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Original research

Immunohistochemical evaluation of VEGF and tryptase for angiogenesis and mast cell counting in giant cell granulomas of the jaws

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ABSTRACT

Objective: Etiology and pathogenesis of giant cell granulomas of the jaws are not well defined. The aim of this study was to evaluate VEGF and tryptase expression for angiogenesis and mast cell counting in peripheral giant cell granulomas (PGCGs) and central giant cell granulomas (CGCGs) with similar histopathological feature but different biological behavior.
Methods: We evaluated the immunoexpression of VEGF and tryptase in 72 giant cell lesions of the jaws including 36 CGCGs and 36 PGCGs. Sixteen blocks of normal mucosa were chosen as control group.
Results: Although the mean percentage of VEGF immunoreactivity of mononucleated cells (MC) and multinucleated giant cells (MGC) was higher in CGCGs, statistical significant difference between two groups was seen for MGC. Mean vessel count was higher in CGCGs than PGCGs with statistically significant difference. Higher mean of tryptase positive mast cells was observed in PGCGs with statistically significant difference. The association between angiogenesis and mast cell count was significant in CGCGs.
Conclusions: The results may explain the different pathogenesis of these two lesions, despite the similarity in histopathologic features. Higher overall expression of VEGF in CGCGs might lead to increased vascularity as well as more destructive nature. More mast cells in PGCGs may explain the different pathogenesis of studied lesions.

1. Introduction

The central giant cell granuloma (CGCG) is a benign intraosseous lesion with variable clinical behavior [1]. The peripheral giant cell granuloma (PGCG) is a reactive exophytic lesion which originates from periodontal ligament or mucoperiosteum of the alveolar ridge. Histologically, the lesions are composed of multinucleated giant cells (MGC) dispersed among spindle mononucleated cells (MC). Despite the similarity in their histopathological feature, they show distinct clinical behavior [2].

Mast cells play a critical role in many pathological, immunological and physiological processes regarding their broad range of function [3].

Mast cells and contents of their granules have strong influence in progress of inflammation and tissue destruction [4]. Tryptase, chymase and histamine, stored in metachromatic granules of mast cells can stimulate production of MMPs 2, 9 and lead to tissue destruction through lysis of ground substance [5–7].

Based on microenviromental circumstances in various lesions, the function, phenotype and number of mast cells can be modified [8].

Vascular endothelial growth factor (VEGF) is an important mediator of angiogenesis [9]. It is a protein that stimulates the growth of new vessels and provides sufficient oxygenation of the tissue when the blood supply is compromised. However when it's concentration in the tissue passes normal limits, it behaves in pathologic manner [10].

Higher vascular concentration in aggressive lesions has been proved by many studies. Some studies have also shown that vas-

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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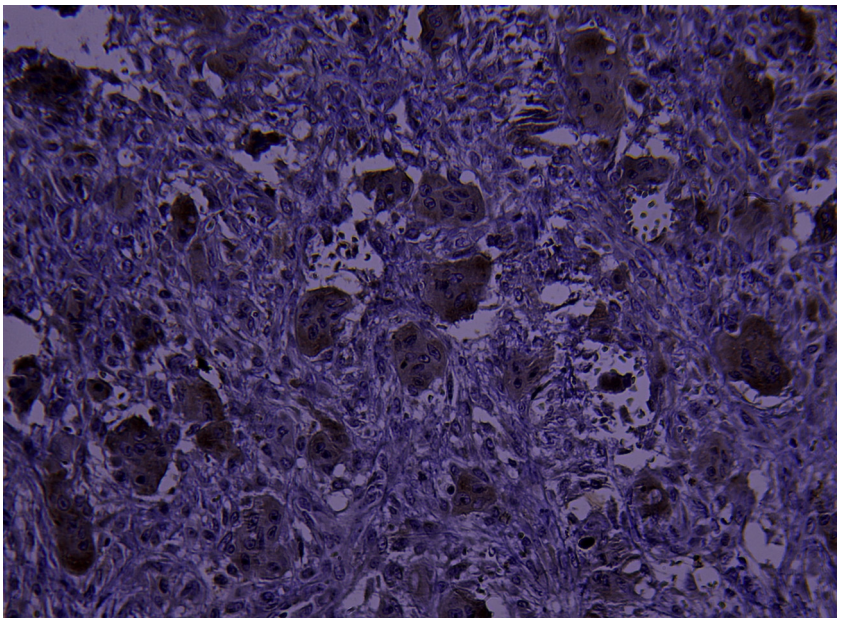

Fig. 1. VEGF staining in MGC and MC of central giant cell granuloma with moderate immunoreactivity (immunohistochemistry staining, original magnification 200×).

cular concentration is considerably higher in aggressive giant cell granuloma of the jaws compared to less aggressive ones [9].

Although the main role of VEGF is blood vessel development and angiogenesis, several studies have shown the importance of this marker in osteoclast formation [11].

Comparison of mast cells and vascular concentrations between these two forms of giant cell granuloma with different clinical behavior and nature might be useful in evaluating the differences in their pathogenesis [3].

The aim of this study was to evaluate VEGF and tryptase expression for angiogenesis and mast cell counting in PGCG and CGCG lesions with different nature and biological behavior.

2. Methods

We examined VEGF and tryptase expression in 72 giant cell lesions of the jaws including 36 CGCGs and 36 PGCGs. Sixteen blocks of normal mucosa were chosen as control group. For the control group, we used normal connective tissue of non-neoplastic lesions like mucoceles without inflammation. Paraffin-embedded tissue blocks were obtained from the files of the Oral and Maxillofacial Pathology Department of Mashhad Dental School, Iran.

Immunohistochemical study was performed on 4 μm-thick, paraffin-embedded sections. Each section was deparaffinized, rehydrated in xylene, graded with ethanol and washed in distilled water. Endogenous peroxide activity was blocked by treatment in 1% H2O2 with methanol for 30 min. For antigen retrieval, the sections were immersed in the citrate solution (0.01 M, pH 6.0) and incubated in the microwave oven under a pressure of 2 atm and a temperature of 120 °C for 15 min. The sections were retained in the same solution for 15 min and subsequently washed with distilled water for 5 min.

Tryptase and VEGF were two target proteins selected in this research. The first one, an enzyme, was chosen for mast cell detection (number of mast cells in 0.2 mm^2) and the second, a growth factor for determination of angiogenesis (number of vessels in 0.2 mm^2) [12].

The applied monoclonal antibodies were mast cell tryptase (clone 10D11, 1:50 dilution, Novocastra Laboratories, Newcastle, UK) and VEGF (clone KLT9, 1:100 dilution Novocastra Laboratories, Newcastle, UK). The antibodies were applied for 60 min at room temperature. Samples were then washed with Tris-Buffered Saline/Tween. For immunohistochemical staining, the Novo link Polymer Detection System was used. This procedure was followed by counterstaining using Meyer's Hematoxylin and dehydration through soaking the sections in 95% ethanol twice, each for 10 s, then in 100% ethanol twice for 10 s.

The immunohistochemical staining was assessed by independent investigators. The reactive cells were considered positive. For mast cell and vessel count, 5 microscopic fields under 400× magnification with the greatest number immunoreactivity were selected as the "hot spots." The number of positive mast cells and micrrovessels in a microscope with field of view of 0.2 mm2 were counted and the mean average of the fields were considered for the counting [12].

For VEGF immunoreactivity in MC and MGC, the slides were examined with a light microscope at a final magnification of 400×. Positivity was considered as the presence of cytoplasmic staining for VEGF. According to the percentage of positive cells in the mean average of five fields, immunohistochemical reactivity for VEGF was scored as follows:

0–5%(−), 5%–25(+), 25%–50%(++), 50%–75%(+ + +),

75%–100%(+ + ++)

Studied groups with VEGF expression graded as ++++, +++, were defined as strong and moderate respectively [12].

The data was analyzed using SPSS version 17. Independent samples test and Mann-Whitney were used for statistical analysis. P value <0.05 was considered statistically significant.

3. Results

3.1. VEGF expression in MC, MGC and assessment of mean vessel count

Most of the MGC in CGCG samples showed moderate immunoreactivity, whereas strong staining was observed in MC.

According to the Mann-Whitney test, percentage of immunostaining was not significant between MC and MGC of CGCGs

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