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Original Article

Expression of CD34 and CD31 in Central and Peripheral Giant Cell Granulomas

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ABSTRACT

Statement of the Problem: There are some differences between clinical features of central giant cell granulomas (CGCGs) and peripheral giant cell granulomas (CGCGs) despite their same microscopic features. The possible role of angiogenesis in this issue is still a matter of debate.

Purpose: The aim of the present study was to compare microvessel density (MVD) between CGCGs and PGCGs of the oral cavity using CD31 and CD34.

Materials and Method: Immunohistochemical staining was performed on 18 PGCGs and 19 CGCGs using a monoclonal antibody against CD34 and CD31. MVD was assessed and compared between the lesions using t-test for statistical analysis. $p< 0.05$ was considered significant.

Results: The expression levels of both CD34 and CD31 were significantly higher in CGCGs compared to PGCGs ($p< 0.002$ and $p< 0.001$, respectively). Significant differences in MVD assessed by both markers were observed between males and females in PGCGs ($p< 0.05$), but not CGCGs ($p< 0.2$).

Conclusion: The combined evaluation of old- and newly-formed vessels by pan-endothelial cell markers showed differences between CGCGs and PGCGs, supporting the possible vascular-proliferative nature of the former. Whether this difference has a part in their diverse biologic behaviors and the role which pre-existent vessels play in comparison to neo-formed vasculature, requires further investigation.

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Introduction

Intra- and extra-osseous lesions occur within the head and neck region, some of which are known counterparts like central and peripheral ameloblastomas and ghost cell odontogenic tumors. [1] However, this relationship is not so clear for giant cell lesions and it is still debatable whether these are separate entities or variants of a single lesion, which can be found at different locations. [2-3]

Peripheral giant cell granulomas (PGCGs) develop in response to local irritation or trauma, occasionally erode the underlying bone, and have a low recurrence rate, especially after adequate treatment. On the other hand, the etiology of central giant cell granulomas (CGCG) is controversial and they are known to demonstrate diverse clinical features and behavior. Some cases demonstrate an indolent behavior and minimal symptoms, while others develop in a younger age group, behave aggressively and tend to recur. Despite their clinical differences, these intra- and extra-osseous lesions have similar histologic characteristics .They are comprised of variable amounts of multinucleated giant cells in a background of the oval to spindle-shaped mononuclear cells. [3] This contradiction has been a major concern among researchers leading to studies on various cytomorphometric, immunohistochemical, and ultrastructural aspects of these lesions. [2, 4-6]

Angiogenesis is an important factor that occurs in both physiological and pathological conditions and it

has been shown that angiogenesis would affect the biologic behavior of various neoplastic and non-neoplastic diseases. This phenomenon is evaluated through assessment of MVD using various endothelial cell markers [7] such as CD34 and CD31. CD34 is a 110-kDa cell surface glycoprotein and functions as a cell-cell adhesion factor. It may also mediate the attachment of stem cells to the bone marrow extracellular matrix or directly to stromal cells. Cells expressing CD34 (CD34+ cell) are normally found in the bone marrow as hematopoietic cells, or in mesenchymal stem cells, endothelial progenitor cells, endothelial cells of blood vessel. [7-8]

CD31 is a 130-kDa glycoprotein that appears on blood endothelial cells, platelets, macrophages and lymphocytes (T cells, B cells, and NK cells) and osteoclast by immunohistochemistry technique, CD31 is used to demonstrate the presence of endothelial cells in histological tissue sections that helping to evaluate the degree of tumor angiogenesis. [7]

Vascular endothelial growth factor (VEGF) has been previously investigated in giant cell lesions and has been suggested that those situated in the jawbones, particularly, lie within the range of primary proliferative vascular lesions. [4] However, this notion was not supported by Kahn *et al.* [9]

Antigenic factors like VEGF and basic fibroblast growth factor (bFGF) have been reported to have a closer relationship with osteoclast genesis than angiogenesis. [4, 9] Microvessel density (MVD) and microvessel count using endothelial cell markers have been evaluated and compared between CGCG and PGCG with contradictory results. [4, 10-13]

Considering the importance of this process and the fact that endothelial cells not only function in angiogenesis-related activities but also have a role in various phenomena, we aimed to evaluate angiogenesis in PGCG and CGCG using CD34 and CD31. We were not able to find previous research in this field using the latter pan-endothelial protein.

Materials and Method

After obtaining ethical approval from the ethics committee of our University, patient records were reviewed from 2004 to 2015 and clinical/demographic data for subjects with a diagnosis of giant cell granuloma were extracted. [3, 9] Considering clinical and radiographic manifestations, all histologic slides were re-evaluated to confirm the diagnosis. [3] Samples with necrotic and/or inadequate tissue, extensive hemorrhage, or incomplete clinical information were excluded. Moreover, other giant-cell-containing lesions like aneurysmal bone cyst, brown tumor of hyperparathyroidism (confirmed by laboratory tests), cherubism, and peripheral ossifying fibroma were excluded from the study.

Paraffin-embedded blocks were retrieved cut into 3µm sections and immunohistochemically stained using the streptavidin-biotin method. All sections were dewaxed, rehydrated, and subjected to endogenous peroxidase blocking. This was followed by immersion in a fresh solution of 10mM citrate buffer at pH 6.0 and placing in a microwave for 10 minutes. After cooling at room temperature, they were rinsed in phosphate buffer saline and incubated in monoclonal antibody against CD31 (Dako, ready-to-use monoclonal mouse anti-human, clone JC70A, Denmark) and CD34 (Novocastra™ ready-to-use mouse monoclonal antibody, product code: RTU-END, Germany) for 50 minutes. The sections were then rinsed in PBS and reacted with biotinylated secondary antibodies for 30 minutes followed by a second rinse in PBS and incubation with streptavidin-peroxidase (30 minutes) and a final rinse in PBS. Color was developed by exposure of the slides to 3-3′ diaminobenzidine after which counterstaining with Harris' hematoxylin was performed. Positive controls included pyogenic granuloma and solitary fibrous tumor for CD31 and CD34, respectively and endothelial cells in normal tissue vasculature were used as internal controls. Primary antibodies were omitted for both groups as negative controls. [14]

In order 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 selected for counting microvessels at 400×, which included all brown-stained cells situated individually or in small clusters and separate from other connective tissue elements in addition to identifiable microvessels 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 microvessels per high power field. Any disagreements

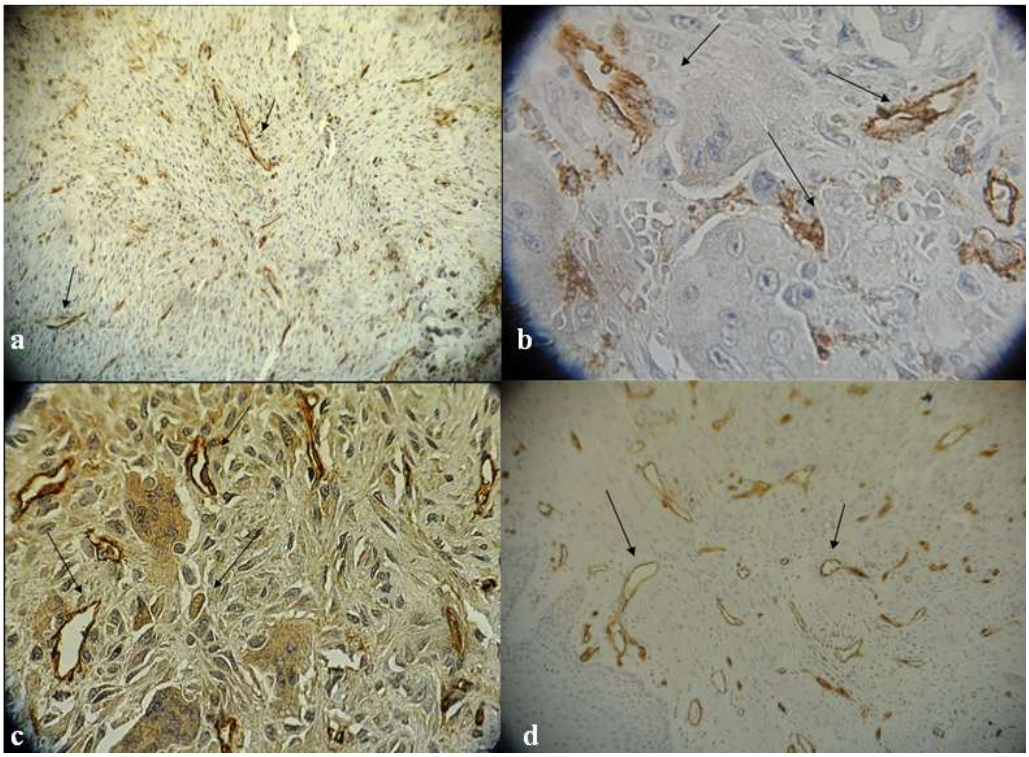

Figure 1a: Blood vessels immunostained by anti-CD34 antigen in CGCG (Nikon light microscope 400×), **b:** Blood vessels and stromal cells immunostained by anti-CD34 antigen in PGCG (Nikon light microscope 1000×), **c:** Blood vessels and stromal cells immunostained by anti-CD31 antigen in CGCG (Nikon light microscope 1000×), **d:** Blood vessels immunostained by anti CD31 antigen in PGCG (Nikon light microscope 400×).

between the observers were resolved by consensus.

Statistical analysis was performed using t-test and $p<0.05$ was considered significant.

Results

According to the inclusion and exclusion criteria, our study sample consisted of 18 PGCGs of which 6 and 12 occurred in males and females, respectively ($p= 0.23$). Of these, 10 were found in the mandible and 8 in the maxilla. The youngest patient was a 13-year-old boy with a lesion on the left posterior mandibular gingiva and the oldest subject was 71 with a maxillary PGCG on the gingiva of the canine region. The number of CGCGs was 19 which occurred in 3 men and 16 women ($p=0.004$). A total of 17 were found in the mandible and 2 in the maxilla. A 17-year-old girl with a right mandibular lesion and a 47-year-old woman with a lesion in the left canine-premolar area constituted the youngest and oldest patients with CGCG in the current investigation. The mean and median ages for individuals with PGCG were 43.2 years and 42.5 years and for CGCG were 33.5 and 34 years, respectively. Median ages were used as the cut-off point to divide patients into younger and older groups as proposed previously. [10]

The expression level of CD34 was 17.6±5.7 and 24.5±6.6 in PGCGs and CGCGs, respectively ($p< 0.002$). (Figure 1a), (Figure 1b) correspondingly, these values were 10.6±2.3 and 19.6±5.3 ($p< 0.001$) for CD31. (Figure 1c), (Figure 1d)

There was an increased intensity/staining of both markers in the peripheral areas of the PGCG samples (Figure 1d).

Table 1 shows immunostaining values of CD34 and CD31 according to the demographic features of patients with both lesions. A significant difference in CD34 ($p< 0.01$) and CD31 ($p< 0.05$) was found between men and women in PGCGs. Expression levels of CD34 were significantly different between the two age groups ($p< 0.05$) in CGCG, while CD31 showed statistically significant difference in lesion size ($p< 0.01$) in PGCGs.

Discussion

In the present study, we evaluated the angiogenesis via assessment of MVD using endothelial cell markers CD34 and CD31. Analysis of our demographic data showed both lesions to be more common in women as compared to men and more prevalent in the mandible compared to the maxilla, which was in agreement with

Table 1: Mean of CD31 and CD34 expression according to clinical and demographic factors

		No	CD34 Mean± SD	*p* Value	CD31 Mean± SD	*p* Value
CGCG						
Sex	Male	3	19±2.1	<0.2	16±3.7	<0.2
	Female	16	25.6±6.6		20.3±5.3	
Age	≤34 years	9	27.7±6.1	<0.05	21.2±5.7	<0.2
	> 34 years	10	21±5.4		17.8±4.4	
Location	Mandible	17	25.6±6	<0.01	20.3±5	<0.4
	Maxilla	2	15.2±2.5		14.2±5.3	
Size	≤2cm	3	19.4±2.5	<0.2	16.6±0.98	<0.4
	>2cm	16	16.2±4.8		20.2±5.6	
PGCG						
Sex	Male	6	12±4.4	<0.01	0.3±1.19	<0.05
	Female	12	20±4.6		12.6±4	
Age	≤34 years	9	19±6.3	<0.4	11.9±5	<0.7
	>34 years	9	16.2±4.8		10.4±2.3	
Location	Mandible	10	19±6.2	<0.4	12.1±4.6	<0.3
	Maxilla	8	15.8 ±4.6	<0.4	10±2.3	
Size	≤ 2 cm	13	18.2±6	<0.5	9.7±1.9	<0.01
	>2 cm	5	16.2±4.6		14.9±5.2	

PGCG: peripheral giant cell granuloma, CGCG: central giant cell granuloma

previous reports. [3, 11-12] The age range of the patients fell within those described formerly; however, the mean age was somewhat higher in the current investigation in comparison to some studies. [3, 13-16]

Histopathologically, similar to other studies, [4, 13] we observed well-formed large vessels in the periphery of the PGCGs as opposed to the microvessels found in the central parts of the lesions.

Based on our results, MVD assessed by both markers was significantly higher in CGCG compared to PGCG. Studies that classified CGCG as a proliferative vascular disease and those compared the angiogenesis between aggressive and non-aggressive forms of this lesion merely confirmed our findings. [4, 13-14] A significantly higher level of vascularity has been reported in aggressive versus non-aggressive forms of CGCG [17-18] that complies with the higher MVD and more aggressive behavior of CGCG reported in the current investigation. In addition, the larger CD68+ cell population has been reported in CGCGs compared to PGCGs cell population. [19]

A higher amount of antigenic cytokines like VEGF, TGFβ1, TGFα, TNFα, PDGF and thymidine phosphorylate in CGCGs, leads to increased endothelial cell proliferation and angiogenesis, [2] which supports our results. Hallikeri *et al.* [14] also observed a significantly higher MVD in CGCGs, similar to the findings of the current investigation. Likewise, Tobón-Arroyave *et al.* [15] reported microvessel counts to be larger in aggressive CGCGs compared to peripheral lesions. Interestingly, microvessel count was similar in PGCGs and non-aggressive CGCGs, but lower in PGCG compared to aggressive CGCG. On the other hand, the results obtained in the current investigation are in contrast to those who have found increased angiogenesis in PGCG compared to CGCG. [4, 11] This could be attributed to differences in the antibody used for assessment of MVD, its clone, or the methodology of measurement.

According to our results, both markers showed significantly higher vasculature in women with PGCG compared to men within the same lesion. By evaluating estrogen and progesterone receptor proteins, Whitaker *et al.* [16] suggested PGCGs to be under hormonal influence, which can help explain this finding.

CD34 and CD31 are panendothelial markers that are known to stain both old- and newly-formed vessels. In contrast, CD105 strongly reacts with newly formed vasculature in angiogenic tissues but weakly or not at all with endothelial cells of normal tissues. [17-18]

The difference in CD105 between CGCG and PGCG was reported to be non-significant, [17] minimizing the impact of neoangiogenesis as a distinguishing factor between these two lesions. Considering the significant difference in CD34/CD31 MVD between central and PGCG found in the present study, it could

be hypothesized that other functions of vascular structures and endothelial cells such as inflammation, vascular tone, permeability may be more pronounced in these lesions and might have a role in the differences found between them. [13-14] Furthermore, due to the fact that plasma cells, monocytes, fibroblasts, and some components of the extracellular matrix may also show reactivity for CD31 and CD34, it may be possible that some of them are counted as positive single endothelial cells during MVD assessment, while possessing an entirely different function. [8, 12, 18, 20]

It is noteworthy that drawing definitive conclusions about the pathogenesis of PGCG and CGCG based on the current investigation would not be possible; however, collecting information from various studies may be a basis for future research evaluating the biologic behavior of these lesions.

According to previous studies, it seems that CD105, p53, MDM2, PCNA, AgNOR, [17, 2-4]; MMP-9 [4] and Cathepsin D Expression [21] have little, if any, impact on the biologic behavior of PGCG and CGCG. On the other hand, VEGF expression in mononucleated and total cells, [4] morphometric parameters of multinucleated giant cells and CD68 immunoreactivity [2] have been shown to differ between these lesions and according to our findings, MVD assessed by panendothelial markers could be added to these factors.

Conclusion

Based on our findings, it seems that combination of old and newly formed vessels are different in PGCGs compared to CGCGs, which could be possibly responsible for the variation in their biologic behavior.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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