroxide and the antigens retrieval was performed in citrate buffer (PH=6.0, 0.01 M). Subsequently, the sections were incubated with anti-MC tryptase (1:100 diluted) and anti-CD31 (1:20 diluted) monoclonal mouse anti-human primary antibodies and rinsed with phosphate buffered saline. The samples were then incubated with the secondary antibody [the Streptavidin–Biotin–Peroxidase HRP complex (Envision/HRP)] to amplify the immune reactions. 3, 3-diaminobenzidine (DAB) was applied for five minutes to visualize the reaction products. The last step was counterstaining the slides in Harris hematoxylin and mounting. Hemangioma and tonsil tissues were considered as positive controls for anti-CD34 and anti-tryptase staining, respectively (1).

Quantitative analysis of MCs count and MVD

Blind immunohistochemical assessment was carried out by two pathologists. The level of agreement was 92%. The slides were scanned at × 100 magnification to detect hot spot fields (the fields most occupied by MCs and micovessels). Consequently, eight high power non-overlapping fields (×400 magnification) limited to hot spot areas were selected. Mast cells and microvessels were counted in the selected fields. Mast cell count and MVD was described as the mean value of eight high power fields (1, 13, 15).

Statistical analysis

Statistical Package for Social Sciences 20.0 (SPSS, Chicago, IL) was used for statistical analysis of the data. Inter-observer reproducibility for both MC count and MVD was based on six double evaluations. Independent t-test was performed for comparison of CD31 and MC tryptase expression among aggressive and aggressive CGCGs. To determine the correlation among MC count and MVD, Pearson correlation coefficient test was carried out. Significance was established at a P value of < 0.05. ❑

RESULTS

Sixteen samples of non-aggressive CGCGs from 17 to 75 (mean 39) year-old subjects and 14 samples of aggressive CGCGs from nine to 60 (mean 29) year-olds were evaluated.

Our findings revealed that aggressive CGCGs occurred in younger adults compared with non-aggressive ones. Microscopically, the classical histopathologic pattern described in the literatures was observed in both aggressive and non-aggressive CGCGs. Multinucleated giant cells were admixed with spindle to ovoid mononuclear mesenchymal cells and round monocyte-macrophages in all of the cases. The morphology, number and size of multinucleated giant cells varied from lesion to lesion and within each lesion. Hemorrhage and numerous capillaries were seen in almost all cases. Reactive bone formation, mostly in depth of the connective tissue, was observed in a number of CGCGs.

Immunohistochemical analysis revealed that MCs were present in all cases of CGCGs. These cells were more commonly observed around microvessels, as brownish round cells. The number of MCs (assessed by MC tryptase expression) was increased significantly in aggressive CGCGs compared with non-aggressive ones, using independent t-test ($p=0.002$). Data are shown in Figures 1 and 2 and in Table 1.

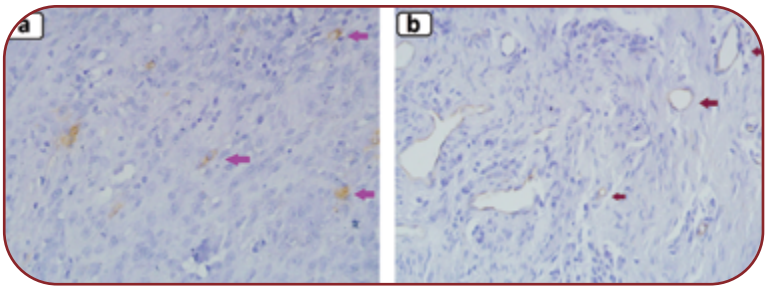

FIGURE 1. a) Mast cells in the stroma of aggressive central giant cell granuloma (CGCG); b) microvessels throughout the aggressive central CGCG (IHC staining×400)

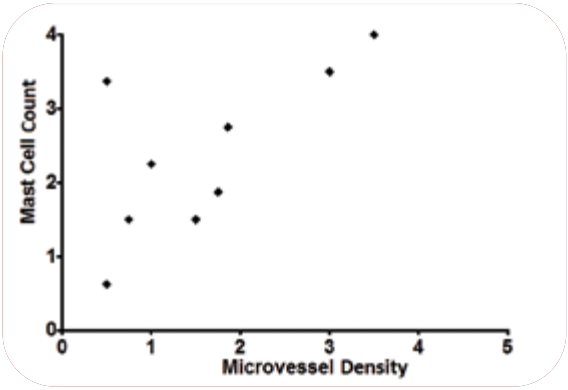

FIGURE 2. Positive correlation between mast cell count and microvessel density in central giant cell granuloma (CGCG)

TABLE 1. Mast cell count and microvessel density in central giant cell granuloma (CGCG)

Studied groups	Mast cell count mean±SEM*	Microvessel density mean±SEM*
Aggressive CGCG	7.48 ± 0.84	5.55 ± 0.89
Non-aggressive CGCG	3.85 ± 0.51	3.19 ± 0.61

*SEM: standard error of mean; CGCG: central giant cell granuloma

Angiogenesis (evaluated by CD31 expression) was significantly higher in aggressive CGCGs in comparison with non-aggressive lesions using independent t-test (p=0.045). Pearson correlation coefficient test revealed a statistically significant positive correlation between MC count and MVD in CGCGs (r=0.451, p=0.012/ r=0.570, p=0.001). ❑

DISCUSSION

Considering the role of MCs in clinical aggressiveness of many lesions, we decided to investigate whether these cells have fundamental role in CGCG behaviour. Our findings revealed that MCs were more commonly observed in aggressive CGCGs than non-aggressive lesions. As previously stated, aggressive CGCGs differ from non-aggressive lesions in the presence of symptoms, higher rate of growth, perforation of the cortex, root resorption and higher recurrence rate (3, 4).

Since the categorization of CGCGs into aggressive and non-aggressive lesions about three decades ago, many researches have been carried out to find valuable biomarkers to predict the clinical behavior of CGCG at the time of initial biopsy. Early studies focused on giant cells (the hallmark of the lesion), *i.e.*, the number and distribution of these cells. The stromal elements as the principal cells involved in the pathogenesis of these lesions and formation of the giant cells received more attention (3, 7, 21, 22). In previous studies, different biomarkers have been investigated to distinguish between aggressive and non-aggressive lesions. Toban-Arroyave *et al* investigated the expression of osteotropic factors as RANK, GRα and CTR in the CGCGs and found a statistically significant difference in CTR expression between the two clinical forms of CGCGs. However, their results did not show a significant difference in RANK and GRα expressions between study groups. They suggested more investigations on these biomarkers with the ultimate goal of achieving to the more effective molecular therapies (1). In another study, these researchers declared that the mean expressions of matrix metalloproteinase-1 and -9 (MMP-1,9), the proteolytic enzymes, were significantly higher in the aggressive lesions than the non-aggressive CGCGs. They expressed that the differences in MMP-1,9 enzymes and their related resorptive functions may cause the distinct clinical behavior of these lesions (6). In a study by Vered *et al*, the correlation between myofibroblast density and biological behavior of CGCG was investigated. The authors found that the myofibroblast density could not distinguish between aggressive and non-aggressive lesions. However, they recommended further investigation to identify the precise role of stromal myofibroblasts in CGCGs of the jaws and their contribution to the pathogenesis of these lesions (7). Mahajan *et al* evaluated nuclear organizing regions (AgNOR) in CGCGs of the jaws and found a positive correlation between this biomarker and the biological behavior of these lesions. They suggested AgNOR as a prognostic indicator of aggressive behaviour in CGCG (23). However, the definite role of cellular and molecular factors in the pathogenesis of CGCG remains uncertain.

Our findings revealed that both clinical types of CGCGs, especially aggressive ones, possessed stromal MCs. Many previous studies showed that MCs were associated with high growth rate, metastasis and poor prognosis in some lesions such as gastric cancer, renal cell carcinoma and lung cancer (13-15, 24). Ammendola *et al* noticed a significant positive correlation between MC count and angiogenesis in gastric cancer. They stated that MCs were associated with bone me-

tastasis in these patients (13). Ullah *et al* found a positive correlation between MC counts, angiogenesis of squamous cell carcinoma of the lung (15). Marech *et al* performed a comprehensive review on the role of MCs in renal cell carcinoma. They stated that MCs were correlated with angiogenesis. This correlation has prognostic significance and possible therapeutic implications in renal cell carcinoma (14).

In the second part of this study, we assessed CD31 expression in CGCGs. Our results revealed that CD31 expression was higher in aggressive CGCG than non-aggressive lesions. Kaban *et al*. introduced interferon α (anti-angiogenic therapy) as a novel treatment strategy for a child with a life-endangering CGCG of the mandible that had recurred multiple times (in 1999) (20). In 2002, Kaban *et al* administered interferon α and β for treatment of eight patients with aggressive CGCG. Seven of these patients completed interferon therapy and no recurrences was noted during one year to six years of follow-up (19). Our results show that MVD reflects the aggressive clinical behavior of CGCG as a rationale behind anti-angiogenic therapy as a strategy for treatment of aggressive CGCGs.

In our study, a positive correlation between MC count and MVD was found. Most of the previous studies have shown a positive correlation between the number of MC and microvessels (14, 15, 25, 26). Moreover, *in vitro* studies have suggested that MCs promoted the formation of new blood vessels. Blair *et al* showed that MCs tryptase directly induced proliferation of the human dermal endothelial cells. They suggested that MCs accumulated near the sites of vessel formation and tryptase acted as a potent angiogenic factor (27). Tryptase is a serine protease that is produced by activated MCs and high levels of this protein is observed in plasma and other body fluids (17). We intentionally selected MC tryptase for IHC staining of CGCGs, because both intact and degranulated MCs are detectable with high degree of precision and sensitivity using this marker. Tryptase is positive in all human MCs and other cell types do not stain positively with this biomarker. Thus, tryptase is the most reliable indicators of MCs (28). It has been suggested that this biomarker directly affects the endothelial cells and stimulates the proliferation and migration of these cells. Furthermore, it indirectly affects the process of angiogenesis and degrades the connective tissue matrix which consequently provides space for the formation of new blood vessels. However, this positive correlation was not always observed in clinical studies (29, 30). Mohseni *et al* did not find a statistically significant correlation between the number of MCs and microvessels in renal cell carcinoma (29). Similarly, in a study on non-small cell carcinoma of the lung, a positive correlation was not observed between the number of MCs and microvessels (30).

Our findings revealed that MCs were an integral component of CGCG and MC count reflected the aggressive clinical behavior of this lesion. Microvessel density was also higher in aggressive lesions and our results confirm the anti-angiogenic therapy as a useful strategy for the treatment of aggressive CGCGs. ❑

CONCLUSION

Mast cell count and MVD might be used as prognostic markers of aggressive behavior of CGCGs at the time of initial biopsy. Our findings might postulate that therapeutic strategies against MC mediators and angiogenesis may benefit in aggressive CGCG as a non-surgical modality of treatment. With further studies, their prognostic significance and therapeutic application can be evaluated in patients suffering from aggressive CGCG. ❑

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