INTRODUCTION

The roaring history about cell and the nucleus has always been a matter of interest and curiosity for the pathologist. The known fact of cell having a single nucleus is universally accepted, but the subject becomes interesting when some tissues show several nuclei sharing the same cytoplasm. The occurrence of polykaryons or multinucleated cells is physiologic in skeletal muscle, placenta and bone; other than this it is considered pathological.

A number of lesions affecting maxilla and mandible contain multinucleated giant cells (MNGC). Such a list would vary from infectious granulomatous process to benign and malignant neoplasms.

Giant cell granulomas is a relatively common tumor-like lesion of the oral cavity and jaws.[1] The lesions can be further designated as either a central giant cell granuloma (CGCG) when it is found within the alveolar bone or a peripheral giant cell granuloma (PGCG) when it occur peripherally from periodontal ligament and periosteum.[2]

The CGCG of the jaws is usually a nonneoplastic bone lesion accounting for fewer than 7% of all benign tumors of the jaws [Figure 1].[3] It mainly occurs in children or in young adults, with a female predilection. It is more common in the mandible.[4] Most giant cell granulomas of the jaws are asymptomatic. A minority of cases, however, may be associated with pain, paresthesia, or perforation of the cortical bone plate, occasionally resulting in ulceration of the mucosal surface by the underlying lesion.[5]

The PGCG is an infrequent reactive, exophytic lesion of the oral cavity. It is the most frequent giant cell lesions
of the jaws [Figure 2].[6] Individual lesions are nodular and pedunculated, frequently with an ulcerated surface, frequently with a red, brown, or bluish hue.[7] Located in the region of the gums or edentulous alveolar margins, commonly in the lower jaw. It is more common in the 5th and 6th decades of life with a slight female predilection.[8] Both lesions share the same microscopic features, including the presence of numerous MNGC amidst a stroma of oval to spindle-shaped mononucleated cells.[9] Both are virtually identical histologically, being characterized by the presence of numerous MNGC and mononuclear cells within a prominent fibrous stroma. However, despite their similarity, CGCG and PGCG have distinct clinical behavior.[10]

The nature of giant cell lesions of oral cavity has been controversial for many years and many histological, immunohistological, ultrastructural and enzymatic studies have been carried out to determine the origin and role of MNGC in giant cell granulomas. However, their main nature has not been known yet.[11] Some investigators believe that MNGC have phagocyte, foreign body or osteoclast origin, but in some studies, an endothelial cell origin is suggested.[12-15] Moreover, some investigations show that the mononuclear cells of stroma have an important role in the development of the giant cells[16,17] and few others suggested that MNGC were formed by fusion and adhesion of stromal mononuclear cells, but the related mechanism remains unknown.[18] Therefore, recent studies of giant cell granulomas have been shifted to mononuclear cells and many investigators believe that mononuclear cells that are a proliferative compartment of these lesions are responsible for their biological activity.[9] The varying clinical behavior and histological parameter have attracted interest of many researchers to understand this diverse group of lesions.

To provide appropriate treatment, the knowledge of their possible origin and nature is very important. Hence, the aim of the study is to assess the expression of CD34 (cluster of differentiation of 34) and CD68 (cluster of differentiation 68) in PGCG and CGCG in order to gain better understanding of the origin and formation of MNGC.

MATERIALS AND METHODS

This laboratory-based study involved the use of buffered formalin-fixed, paraffin-embedded tissues of histopathologically diagnosed cases of selected giant cell granulomas of jaws, retrieved from archives of our Department.

A total of 40 cases were evaluated immunohistochemically for CD34 and CD68 protein expression. These included 20 cases of CGCG (Group I) and 20 cases of PGCG (Group II) and sections of pyogenic granuloma and sections of normal human lymph node were taken as positive controls for CD34 and CD68, respectively.

The patient’s details regarding age, gender and location of lesions were recorded [Table 1]. Each section was stained with hematoxylin and eosin and also for immunohistochemical stain to assess the nature and origin of mononuclear stromal cells and MNGC.

The immunohistochemical procedure followed here was based on instructions provided by the manufacturer (BioGenex, USA). The sections of 3μm thickness were cut and mounted on the 3-aminopropyltriethoxysilane coated glass slides. The slides were incubated at 55–60°C overnight before the day of staining. The sections were deparaffinized in two changes of xylene for 5 min each and then the slides were hydrated through different grades of isopropyl alcohol (100–50% for 3 min each) and brought to distilled water.

The tissues were then incubated with 3% hydrogen peroxide block for 5–10 min at room temperature to block the endogenous peroxidase activity. The tissues were then

Figure 1: Photomicrograph showing giant cells in the background of stromal cells with areas of hemorrhage in central giant cell granuloma (CGCG) (H&E stain, x100)

Figure 2: Photomicrograph showing surface stratified squamous epithelium. The underlying connective tissue shows numerous giant cells in the background of mononuclear stromal cells in peripheral giant cell granuloma (PGCG) (H&E stain, x40)
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Assessment of CD34 and CD68 expression

The immunoeexpression at the site of target antigen (cytoplasm) was considered as positive for CD34 and CD68, which was appreciated in our positive control.

Immunopositivity for CD34 was seen in cytoplasm of endothelial cells of blood vessels and was not seen in MNGCs nor in stromal mononuclear cells [Figure 3 and 4]. On the other hand, all 40 cases showed a consistent cytoplasmic immunopositivity for CD68 in the MNGCs and few stromal cells suggesting that these cells belong to monocyte/macrophage lineage [Figure 5 and 6]. These were quantitatively assessed by counting them using eyepiece graticule which hauls a grid with 100 blocks. Eight fields were selected randomly for each slide at a magnification of × 40.

In each high power field, the expression of CD68 were assessed under light microscope for following parameters:

- Number of CD68 positive giant cells, number of nuclei in each giant cell, CD68 expression (stained cells/total no of cells), proportion of stained cells, staining intensity, staining intensity distribution (SID), number of CD68 macrophages and ratio of macrophages to MNGC.

In each slide cells were counted in step ladder pattern and the count was divided by the total number of cells in each field. The mean of the eight fields was the marker expression estimation for each sample.

Furthermore, each field was evaluated for the proportion of stained cells and the intensity of overall staining. 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, >50% stained cells. Staining intensity was graded as: 0, negative staining; 1, light staining; 2, moderate staining; and 3, intense staining. A SID score was computed for each sample as follows. The mean of the eight fields was the SID score for the sample.

SID score = the score of the proportion of stained cells for each field × the score of the staining intensity in that field. The mean of the eight fields was the SID score for the sample.

A single trained research associate performed specimen assessment. The results were then tabulated and subjected to statistical analysis.

Statistical methods

Descriptive statistical analysis was performed using Chi-square and Mann–Whitney U test and significance determined at 5% level of significance.

Statistical software

The statistical software namely Statistical Package for Social Sciences (SPSS) 5.5 was used for analysis of the

**Figure 3:** Photomicrograph showing CD34 stained blood vessels in CGCG (IHC stain, x400) CGCG = Central giant cell granulomas

**Figure 4:** Photomicrograph showing CD34 stained blood vessels in PGCG (IHC stain, x100). PGCG = Peripheral giant cell granulomas
data and Microsoft Word and Excel were used to generate tables.

**RESULTS**

Demographic details of study samples are as mentioned in Table 1. Pairwise comparison of quantitative expression of CD68 positive MNGC among two groups by Mann Whitney U test is tabulated in table 2.

The numbers of CD68 positive MNGC in CGCG were less in comparison to PGCG. The values were statistically significant ($P=0.0193$). The number of nuclei in MNGC among two groups was compared using Chi-square test [Table 3].

The number of nuclei among both groups was compared. In Group I, 70% of cases showed more than 20 nuclei per MNGC and remaining 30% showed less than 20 nuclei per MNGC. In Group II, 100% showed less than 20 nuclei per MNGC. The values were statistically significant ($P=0.0000$).

Pairwise comparison among CD68 expression (stained/total no. of cells) of two groups by Mann–Whitney U test is listed in table 4. Comparison of CD68 expression between Groups I and II showed statistical significance ($P=0.0036$).

Comparison of proportion of stained cells among two groups by Chi-square test [Table 5]. Both group of lesions showed varying degrees of proportion of stained cells. In Group I, 80% of cases showed less than 25% of stained cells and 20% showed 25–50% of stained cells. In Group II, 95% of cases showed less than 25% of stained cells and only 5% showed 25–50% of stained cells. There was no statistical significant difference ($P=0.1515$).

Comparison of intensity of stained cells among two groups by Chi-square test [Table 6]. The staining intensity in both groups ranged from moderate to intense. In Group I, 70% of cases showed intense staining and 30% showed moderate staining. In Group II, 75% of cases showed intense staining and 25% showed moderate intensity staining. There was no statistical significant difference ($P=0.7233$).

**Table 1: Demographic details of two groups**

| Group I | Age | Gender | Site | Group II | Age | Gender | Site |
|---------|-----|--------|------|----------|-----|--------|------|
| CGCG    | 3   | 2      | 2    | PGCG     | 5   | 2      | 2    |
| CGCG    | 1   | 1      | 4    | PGCG     | 6   | 2      | 4    |
| CGCG    | 3   | 2      | 2    | PGCG     | 6   | 2      | 2    |
| CGCG    | 4   | 2      | 4    | PGCG     | 4   | 1      | 4    |
| CGCG    | 1   | 4      | 3    | PGCG     | 2   | 2      | 4    |
| CGCG    | 2   | 4      | 2    | PGCG     | 6   | 2      | 2    |
| CGCG    | 3   | 3      | 3    | PGCG     | 4   | 2      | 3    |
| CGCG    | 3   | 1      | 4    | PGCG     | 4   | 2      | 4    |
| CGCG    | 2   | 2      | 4    | PGCG     | 4   | 2      | 4    |
| CGCG    | 2   | 2      | 2    | PGCG     | 5   | 2      | 1    |
| CGCG    | 2   | 2      | 3    | PGCG     | 4   | 2      | 2    |
| CGCG    | 3   | 2      | 4    | PGCG     | 4   | 2      | 1    |
| CGCG    | 3   | 2      | 2    | PGCG     | 4   | 1      | 2    |
| CGCG    | 1   | 1      | 4    | PGCG     | 1   | 2      | 4    |
| CGCG    | 2   | 2      | 4    | PGCG     | 4   | 1      | 4    |
| CGCG    | 5   | 2      | 4    | PGCG     | 5   | 1      | 2    |
| CGCG    | 2   | 2      | 4    | PGCG     | 4   | 1      | 4    |
| CGCG    | 4   | 1      | 2    | PGCG     | 5   | 1      | 2    |
| CGCG    | 1   | 1      | 2    | PGCG     | 5   | 2      | 2    |

**Figure 5:** Photomicrograph Showing CD68 expression in giant cells and macrophages of CGCG, with more than 20 nuclei evident in one of the giant cell (IHC stain, x400)

**Figure 6:** Showing intense cytoplasmic staining of CD 68 in macrophages and MNGC in PGCG (IHC stain, x40). MNGC = Multinucleated giant cells

CGCG=Central giant cell granulomas, PGCG=peripheral giant cell granulomas
Comparison of staining intensity distribution of CD68 positive cells among two groups by Mann–Whitney U test [Table 7]. Comparison of staining intensity distribution score between Groups I and II showed no statistical significance (P = 0.7251).

Pairwise comparison of ratio of macrophages to MNGC among two groups by Mann–Whitney U test [Table 8].

Comparison of ratio of macrophages to MNGC between Groups I and II showed statistical significance (P = 0.0000).

DISCUSSION

The giant cell granuloma of oral cavity and jaws are lesions that arise either centrally in the bone or peripherally in periodontal ligament and mucoperiosteum of the alveolar ridge.[5] Although histologically similar features are seen in both CGCG and PGCG, they differ clinically in terms of their behavior. Particularly in the case of CGCG and to a lesser intensity and frequency in PGCG, there may be bone resorption.[19]

To provide appropriate treatment, the knowledge of their possible origin and nature is very important. Therefore, in this study CD68 (PG-M1) which is a specific macrophagic/histiocytes marker and CD34, endothelial marker were performed in CGCG and PGCG in order to gain better understanding of the origin and formation of MNGC.

Our study result supports the hypothesis that MNGC in giant cell granulomas arise from the fusion of the stromal macrophages in both CGCG and PGCG. Several studies also support these finding suggesting MNGC arise from monocyte/macrophage lineage.[20,21] In a study; acid phosphatase, nonspecific esterase, lysozyme and alpha-1-antitrypsin were employed as markers for cells of histiocytic origin. The giant cells and the macrophage-like cells were identical in their

### Table 2: Pairwise comparison of quantitative expression of CD68 positive giant cells among two groups by Mann-Whitney U test

| Variable           | Group | Mean  | SD    | Sum of ranks | U value | Z value | P value | Significance |
|--------------------|-------|-------|-------|--------------|---------|---------|---------|-------------|
| No. of giant cells | I     | 16.6995 | 4.4959 | 323.50       | 113.50  | -2.3398 | 0.0193  | S           |
|                    | II    | 19.8250 | 4.6031 | 496.50       |          |         |         |             |

SD=Standard deviation, S=Significant

### Table 3: Comparison of number of nuclei in giant cell among two groups by Chi-square test

| No. of nuclei in giant cells | Group I | %   | Group II | %   | Total | %   | Chi-square | df  | P value | Significance |
|-----------------------------|---------|-----|----------|-----|-------|-----|------------|-----|---------|-------------|
| 1                           | 6       | 30  | 20       | 100 | 26    | 65  | 21.5385    | 1   | 0.0000  | S           |
| 2                           | 14      | 70.00 | 0       | 0.00 | 14    | 35.00|           |     |         |             |
| Total                       | 20      | 100.00 | 20     | 100.00 | 40    | 100.00|           |     |         |             |

Number of giant cell nuclei: Less than 20=1, more than 20=2. df=Degrees of freedom, S=Significant

### Table 4: Pairwise comparison of among CD68 expression (stained/total no. of cells) two groups by Mann–Whitney U test

| Variable                   | Group | Mean    | SD    | Sum of ranks | U value | Z value | P value | Significance |
|----------------------------|-------|---------|-------|--------------|---------|---------|---------|-------------|
| CD68 expression (stained/total no cells) | I     | 0.1730  | 0.0515 | 517.50       | 92.50   | -2.9079 | 0.0036  | S           |
|                            | II    | 0.1328  | 0.0287 | 302.50       |         |         |         |             |

SD=Standard deviation, S=Significant

### Table 5: Comparison of proportion of stained cells among two groups by Chi-square test

| Proportion of stained cells | Group I | %   | Group II | %   | Total | %   | Chi-square | df  | P value | Significance |
|-----------------------------|---------|-----|----------|-----|-------|-----|------------|-----|---------|-------------|
| 1                           | 16      | 80.00 | 19      | 95.00 | 35    | 87.50 | 2.0571     | 1   | 0.1515  | NS          |
| 2                           | 4       | 20.00 | 1       | 5.00  | 5     | 12.50 |           |     |         |             |
| Total                       | 20      | 100.00 | 20     | 100.00 | 40    | 100.00|           |     |         |             |

Proportion of stained cells: No stained cells=1, <25% stained cells=2, 25%-50% stained cells=3, >50% stained cells=4. df=Degrees of freedom, NS=Not significant

### Table 6: Comparison of intensity of stained cells among two groups by Chi-square test

| Intensity of stained cells | Group I | %   | Group II | %   | Total | %   | Chi-square | df  | P value | Significance |
|----------------------------|---------|-----|----------|-----|-------|-----|------------|-----|---------|-------------|
| 2                           | 6       | 30.00 | 5       | 25.00 | 11    | 27.50 | 0.1254     | 1   | 0.7233  | NS          |
| 3                           | 14      | 70.00 | 15      | 75.00 | 29    | 72.50 |           |     |         |             |
| Total                       | 20      | 100.00 | 20     | 100.00 | 40    | 100.00|           |     |         |             |

Staining intensity: Negative=0, light staining=1, moderate staining=2, intense staining=3. df=Degrees of freedom, NS=Not significant
staining characteristics and showed positive staining for all histiocytic markers tested.\[21\]

Because of the presumed participation of macrophages in the development of MNGC in the giant cell lesions, antigenic markers such as muramidase, alpha-1 antitrypsin and alpha-1-antichymotrypsin associated with cells of the mononuclear–phagocyte system were evaluated. Both CGCG and PGCG showed a consistent staining pattern in round macrophage-like mononuclear tumor cells and giant cells. Based on above findings, he suggested that MNGC are derived from macrophages.\[21\]

In our study, the greater ratio of macrophages to MNGC was observed in CGCG than PGCG. Mean values for ratio of macrophages to MNGC in CGCG and PGCG were 2.7600 and 1.521, respectively. Pairwise comparison of ratio of macrophages to MNGC in CGCG and PGCG by Mann–Whitney U test showed statistical significance. Stained cells include total number of macrophages and MNGC, thus increased CD68 expression suggested that higher frequency of cells of the macrophage lineage in CGCG when compared to PGCG.

In the light microscope and with conventional stains, macrophages are difficult to identify unless they display obvious evidence of phagocytic activity.\[23\] Thus by using an immunohistochemical stain it was easy to identify the macrophage amidst fibrocellular stroma.

It is well known that macrophages are ubiquitous in all tissues. Besides other functions, such as endocytosis and cytotoxicity, there is increasing evidence suggesting that macrophages are involved in autocrine mechanisms amplifying inflammation, angiogenesis and invasive depth by secretion of vascular endothelial growth factor (VEGF), basic fibroblast growth factor, activation of humoral and cellular immune responses in diverse diseases by secreting cytokine like transforming growth factor (TGF)-β and tumor necrosis factor.\[24\] The expression of VEGF and matrix metalloproteinase (MMP)-9 may be related to the process of osteoclastogenesis in which the formation of incomplete vascular barriers during angiogenesis provides a source of monocytes from circulation. The latter are then recruited into the lesion by local production of monocyte chemoattractant protein-1 and TGF-β from the stromal cells. The stromal cells further stimulate monocyte/macrophage towards the formation of MNGC. It is fundamental for the growth of these lesions.\[24\]

Cells of monocyte–macrophage lineage have been shown to be an important source of MMP-9. It exerts important

| Variable | Group | Mean | SD  | Sum of ranks | U value | Z value | P value | Significance |
|----------|-------|------|-----|--------------|---------|---------|---------|-------------|
| Staining intensity distribution of CD68 positive cells | I     | 3.3000 | 1.4546 | 423.00 | 423.00 | 187.00 | -0.3517 | 0.7251 |
|          | II    | 2.9000 | 0.8522 | 397.00 | 397.00 | 187.00 | -0.3517 | 0.7251 |

SD=Standard deviation, NS=not significant

| Variable | Group | Mean | SD  | Sum of ranks | U value | Z value | P value | Significance |
|----------|-------|------|-----|--------------|---------|---------|---------|-------------|
| Ratio of macrophages to giant cells | I     | 2.7600 | 1.0107 | 571.50 | 571.50 | 187.00 | -4.3686 | 0.0000 | S |
|          | II    | 1.5210 | 0.5012 | 248.50 | 248.50 | 187.00 | -4.3686 | 0.0000 |

SD=Standard deviation, S=Significant

**Table 7: Comparison of staining intensity distribution of CD68 positive cells among two groups by Mann-Whitney U test**

**Table 8: Pairwise comparison of ratio of macrophages to giant cells among two groups by Mann-Whitney U test**
functions in the processes of angiogenesis and bone remodeling. In bone remodeling, MMP-9 is implicated in the degradation of bone tissue because of its effective proteolytic action against organic components of the nonmineralized bone matrix, such as collagen types I and II. Therefore, the overexpression of CD68 and the greater ratio of macrophages to MNGC observed in CGCG compared with PGCG might be related to their clinical behavior, such as increased growth rate, associated symptoms and bone/tooth resorption.

Quantitative analysis of CD68 positive MNGC and mononuclear cells in the present study showed predominance of score 1 (<25% stained cells) in both CGCG (80%) and PGCG (95%). Only few number of cases, four cases (20%) of CGCGs and one case (5%) of PGCGs showed score 2 (25–50% stained cells). There was no statistically significant difference in proportion of stained among CGCG and PGCG.

In the present study, immunoreactivity for CD68 in MNGC and few of mononuclear cells varied from moderate to intense staining in both CGCG and PGCG and was found in cytoplasm of MNGC and macrophages. Maximum number of cases showed intense staining, 14 cases (70%) of CGCGs and 15 cases (75%) of PGCG. Only few cases showed moderate staining intensity, six (30%) of CGCG and five (25%) of PGCG. This is in accordance with few studies. Thus, it can be said that giant cells and a group of mononuclear cells have macrophagic characteristics. There was no statistical significance seen in the intensity of staining among CGCG and PGCG.

In present study, the low proportion of stained cells (average <25%) together with mild variation of staining intensity (moderate intense) detected within CGCG and PGCG, led to statistically similar SID score between them when subjected to Mann–Whitney U test. Mean values of SID score in CGCG and PGCG were 2.9(SD-0.8522), respectively. This finding is consistent with a study by Flórez-Moreno et al. These finding could once again indicate that MNGC in CGCG and PGCG are formed by fusion of macrophages.

In the present study, a consistent cytoplasmic immunopositivity for CD68 in the MNGC and few stromal cells suggested that these CD68 positive mononuclear cells are macrophages and the MNGC are probably derived from monocyte/macrophage lineage.

Despite the fact that CGCG share some clinical and histopathological similarities with PGCGs, differences in number of nuclei in MNGC and CD68 immunoreactivity may underlie the distinct clinical behavior. Thus, concluding that macrophages play important role in the pathogenesis of giant cell lesions and more studies should be conducted to determine the role of mononuclear cells and their products that can affect the growth of giant cell granulomas.

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