Original Article

Evaluation of microvessel density in central and peripheral giant cell granulomas

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Abstract

Background: Angiogenesis in the physiological status, balance is maintained between the pro- and anti-angiogenic factors and it is required for a tumor to grow beyond 1-2 mm in diameter. In central giant cell granuloma (CGCG) and peripheral giant cell granuloma (PGCG) the origin of giant cells, clinicopathological features overlap, but still there is a difference in the biological behavior. We aimed to assess the possible role of angiogenesis and presence of macrophages by the expression of CD34 and CD68 respectively.

Materials and Methods: Eighty cases each consisting of each forty CGCG and PGCG were evaluated clinically and immunohistochemically using CD34 and CD 68. Microvessel density (MVD) was expressed as the mean of blood vessels and macrophages in four high-power-fields. Statistical analysis was performed using Kruskal–Wallis and Mann–Whitney U-tests.

Results: The peak incidence of 21-30 years and 31-40 years was seen in CGCG’s and PGCG’s respectively with a statistical significant difference. Both groups showed female predominance with posterior mandible and maxilla being the common site. Number of mononuclear cells was more in the CGCG than PGCG with an ovoid morphology. CD34 positive microvessels and macrophages were more in CGCG compared to PGCG with statistically significant difference.

Conclusion: Clinicopathological features were similar to the previous studies. Statistically significant difference was evident in the MVD and macrophages in CGCG and PGCG. This new data about the microvessel count in the CGCG and PGCG adds to the literature. Importance of angiogenesis can add insight to the clinical behavior and also in understanding its histogenesis.

Introduction

Angiogenesis refers to the formation of new blood vessels, and for tumors to grow beyond 1-2 mm in diameter. The process of new vessel formation combines endothelial proliferation, proteolytic degradation of basement membrane and extracellular matrix and migration to form of endothelial cells that then combine to form a loop and to develop a lumen. Angiogenesis is influenced by the angiogenic growth and inhibitory factors. The significance of angiogenesis in the process of tumor growth and behavior is well studied in cancer such as breast, oral cavity, leukemia, multiple myeloma, lymphoma.[1-3]

Central giant cell granuloma (CGCG) and Peripheral giant cell granuloma (PGCG) of jaws are relatively uncommon benign reactive disorders. These are characterized by the presence of numerous multinucleated giant cell granuloma (MNGC) and mononuclear cells within the stroma. The biological behavior of this giant cell granuloma of the jaws vary, is known to range from quiescent and slow growing to aggressive and destructive.[4]

To understand the difference in behavior initial importance was given to understand the origin of giant cells. However, there was no difference in the origin of giant cells among the CGCG and PGCG; these may develop through a fusion of fibroblast, myofibroblast, pericytes, osteoprogenitor cells and macrophages.[5] Authors have confirmed that mononuclear cells are the proliferative components by the demonstration immunohistochemical (IHC) expression of PCNA, Ki-67
and histochemical expression of AgNOR count in CGCG and PGCG. Immunoreactivity to Ki-67, PCNA and AgNOR expression of was mainly restricted to ovoid to spindle-shaped mononuclear cells and not in the MNGC suggesting mononuclear cells are proliferating components in both lesions. Ki-67 immunoreactivity was greater in the mononuclear cells of PGCG compared to CGCG. PCNA and AgNOR staining were similar in PGCG and CGCG.[6,7]

Although there are similar features but these two lesions vary in the behavior, with this background we took up this study to analyze if there is any difference in the microvessel density (MVD). Adequate visualization of the microvessels for the purpose of counting is accomplished with endothelial immunostaining. Endothelial cells express a number of markers for which immunostaining techniques have been developed including Factor VIII related antigen, CD31, CD34 and CD36.[8]

To provide appropriate treatment, the nature of the lesions is very important. We aimed to assess the possible role of angiogenesis in CGCG and PGCG by the expression of CD34. To demonstrate the presence of macrophages using CD68 in CGCG and PGCG and also its possible role in angiogenesis is discussed briefly.

Materials and Methods

This laboratory-based study involved the use of buffered formalin fixed, paraaffin embedded tissues of previously histopathologically diagnosed cases of selected giant cell granuloma of jaws, retrieved from the Department of Oral and Maxillofacial Pathology, Sri Dharmasthala Manjunatheshwara College of Dental Sciences and Hospital, Dharwad. A total of 80 cases were evaluated clinically, histopathologically and immuno histochemically for CD34 and CD68 protein expression.

The patient’s details regarding age, gender and location of lesions were recorded. Each section was stained with hematoxylin and eosin stain and also for IHC staining. In each high power field (HPF) the slides were assessed for following parameters: Morphology and number of mononuclear stromal cells, CD34 positive endothelial cells and CD68 positive macrophages.

The IHC procedures were carried out using anti-CD34 monoclonal mouse anti-human antibody (AM236-5M) with super sensitive polymer – horseradish peroxidase IHC detection kit (Biogenex Life Sciences Limited, CA, and USA). Antihuman macrophage CD68 monoclonal antibody (Clone PG-M1, Dako) diluted 1:50 in TRIS-Hcl (pH 7.6) followed by 45 min in secondary antibody. It was then immersed in streptavidin-biotin peroxidase conjugate and incubated for 10 min with 3, 3-diaminobenzidine chromogen. Harris Hematoxylin was used as counter-stain. All the steps were carried out at room temperature and after each step the sections were washed with TRIS-Hcl buffer (pH 7.6). Omission of primary antibody was used as a negative control.

A successful staining procedure resulted in a brown colored end product at the site of target antigen and was considered as the immunoreactivity for CD34. Four areas with the highest amount of vascularization (hotspots) were selected under a magnification of ×100. Microvessels were counted in each of the four fields at ×400 magnification, and the mean density was noted.

The generally accepted criterion for a microvessel profile is an endothelial marker-stained cell or cluster that is separate from adjacent microvessel profiles, and present within the tumour but not in necrotic or sclerotic zones as given by Weidner et al., (1991).[1] MVD quantification was carried out using a light microscope and counting done using high power magnification in four HPFs, according to the method suggested by Weidner et al. (1991).[1] MVD was expressed as the mean number of microvessels per HPF. Representative fields were selected in each case to count CD68 positive macrophages under HPF (×40).

All slides were evaluated by two observers and both had to agree on each of the individual microvessel before being included in the count. The field size for ×400 magnification was approximately 0.1885 mm². Statistical analysis was performed using Kruskal–Wallis and Mann–Whitney U-tests.

Results

The peak incidence of CGCG’s were seen in patients with age range of 21-30 years (45%), followed by 11-20 years (30%), 31-40 years (20%) and least in the age group of 1-10 years (5%). The age group distribution in PGCG showed wide age distribution with the highest incidence at the age of 31-40 years (45%), and no case were recorded in the third decade. The mean age was 23 years and 34 years in CGCG and PGCG respectively with statistical significant difference between two groups [Table 1].

Graph 1 shows detailed sex distribution among both the groups; predominantly females were affected than the males in CGCG and PGCG. Site distribution of both lesions according to location is shown in Graph 2. The most common location of the CGCG and PGCG is posterior region of mandible i.e. 62.5% and maxilla, 55% respectively. Other sites involved in the CGCG were posterior region of the maxilla (25%) and anterior region of mandible (12.5%) in respective lesions. Similarly, PGCG had second common site of involvement was maxillary posterior (35%), anterior region of maxilla and mandible (5%).

<p>| Table 1: Comparison of age distribution among two groups |</p>
<table>
<thead>
<tr>
<th>Age</th>
<th>Group I (%)</th>
<th>Group II (%)</th>
<th>Total (%)</th>
<th>Chi-square</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4 (10)</td>
<td>6 (7.5)</td>
<td>351.000</td>
<td>5</td>
<td>0.000</td>
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<tr>
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<td>12 (30)</td>
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<td>16 (20)</td>
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</tr>
<tr>
<td>3</td>
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<td>18 (22.5)</td>
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<td>5</td>
<td>0</td>
<td>10 (25)</td>
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<tr>
<td>6</td>
<td>0</td>
<td>4 (10)</td>
<td>4 (5)</td>
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</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>40</td>
<td>80</td>
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</tbody>
</table>

Age range: 1-10 years=1, 11-20 years=2, 21-30 years=3, 31-40 years=4, 41-50 years=5, Above 51 years=6.
Both the groups of lesions had predominantly ovoid cells than spindle-shaped cells in the stroma. The number mononuclear cells in CGCG were more in comparison to PGCG [Graph 3]. The mean numbers of mononuclear cells in CGCG were 58.45 (standard deviation [SD] = 12.123) and in PGCG the mean numbers of mononuclear cells were 42.63 (SD = 7.482). The statistical significant difference was evident in the number of mononuclear cells in both the groups (P = 0.000).

CD 34 positive microvessels were more in CGCG [Figure 1] compared to PGCG [Figure 2]. Mean number of vessels in CGCG and PGCG were 34.70 (SD = 7.140) and 30.05 (SD = 8.006) respectively. Statistical significance was evident in the number of CD 34 immunoreactive vessels in CGCG and PGCG (P = 0.008).

Pairwise comparison number of macrophages immunostained by CD68 expression in between CGCG [Figure 3] and PGCG (P = 0.000), by two-tailed t-test showed statistical significance at 5% level [Table 2 and Graph 4], mean ± SD values for CD68 expression in CGCG and PGCG were 49.95 ± 6.891 and 34.15± 6.632 respectively.

Discussion

In the present study, CGCG showed a wide age range of distribution, with the second and third decade being the common age group affected. This finding is in close agreement with study Motamedi et al. An early age of presentation of CGCG (80% before the age of 30 years) and peak incidence (45%) in third decade of life are also in agreement with previous studies done by Farrier et al. and Whitaker et al. The occurrence of the lesion beyond age 50 years is unusual as reported by Choung et al., no cases were reported in the present study. Whereas PGCG can be found in all age groups as reported in previous literature by Katsikeris et al. In our study, age of patients ranged from 9 to 68 years that was in close harmony with Niloofar et al. and the highest prevalence (45%) in the fourth decade of life this in agreement with Whitaker and Bouquot. In their study, they mentioned that hormonal changes will be most pronounced during first four decades of life. An age of presentation before the age of 50 years (90%) is common and is in accord with the study by Motamedi et al. There was statistically significant difference in age distribution among CGCG and PGCG.

Our study showed significant female predominance in both CGCG (75%) and PGCG (80%). This finding is in agreement with the data presented in previous reported series of cases. There was no statistically significant difference in sex distribution among CGCG and PGCG. Predominant incidence in female suggests that both CGCG and PGCG could be under influence
of ovarian hormones as mentioned by Whitaker et al. (1994). It is well-known that estrogen and progesterone may decrease one’s immune response and may leave a person at increased risk for the development or progression of certain lesions.

Previous studies have shown that the CGCG occurs at least twice as often in the mandible as in maxilla. The present study confirms these findings since 74% of the CGCG were seen in the mandible. The distribution of the lesions within jaws in our study is not in agreement with previous report by Motamedi et al. (2007) and Whitaker et al. who suggested that CGCG’s are commonly seen in the anterior region of mandible and maxilla. In our study, 62.5% of cases were seen in posterior mandibular region, and 25% and 5% of cases were seen in maxillary posterior region and anterior region of mandible respectively. The reason for the occurrence of the lesion in the anterior and posterior region has not yet been elicited. PGCG’s in our study was equally distributed in both maxilla and mandible. Our finding is in contrast with the previous reported data by Shadman et al. (2009) who state that PGCG is more common in the lower jaw rather than the upper jaw (2.4:1). In mandible, posterior region (55%) is more commonly affected than the anterior region (5%). This finding is in agreement with previous report by Mighell et al., Cooke, and Katsikeris et al. According to Mighella et al. PGCG is equally distributed between anterior and posterior region of maxilla but in our study maxillary posterior region (35%) is the most commonly involved region than anterior region (5%). According to Cooke (1952), the area anterior to molars is mainly prone to be affected, since the transition of deciduous to permanent dentition takes place in this area, which results in high osteoclastic activity. However, the high incidence of lesions in the area of permanent molars makes this assumption questionable. Probably other factors such as trauma and hormonal changes can be considered as causative agents in permanent molar region.

Histologically both CGCG and PGCG showed fibrocellular stroma in the present study. This is consistent with the finding in a study done by Austin et al. and Chaparro et al. The shape of mononuclear cells in the stroma were evaluated and categorized as predominantly spindle, predominantly ovoid or mixed. In our study all both CGCG (85%) and PGCG (75%) were characterized by predominant ovoid cells than spindle-shaped cells. The ovoid shape of the mononuclear cells indicates that they are in active or proliferating state. This finding is confirmed in the lower jaw rather than the upper jaw (2.4:1). In mandible, posterior region (55%) is more commonly affected than the anterior region (5%). This finding is in agreement with previous report by Mighell et al., Cooke, and Katsikeris et al. According to Mighella et al. PGCG is equally distributed between anterior and posterior region of maxilla but in our study maxillary posterior region (35%) is the most commonly involved region than anterior region (5%). According to Cooke (1952), the area anterior to molars is mainly prone to be affected, since the transition of deciduous to permanent dentition takes place in this area, which results in high osteoclastic activity. However, the high incidence of lesions in the area of permanent molars makes this assumption questionable. Probably other factors such as trauma and hormonal changes can be considered as causative agents in permanent molar region.

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<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Standard error mean</th>
</tr>
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<tr>
<td>Number of mononuclear cell</td>
<td></td>
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<tr>
<td>CGCG</td>
<td>40</td>
<td>58.45</td>
<td>12.123</td>
<td>1.917</td>
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<td>PGGC</td>
<td>40</td>
<td>42.63</td>
<td>7.482</td>
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<td>CD34 BV</td>
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<td>CGCG</td>
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<td>PGGC</td>
<td>40</td>
<td>34.15</td>
<td>6.632</td>
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PGCG: Peripheral giant cell granuloma, CGCG: Central giant cell granuloma, SD: Standard deviation
by demonstration of proliferative markers like PCNA, Ki-67 and histochemical expression of AgNOR counts in the stromal cells of CGCG and PGCG.\textsuperscript{[6,7]} Immunoreactivity to Ki-67, PCNA and AgNOR expression of was mainly restricted to ovoid to spindle-shaped mononuclear cells and not in the MNGC suggesting mononuclear cells are proliferating components in both lesions. The mean number of these mononuclear cells was evaluated in the fibro cellular stroma of CGCG and PGCG. The mean value was slightly more in CGCG 58.45 (SD = 12.123) than PGCG 42.63 (SD = 7.482). When pair wise comparison among two groups was done there was statistically significant difference (P = 0.000). Based on this finding, it can be suggested that CGCGs is more cellular than PGCGs.

MVD is the most widely used method to estimate angiogenesis; MVD count help to assess the presence of blood vessels. The mean MVD was more in the CGCG (34.70) compared with PGCG (30.05), with a statistical significant difference (P = 0.0361) between both the groups. The variation of mean MVD among the groups also suggests that angiogenesis may be one of the mechanisms possibly contributing to the different biological behavior. The mean MVD of total blood vessels in CGCG and PGCG suggests that angiogenesis could affect the architecture or pattern of growth in. Furthermore, study revealed number of macrophages in the CGCG compared PGCG with the statistical significant difference. The macrophages are considered as the key angiogenic effector cells in the stroma. They produce a number of growth stimulators and inhibitors, proteolytic enzymes and cytokines that modulate the angiogenic process. The pair-wise comparison of the ratio of macrophage to blood vessels didn’t show statistically significant difference.

However in Ameloblastoma, odontogenic keratocyst or keratinizing cystic odontogenic tumor increased angiogenesis was seen compared to the other odontogenic cysts like dentigerous cyst, and follicular cyst. The demonstration of and demonstration of angiogenesis was done using the markers such as VEGF, CD31, and CD105 and it is well-correlated with the invasive growth pattern of the lesions.\textsuperscript{[21,22]} Torisu et al. demonstrated increase in the numbers of macrophages and microvessels with increasing depth of tumor with tumor angiogenesis. Thus, this can be used as useful diagnostic marker for the progression of cutaneous melanoma.\textsuperscript{[23]}

Angiogenesis is a key process in tumor growth and metastasis and is a major independent prognostic factor in breast cancer. A range of cytokines stimulate the tumor neovascularature, and tumor-associated macrophages (TAM) have been shown recently to produce several important angiogenic factors.\textsuperscript{[24]} In the malignant tumor-like breast cancer the TAM are regulated by number of cytokines and chemokines in particular macrophage chemotactic protein-1 (MCP-1). This MCP-1 was associated with macrophage accumulation and correlated with the concentration of various angiogenic factors. The increased macrophage counts reduced overall survival. Thus it can be used as an independent prognostic indicator in breast cancer. MCP-1 is known to regulate the balance between angiogenesis and immune system. Thus, the TAM/MCP-1 are a new target of treatment in breast cancer using antiangiogenic and immune inhibitory therapy.\textsuperscript{[24,25]}

Further sub-classification blood vessels into immature, intermediate and mature is required as this important by using the combination of IHC CD34 and SMA stain to demonstrate pericyte. This is required to differentiate the intermediate blood vessels from the mature blood vessels. Since immature and intermediate blood vessels are considered as an indicator of the degree of angiogenic activity. Since immature and intermediate vessels are the main target of antiangiogenic therapy and not the mature blood vessels, quantification of immature blood vessels may be helpful in estimation of prognosis especially for agents that do selectively target angiogenic endothelial cells, information may provide additional evidence of therapeutic anti-vascular effect for the control and prevention of the growth by inhibition of angiogenesis by anti-angiogenic therapy could be a potent therapeutic strategy.\textsuperscript{[26]}

**Conclusion**

In the present study, clinical data reported was similar to the previous studies and also histopathologically, there were no differences among the CGCG and PGCG. But statistically significant difference was evident in the MVD of CGCG and PGCG. This new data about the microvessel count in the CGCG and PGCG adds to the literature. Importance of angiogenesis can add insight to the clinical behavior and also in understanding its histogenesis.

**References**


