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Mononuclear cell phagocytosis in giant cell lesions of the jaws

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SUMMARY

Background and objective. The central giant cell lesion (CGCL) is a benign intraosseous lesion that may resemble other giant cell-rich lesions, such as the peripheral giant cell lesion (PGCL). This lesion may be classified as aggressive or non-aggressive according to clinical and radiographic criteria, however, there is no biological marker that may help to define the CGCL based on the biological behavior. In this context, stromal cell phagocytosis has been described as an event related to aggressive lesions, however, only a few studies have investigated this phenomenon in CGCL. The aim of this study was to compare mononuclear cell phagocytosis by the multinucleated giant cells between aggressive, non-aggressive CGCL, and PGCL.

Material and methods. Thirty-three cases of CGCL, 10 aggressive and 23 non-aggressive, and 20 cases of PGCL were included in this study. Phagocytosis events were evaluated in five consecutive microscopic fields from histological sections, stained with hematoxylin and eosin. The ratio between the numbers of phagocytosis/mm^2 and the total number of multinucleated giant cells/mm^2 was calculated.

Results. Phagocytosis was observed in the multinucleated giant cells of all cases. The density of phagocytosis/mm^2 in relation to giant cells/mm^2 was higher in the CGCL than in the PGCL. In addition, aggressive CGCL showed higher phagocytosis events when compared to the non-aggressive variant.

Conclusion. Our results suggest that the aggressiveness of multinucleated giant cell lesions of the jaws may be related to the number of phagocytosis events in the lesion.

Keywords: fibro-osseous lesions, giant cell granuloma, peripheral giant cell lesion, central giant cell granuloma, phagocytosis.

INTRODUCTION

The central giant cell granuloma (CGCL) is a rare benign lesion of unknown origin and usually occurs in the mandible, of female patients, in 25-30 years old (1, 2). Microscopically, it consists of vascularized fibrous connective tissue with multiple hemorrhagic and hemosiderin pigment foci, multinucleated giant cells, and, occasionally, bone trabeculae and fibrous septa (3). These histological aspects of CGCL are similar to other giant cell lesions that affect the maxillofacial region, for example, hyperparathyroidism brown tumor, aneurysmatic cyst, and peripheral giant cell lesion (PGCL) (4). The PGCL shows morphologic and microscopic aspects similar to CGCL (5). Despite these histological similarities, PGCL is considered a reactive lesion and may have an indolent biological behavior when compared to CGCL, PGCL is easily treated by surgery and presents low recurrence rates (6, 7).

Chuong *et al.* (8), based on clinical and radiographic criteria, were the first authors to classify CGCL into two variants, the aggressive and non-aggressive, and this classification has been widely used (1, 2, 4, 8, 9). Aggressive lesions usually affect younger patients than non-aggressive lesions, show symptoms, rapid growth, expansion and resorption of the cortical bone, displacement and dental resorption, and a high rate of recurrence (8).

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The biological behavior regarding the aggressiveness of giant cell cells of the maxillofacial complex, especially of the CGCL, has been studied through histological and immunohistochemical investigations; however, the results of the studies are controversial and a real biomarker for the differentiation between the variants, as well as the differences between CGCL and PGCL, is not well defined (1, 2, 9-14).

The phagocytic activity of multinucleated giant cells, present in both CGCL and PGCL, has been raised as an important histological finding that may be related to the aggressiveness of these lesions (5, 15, 16). Phagocytosis of mononuclear cells by multinucleated giant cells has been shown to be more frequent in aggressive CGCL than in the non-aggressive variant or in PGCL (5, 15). However, the number of cases that were investigated is limited, and studies on this phenomenon should be continued, considering the promising results that indicate that phagocytic activity may help in determining the biological behavior of CGCL (5, 15).

The diagnosis of the aggressive CGCL is important for predicting the prognosis; however, considering only the routine histopathological examination, this prediction is still imprecise. Investigations that aim to identify a biomarker that indicates the aggressiveness of CGCL may contribute to the choice of treatment based on histological findings. Therefore, the aim of this study was to compare the phagocytic activity of the multinucleated giant cells of the aggressive and non-aggressive CGCL compared to PGCL.

MATERIAL AND METHODS

Sample

For this study, formalin-fixed paraffin-embedded samples of 33 CGCL and 20 PGCL were selected from the archives of the Laboratory of Oral Pathology of the School of Dentistry of the Universidade Federal de Goiás. Inclusion criteria were: well-preserved paraffin blocks of patients who had primary (patients who did not receive the prior treatment) intraoral lesions of CGCL or PCGL, confirmed by microscopic diagnosis and through the investigation of clinical data; patients who have not undergone drug treatment previously the surgical treatment; properly filled out medical records; blocks with enough tissue for analysis. Samples obtained from excisional biopsy were used in cases of PGCL. In cases of CGCL, the samples were obtained from the curettage of the lesion. For CGCL treated with drugs, the sample obtained from the incisional biopsy was evaluated.

Clinical-demographic and radiologic data were collected from patient records. Data on age, sex, location, time of evolution, symptoms, presence of tooth displacements, or tooth resorption were collected. This research was approved by the Research Ethics Committee of the Universidade Federal de Goiás (number 32992320.3.3001.5083).

CGCL Classification

Clinical and radiographic data were assessed to confirm the diagnosis of CGCL or PGCL. These data were also useful for classifying the lesions, based on the criteria established by Chuong *et al.* (8) and Martins *et al.* (9). Lesions that had little or no symptoms, no evidence of bone or root resorption or tooth displacement, with slow growth and low recurrence rates were considered non-aggressive CGCL. The lesions considered aggressive presented pain, root resorption, tooth displacement, cortical bone perforation, rapid growth, and high recurrence rates.

A rapid growth rate was considered when patient data indicated that the lesion evolved over a six-month period (9). A high recurrence rate was defined as at least one recurrence within 24 months of the previous treatment (this period was established based on reports by Chuong *et al.* (8). In this specific study, patients were followed for 24 up to 120 months.

Definition and quantification of phagocytosis

The phagocytosis event was evaluated in the multinucleated giant cells of the CGCL and PGCL. Histological sections stained with hematoxylin and eosin (HE) were used, according to the methodology adapted from the study by Sarode *et al.* (16). The samples available in paraffin blocks were sectioned in a microtome (Leica RM2165, Leica Microsystem, Wetzlar, Germany), obtaining 5μm slides from each block, which were placed on histological slides and stained with HE.

A phagocytosis event was considered when the multinucleated giant cell demonstrated pseudopod formation delimiting a stromal mononuclear cell. In this type of classification of cannibalism, a small concavity is observed at the interface of the mononuclear cell and the membrane of the multinucleated giant cell. The other form of phagocytosis is observed when the mononuclear cell is completely within the cytoplasm of the multinucleated giant cell (16).

Five consecutive microscopic fields were evaluated at 400x magnification. Photographs of the five microscopic fields were obtained using a digital camera coupled to an optical microscope (Carl Zeiss, Oberkochen, Germany). The total area of each photographed field was 0.059 mm^2. The ImageJ software (17) was used for the quantification of multinucleated giant cells and phagocytosis events. The multinucle-

ated giant cells that showed and did not show phagocytic activity were quantified. The average of the five fields was used to calculate the phagocytosis density/mm^2. In cases of CGCL, multinucleated giant cells close to bone trabeculae were not counted, in order not to include osteoclasts in this assessment. Red blood cell phagocytosis was not quantified.

The phagocytosis event of each case was obtained by the ratio between the number of phagocytosis/mm^2 divided by the total number of multinucleated giant cells/mm^2 was calculated according to the formula below:

$$\text{Phagocytosis} = \frac{\text{Phagocytosis/mm}^2}{\textit{Multinucleated giant cells/mm}^2}$$

Statistical data analysis

For the hypothesis tests, a significance level of 5% was adopted for the rejection of the null hypothesis. The Mann-Whitney test was used to compare phagocytosis density and age between groups. The Chi-square test was used to compare nominal variables. The correlation between multinucleated giant cell numbers and phagocytosis was calculated by Spearman's Correlation Test. The tests were performed using the statistical software IBM SPSS 20.0 (IBM, New York, USA). The results are expressed as median (Q25-Q75).

RESULTS

Based on our sample, the female sex was more affected in both CGCL (63.6%) and PGCL (55%). The alveolar ridge of the mandible was affected in 63.2% of the PGCL cases. Likewise, the mandible was also the most affected anatomical site by CGCL (60.6%).

Regarding the variants of CGCL, female patients represent 65.2% of the non-aggressive cases and 60% of the aggressive lesions. In non-aggressive lesions, the mandible was affected in 52.2%, while this location was affected in 80% of the aggressive CGCLs. Meanwhile, for PGCL, Table 1 summarizes the clinical and pathological data of the cases.

Multinucleated Giant Cells

The total number of multinucleated giant cells was higher in PGCL (81.35 cells per mm^2) than in aggressive CGCL (57.62 per mm^2, p=0.307, Mann-Whitney test) or than in the non-aggressive CGCL (74.57 per mm^2, p=0.188, Mann-Whitney test), however, there was no statistically significant difference between the groups (Table). No statistically significant differences were also found for the comparison between aggressive and non-aggressive variants (p=0.98, Mann-Whitney test).

Phagocytosis

The phagocytosis of mononuclear cells by the multinucleated giant cells was observed in all cases (Figure 1). Considering all cases of CGCL it was observed a higher number of phagocytic events (0.48; 0.33–0.56) than the PGCL (0.32; 0.21–0.43; p=0.003, Mann-Whitney test).

Considering the variants of the CGCL, the density of phagocytosis in relation to the density of giant cells

Table. Clinical demographic and pathological data

		PGCL (n = 20)	CGCL (n = 33)	Non-aggressive CGCL (n = 23)	Aggressive CGCL (n = 10)
Age in years (mean ± SD)		41.90±22.15*	28.36±21.03	33.17±23.32	17.30±9.99
Evolution in months (mean ± SD)		5.51±3.22*	6.00±8.58	7.26±9.39	1.85±2.48
Sex	Male	9 (45%)	12 (36.3%)	8 (34.8%)	4 (40.0%)
	Female	11 (55%)	21 (63.6%)	15 (65.2%)	6 (60.0%)
Site	Maxilla	5 (25%)	13 (39.3%)	11 (47.8%)	2 (20.0%)
	Mandible	15 (75%)	20 (60.6%)	12 (52.2%)	8 (80.0%)
Recurrence		0	2 (6.06%)	0	2 (20%)
Treatment	Surgical curettage	0	33 (100%)	23 (100%)	10 (100%)
	Additional treatmenta	0	2 (6.06%)	0	2 (20.0%)
	Surgical excision	20 (100%)	0	0	0
Phagocytosis/mm² (median Q25-Q75)		27.11#† (16.95–36.44)	29.66† (16.95–45.76)	29.66# (16.95–44.07)	32.76# (23.72–55.08)
MGC/mm² (median Q25-Q75)		81.35 (61.86–97.45)	61.01 (47.45–91.97)	74.57 (50.84–91.52)	57.62 (44.96–104.23)

* – statistically significant different when compared PGCL vs. Aggressive CGCL (p = 0.005 and p = 0.014 for age and evolution respectively, Mann-Whitney Test). # and † Indicates statistically significant difference between groups with same symbol. [a] – the additional treatment was performed with intranasal spray of calcitonin and intralesional corticosteroids. Abbreviations: CGCL – central giant cell lesion; MGC - multinucleated giant cell; PGCL – peripheral giant cell lesion.

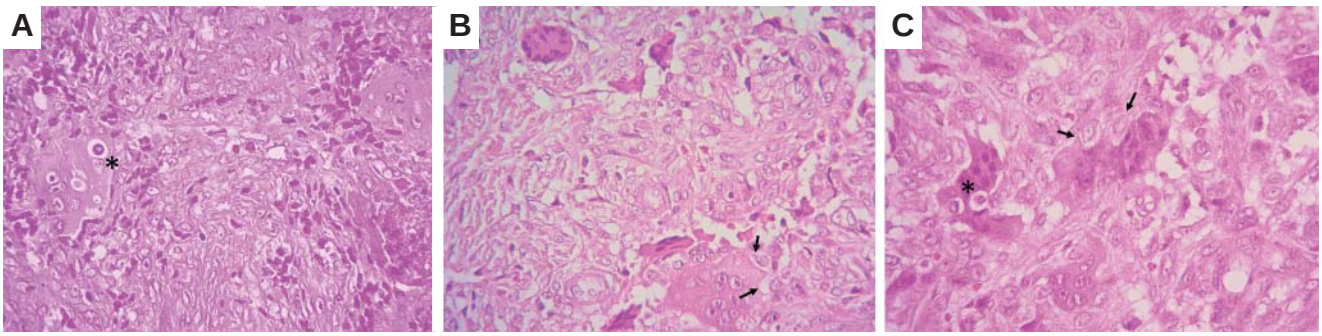

Fig. 1. Phagocytosis in multinucleated giant cells. A – Peripheral Giant Cell Lesion; B – aggressive Central Giant Cell Lesion; C – non-aggressive Central Giant Cell Lesion. Asterisk shows a stromal mononuclear cell internalized by the multinucleated giant cell. The arrows indicate a pseudopod formation by the multinucleated giant cell. Hematoxylin and eosin, 400×.

was higher in the aggressive lesions, 0.56 (0.45–0.63) when compared to non-aggressive lesions, 0.43 (0.31–0.54; p=0.025; Mann-Whitney test). The aggressive and non-aggressive variants also had a higher number of phagocytosis than the PGCL (p <0.0001 and p=0.038 respectively; Mann-Whitney test) (Figure 2).

Correlation between multinucleated giant cells and phagocytosis

Positive moderate to strong correlations were observed in all groups. The correlation coefficient (r) between the number of multinucleated giant cells per mm^2 and the number of phagocytosis per mm^2 was 0.66 (p=0.002; Spearman's correlation test) for the PCGL. For the aggressive CGCL the correlation was 0.83 (p=0.003) and for the non-aggressive lesions the correlation coefficient (r) was 0.66 (p=0.001). Figure 3 shows the dispersion of the number of multinucleated giant cells versus the number of phagocytosis per mm^2.

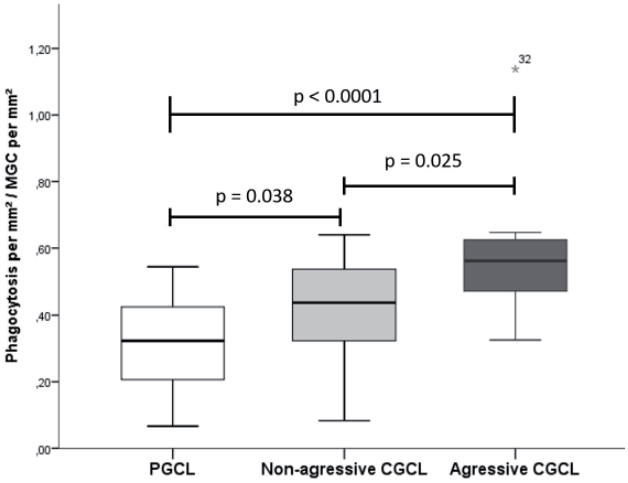

Fig. 2. Phagocytosis in multinucleated giant cell-rich lesions. Box-plot of the phagocytosis ratio in Peripheral Giant Cell Lesion, aggressive Central Giant Cell Lesion, and Non-aggressive Central Giant Cell Lesion. Abbreviations: PGCL – Peripheral Giant Cell Lesion; CGCL – Central Giant Cell Lesion.

DISCUSSION

This study shows that the phagocytic activity of multinucleated giant cells is higher in aggressive CGCL than in non-aggressive CGCL, and then in PCGL, suggesting that the phagocytosis observed in the multinucleated giant cells may indicate higher aggressiveness of a giant cell lesions, mainly in cases of CGCL.

Phagocytic activity in CGCL was first described as a process of cellular cannibalism (5, 16). Cannibalism is commonly observed in mononuclear cells of malignant lesions and is related to the greater aggressiveness of these neoplasms (18-21). However, this biological event may also be present in benign lesions of the maxillofacial complex (22).

Sarode and Sarode (5) observed phagocytosis in about 20% to 56% of the giant cells of the CGCL, and aggressive lesions present a higher frequency of giant phagocytic cells. Likewise, Sarode *et al.* (16) also observed that the CGCL of the aggressive variant has more phagocytosis figures. Finally, Urs *et al.* (15), evaluating 25 cases of PGCL, 18 of aggressive CGCL, and 22 of non-aggressive CGCL, showed that the number of phagocytosis is higher in CGCL than in PGCL and that the aggressive CGCL has a higher number of phagocytic cells than the non-aggressive ones. Our findings corroborate these previous studies and suggest that the number of multinucleated giant cells with phagocytic activity may present a reliable biomarker for predicting the aggressiveness of CGCL. However, it is important to emphasize that in this study the methodology considered not only the number of giant cells but also the surface that these cells occupy. We believe that this reflects the real cannibalism in the case evaluated since the number of phagocytoses takes into consideration the number of multinucleated giant cells per mm^2.

Sarode *et al.* (22) suggest that the term cell cannibalism should be used to refer to the phagocytosis

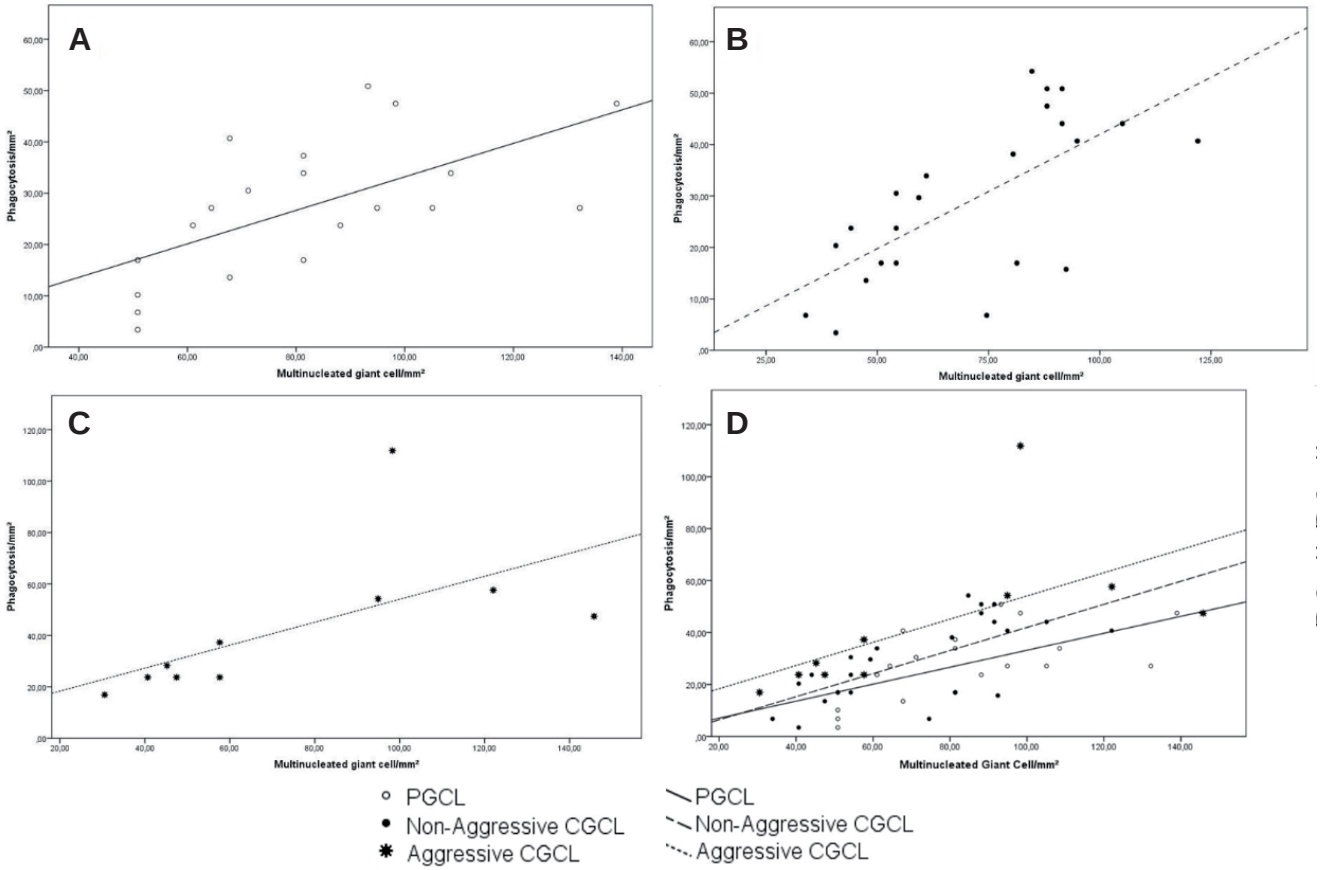

Fig. 3. Dispersion of the number of multinucleated giant cells versus the number of phagocytosis per mm^2.
A – the number of multinucleated giant cells and phagocytosis per mm² of Peripheral Giant Cell Lesion is illustrated; B and C refers to non-aggressive and aggressive Central Giant Cell Lesion, respectively. D – the dispersion of the number of multinucleated giant cells versus the number of phagocytosis per mm^2 in all three groups. Abbreviations: PGCL – Peripheral Giant Cell Lesion; CGCL – Central Giant Cell Lesion.

of a mononuclear cell by another mononucleated one. The event that occurs in multinucleated giant cells is, in fact, phagocytosis (22). Studies have shown that multinucleated giant cells of CGCL have phagocytic cell lineage, due to the CD68 expression (5, 23, 24). Phagocytosis, under physiological conditions, is a common phenomenon among cells and is important in the resolution of inflammation, antigen presentation, and elimination of apoptotic cells (21). However, we believe that phagocytosis in CGCL and PGCL is not related to the resolution of the lesion, but rather to aggressiveness and lesion progression.

The distinction of the CGCL variants using immunohistochemistry staining has been thoroughly studied (9, 10, 13, 14, 25-27). However, the results are controversial and a biological marker of CGCL aggressiveness has not yet been demonstrated (9, 10, 13, 14, 25-27). However, despite being a simple technique, it presents higher costs for pathology services. Thus, studies using routine staining, HE, which differentiates aggressive from non-aggressive CGCLs are important.

Surgery is still the most widely adopted treatment of CGCL and considering the high relative rate of recurrence, mainly in the aggressive variant, this surgical treatment may reflect on several surgical approaches that can cause important functional and aesthetic defects (28). The predictability of the aggressiveness of the lesion is important to prevent multiple surgeries, favoring the patient's prognosis and quality of life.

The small number of cases evaluated can be considered a limitation of the study, however, our findings corroborate the results of the literature data, and the sample included in this specific study is similar to previously published studies (1, 4, 15, 16). Further studies with a higher number of cases should be carried out, in order to assist in predicting the aggressiveness of CGCL by phagocytosis evaluation.

CONCLUSIONS

The phagocytosis of mononuclear cells by multinucleated giant cells is higher in the aggressive CGCL when compared to the non-aggressive CGCL and PGCL. This finding suggests that mononuclear cell phagocytosis by the multinucleated giant cells can aid in determining the aggressiveness of CGCL.

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