Tumor Associated Macrophages in Relation to Collagen Quality and Their Role in Aggressiveness of Giant Cell Granulomas of the Jaws

Original Article

Nermeen S. Afifi[1,2], Inas Helwa[2], Randa H. Mokhtar[2] and Ismail M. Shebl[3]

[1]*Department of Oral Pathology, Faculty of Dentistry, Ain Shams University, Egypt*

[2]*Department of Oral Biology,* [3]*Department of Oral Pathology, Faculty of Oral and Dental Medicine, Misr International University, Egypt*

ABSTRACT

Background: An ambiguity persists regarding variation in clinical aggressiveness of different oral lesions, among which are the giant cell granulomas. Different types of giant cell granulomas can develop in the jaws, including peripheral giant cell granuloma, and central giant cell granuloma, whether aggressive or non-aggressive, each of which show different clinical behavior. Although they show great resemblance histologically on the cellular level, still they may show variation in other histological components as those regarding collagen fibers present. This raises the question about any possible relationship between the clinical behavior and histological components of these lesions.

Aim of the Work: This study aimed to investigate and explore the possible correlation between immunohistochemical detection of tumor associated macrophages (TAMs) using CD163 and the density, packing and type of collagen in giant cell granulomas of the jaws and the role of this correlation in the variation of clinical aggressiveness of these lesions.

Methods: We measured the immunohistochemical expression of CD163 and quality of collagen fibers, using picrosirius red stain in normal oral mucosa, peripheral giant cell granuloma, non-aggressive and aggressive central giant cell granulomas (10 samples each).

Results: Our results indicate that the expression of CD163 showed a statistically significant increase in aggressive central giant granuloma as compared to the non-aggressive samples. Moreover, picrosirius staining revealed that samples from aggressive lesions showed predominant green-yellow birefringence indicating higher expression of collagen type III in these lesions.

Conclusion: These findings suggested a possible influencing role of TAMs and increased deposition of collagen type III in the progression and aggressive behavior of giant cell granulomas of the jaws.

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Corresponding Author: Ismail M Shebl, PhD, Department of Oal Biology, Faculty of Oral and Dental Medicine, Misr International University, Egypt, **Tel.**: +20 10 0603 8514, **E-mail:** ismail.shebl@miuegypt.edu.eg

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INTRODUCTION

Giant cell granulomas of the mandible and maxilla comprise a group of lesions with diverse clinical behavior, ranging from asymptomatic non- aggressive lesions to aggressive lesions. These lesions continue to show a degree of vagueness, whether if there is an association between their clinical behavior and their histological elements or not[1].

Central giant cell granuloma is an osteolytic, non-odontogenic lesion of unidentified cause. It usually affects the bones of the craniofacial area. It is histologically benign, although locally proliferative with variable clinical behaviors[2]. This lesion was initially introduced in 1953 by Jaffe who called it "giant cell reparative granuloma". The "reparative" term was soon stopped after apparent contradictions between the clinical course of such lesions and the reparative process[3]. Some cases of central giant cell granulomas are asymptomatic with slow rate of growth and low rate of recurrence, others are rapidly growing, aggressive, accompanied with quick bone destruction, cortical bone thinning and perforation, nerve displacement, teeth displacement or root resorption, and frequent association with pain[4]. Management of such lesions could depend on surgical or non-surgical methods, depending on the clinical behavior and extent of the lesion. However, non-surgical management is usually linked with 11% to 49% recurrence rates and reaching up to 72% in case of aggressive lesions[5].

Alternatively, peripheral giant cell granuloma is a reactive intra oral lesion which appears in the form of gingival soft tissue mass. Among giant cell granulomas of the jaws, it is the most common one. It originates from mesenchymal cells in the periodontal membrane or periosteum, as a reaction to local irritant or chronic trauma[6].

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The differences in the clinical behavior between peripheral giant cell granuloma, aggressive and non-aggressive cases of central giant cell granulomas have been traced and investigated from different points of view in the previous studies, including the possible role of macrophages in such differences[1,3,7]. Macrophages are recruited to the microenvironment of the tumor and other lesions as a result of the secretion of various chemokines, cytokines and growth factors by the lesional cells. Upon activation, macrophages develop into two main groups, M1 and M2. The subpopulation M2, also called tumor associated macrophages (TAMs) improves tumor growth and survival by stimulating angiogenesis and tissue regeneration and hence favors tumor survival[8].

TAMs are present in several tumors, contributing to the foundation of the tumor microenvironment in terms of promoting tumor development, angiogenesis, invasion, metastasis, and drug resistance[7]. They promote tumor development by expressing several cytokines such as platelet derived growth factor (PDGF), epithelial growth factor (EGF), and others which support survival and proliferation of tumor cells. The presence of receptors for vascular endothelial growth factor (VEGF) on TAMs surface further support these functions. In addition, TAMs secrete MMPs, serine proteases and other components that can help in stromal collagen modification and degradation[9–11].

Studies have consistently confirmed that the microenvironment of the lesion is a major contributor in its development and behavior. One of the key components of this microenvironment is the extracellular matrix[12]. Collagen is the most abundant component of the extracellular matrix. Collagen, with its 28 different types, is involved in diverse normal biological functions such as cell adhesion, cell division, cell migration, angiogenesis, and tissue repair. Thus, the role of collagen in influencing lesion behavior and progression is of prime importance. The concept of "tumor-associated collagen signature, TACS" has been introduced as a model for the progression capacity of aggressive lesions. The analysis of the collagen distribution pattern of certain lesions can be a credible and reproducible method for predicting its behavior and can hence be reflected in its management[13–15]. Provenzano *et al*.[16] characterized in situ well-defined TACS that can be regarded as signature to characterize breast tumors. They suggested that these collagen patterns can be indicative of the invasive and metastatic potential of the tumor in fresh biopsies.

Histochemical staining of thin sections using picrosirius red (PSR) is a sensitive technique to visualize and evaluate collagen fibers. As demonstrated by Segnani *et al*.[17] the double-staining of the PSR technique allows the collagen fibers to stand out over tissue background allowing better visualization than sirius red alone or other staining techniques such as Van Gieson. The stained fibers show a range of colors when examined by polarized light based on the fiber size, density of fibers packing, and therefore demonstrates clear arrangement of collagen fibers. Since PSR molecules associate with collagen fiber, parallelly to the long axis of each of them, it improves the birefringence of collagen under polarized light microscopy. Thick collagen (type I) would show a red-orange color, while thin collagen (type III) would be yellow-green[18,19]. Additionally, more hydration and substandard alignment and orientation is present with young collagen fibrils when compared to the more mature ones. Moreover, intensity of birefringence is decided by the cross links among the fibrils. The yellow-green color of thin fibers suggest that the collagen packing is loose, and possibly will not be composed of tightly packed normal fiber, but rather from procollagens, intermediates, or pathological collagen. Also, orange-red color reflects the tightness of fibers packing and molecules with better alignment, which causes shift to the longer wavelength of polarization colors[20].

Stromal collagen density, packing and type can influence the clinical behavior of different lesions through its effect on some signaling pathways. Also, collagen-rich conditions may cause hypoxia which can lead to enhanced progression of different lesions. Additionally, components of the extracellular matrix such as MMPs, hyaluronic acid and fibronectin can interact with collagen and consequently influence activity of lesional cells. Some of the connective tissue cells such as lymphocytes, fibroblasts and macrophages play an important role together with stromal collagen in lesion progression. On the other hand, stromal and epithelial cells can affect the formation of collagen through alteration of several genes and by altering transcription factors involved in the process of collagen biosynthesis[21]. The role of TAMs and their relation to collagen quality, and whether this relation affects clinical behavior has been proved in different lesions[22–24]. However, this relation was not thoroughly explored in different giant cell granulomas of the jaw.

AIM OF THE WORK

The aim of this study was to inspect the possible association between immunohistochemical detection of CD163 positive TAMs and stromal collagen quality (density, packing and type) in giant cell granulomas of the jaws, and the role of this correlation in the variation of clinical aggressiveness of such lesions.

MATERIALS AND METHODS

Cases selection

Blocks of peripheral giant cell granuloma, aggressive and non-aggressive central giant cell granuloma (ten specimens of each entity) as well as ten specimens of regular oral mucosa were used in this study. The clinical data sheet corresponding to each block was available, to which the aggressiveness and clinical manifestations of each lesion was determined by. Total number of blocks was retrieved from the Faculty of Dentistry, Ain Shams University and Misr International University, Egypt, from the Oral Pathology department archives. The protocol

of this research has been approved by the Ain Shams University ethical committee (Exemption code, FDASU-Rec ER122225).

The aggressive cases were selected based on criteria described by Chuong *et al*.[25] which are pain, paresthesia, root resorption, rapid growth, more than 5 cm in size, destruction of the cortical bone and a elevated level of postoperative relapses.

Immunohistochemical staining and assessment

Four μm thick sections were prepared from each paraffin-embedded block and mounted on glass slides. Section deparaffinization was made using xylene, then were inserted in different grades of ethyl alcohol for hydration. Later, samples were submerged in a solution of citrate buffer of pH 4.8, before the process of staining takes place. Universal kit (Lab Vision, Thermo fisher scientific USA) was used for immunostaining. Peroxidase anti-peroxidase technique of immunostaining using the streptavidin biotin system was accomplished, and blocking endogenous peroxidase activity was made by adding 3% hydrogen peroxide. Samples were then rinsed with phosphate buffered saline solution, then incubated overnight with anti-CD163 primary monoclonal (Lab Vision, Thermo fisher scientific USA). Sections were protected by the use of link antibody, then by the streptavidin biotin labeling antibody. Phosphate buffer saline was then used to rinse the sections, then 3,3'-Diaminobenzidine (DAB) chromogen was added to the specimens subsequently for counter staining. Sections dryness was achieved by the use of different grades of alcohol, afterwards clearance using xylene. All chemicals were purchased from Sigma-Aldrich, USA. Immunohistochemical staining was performed at Ain Shams University Specialized Hospital histology laboratory, Egypt.

For measurement of immunohistochemical expression, four areas under the microscope revealing the greatest immunopositive parts were selected from each positive section. Digital camera (LEICA DFC295) attached to a light microscope (LEICA DM LS2) was used to capture photomicrographs of the different areas, at a magnification of 40X. Area fraction of CD163 immunopositivity was measured using Image J, 1.41a software. Photomicrographic images and their analysis was completed at Misr International University research unit.

Picrosirius red staining

Tissue blocks inserted in paraffin were cut at thin sections (thickness of five μm); the sections were then placed onto the glass slides by floating. Slide warmer was then used to incubate the slides at 60° C, to ensure sections adhered properly to the slides. Paraffin was then removed from, and sections were then hydrated and stained with picrosirius red (PSR) stain (Sigma-Aldrich) according to the manufacturers protocol. Polarized light microscope was then used to examine the stained slides in the Precision Measurements Unit, Faculty of Dentistry, Ain Shams University. At a magnification 20X, photomicrographs of different fields of each slide were taken. Collagen fibers displayed polarizing colors ranging from red-orange to yellow-green birefringence. The proportions of yellow-green fibers birefringence were assessed in relation to the entire area of the fibers via image analysis software (Image J, 1.41a).

Statistical Analysis

Statistical data were studied for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Data indicated non-normal (non-parametric) distribution. Data were displayed as median and range values. Kruskal-Wallis test was applied to compare between the groups. Dunn's test was used for pair-wise comparisons when Kruskal-Wallis test is significant. Spearman's correlation coefficient was used to verify the correlation between area fraction of CD163 immunopositivity and area fraction of yellow-green fibers birefringence. The significance level was set at $P \leq 0.05$. Statistical analysis was completed with IBM SPSS Statistics for Windows, Version 23.0.

RESULTS

Immunohistochemical expression of CD163

All specimens used in this study confirmed immunopositivity for CD163. The reaction was cytoplasmic and granular in the stromal cells. The connective tissue of normal oral mucosa showed lowest expression of CD163 (Figure 1a), which was significantly lower than the other groups (Table 1, Figure 3). Both peripheral giant cell granuloma and non-aggressive central giant cell granuloma cases showed moderate immunopositivity in the stromal cells (Figures 1b, c) with no statistical difference between them (Table 1, Figure 3). Aggressive central giant cell granuloma showed the highest statistically significant immunopositivity for CD163 (Figure 1d, Table 1, Figure 3).

Picrosirius red staining results

The color birefringence of PSR-stained collagen examined by polarized light microscope was different among different groups (Figure 2). The collagen fibers of normal oral mucosa showed minimal yellowish birefringence with predominance of red birefringence. The median area fraction of yellow-green birefringence in this group was significantly lower than the other groups (Table 1, Figure 3). In peripheral giant cell granuloma and non-aggressive central giant cell granuloma the yellowish birefringence was more obvious than normal oral mucosa, but it was still non-significant as compared to the two other groups. The collagen birefringence in aggressive central giant cell granuloma was predominantly yellow-green with statistically higher significant median value than other groups (Table 1, Figure 3).

Correlation between CD163 and yellow-green fibers

Statistically, there was a significant direct correlation

between area fraction of CD163 immuno-expression and area fraction of yellow-green fibers (Correlation coefficient = 0.749, *P-value* <0.001). Rise of CD163 area fraction is associated with an increase in yellow-green fibers area fraction and vice versa (Figure 4).

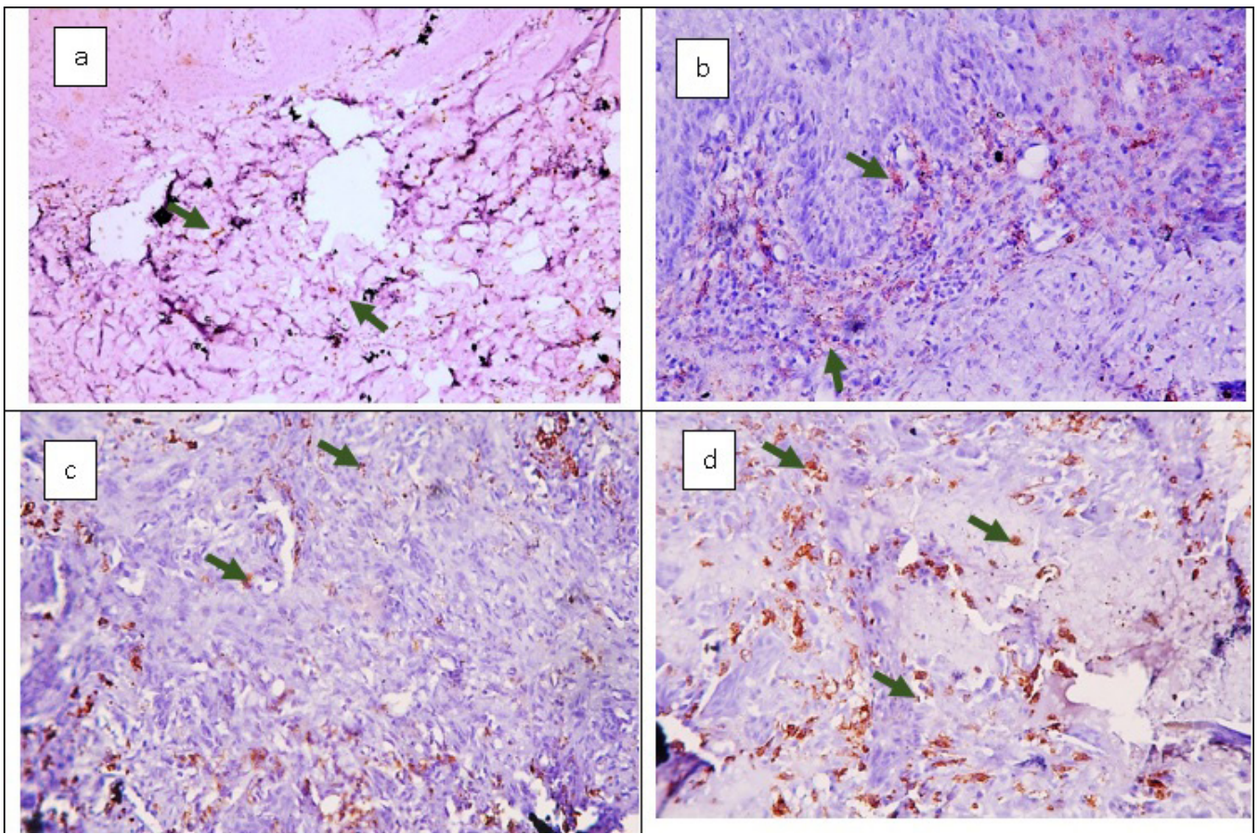

Fig. 1: (a) normal mucosa. (b) Peripheral giant cell granuloma, (c) non-aggressive central giant cell granuloma, and (d) aggressive central giant cell granuloma showing granular cytoplasmic immunohistochemical expression of CD163 in the stromal cells.

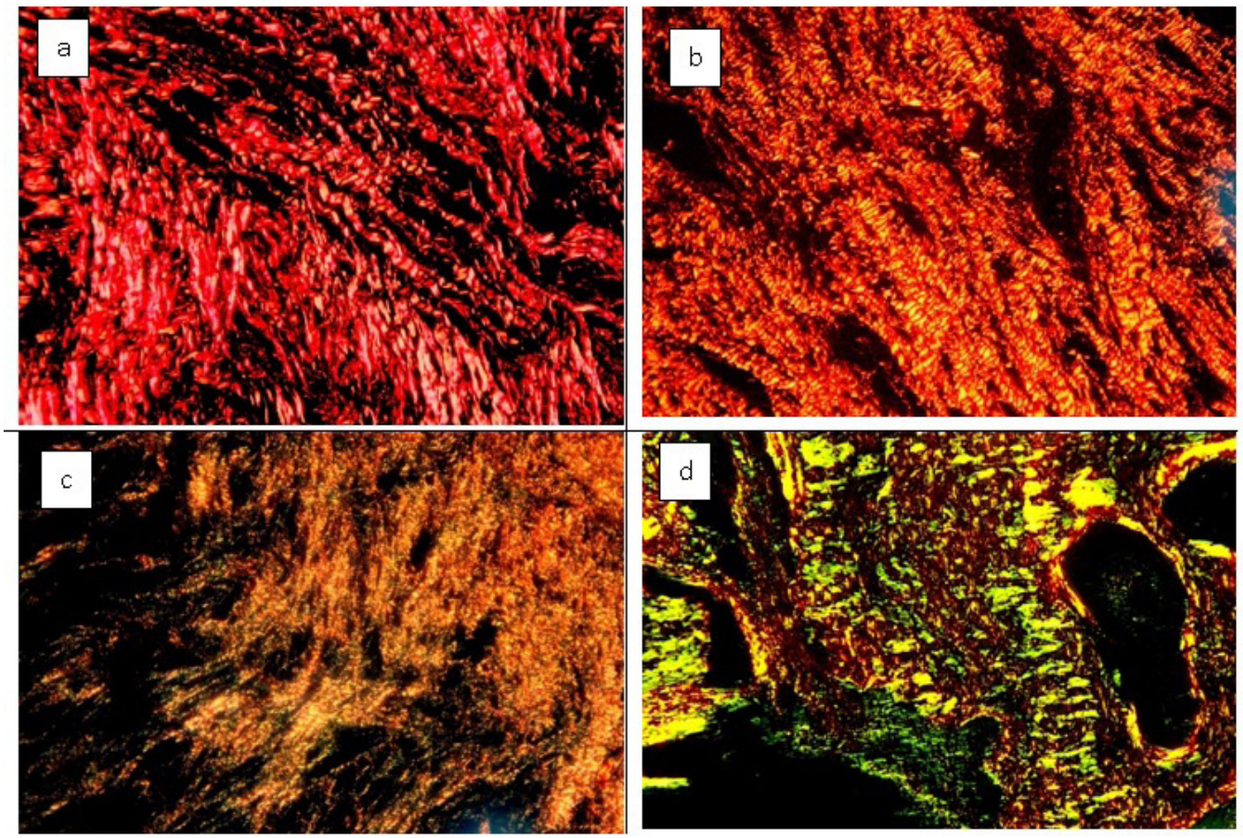

Fig. 2: (a) normal mucosa. (b) peripheral giant cell granuloma, (c) non-aggressive central giant cell granuloma, and (d) aggressive central giant cell granuloma. Predominance of yellow-green birefringence in aggressive central giant cell granuloma is noted.

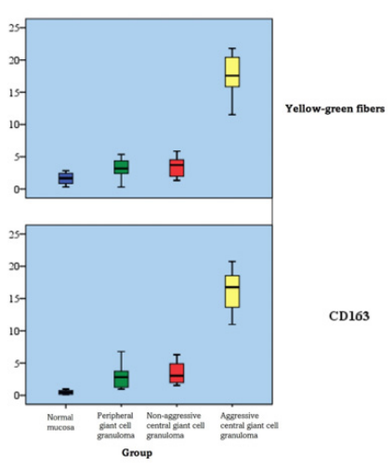

Fig. 3: Box plot representing median and range values for area fraction of yellow-green birefreingence of collagen fibers and area fraction of CD163 immunopositivity in different groups.

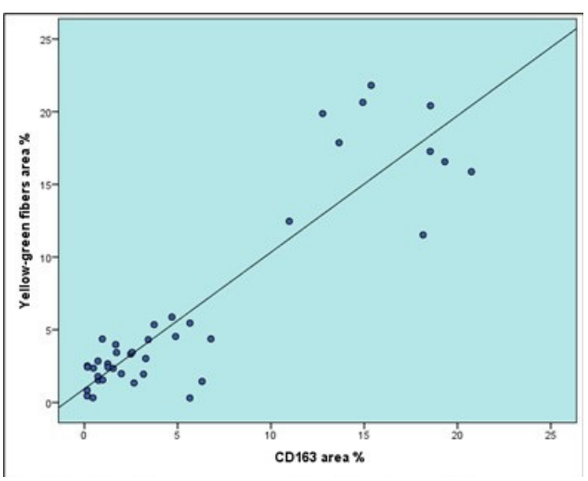

Fig. 4: Scatter diagram representing direct correlation between area fraction of CD163 and area fraction of yellow-green birefringence of collagen fibers.

Table 1: Descriptive statistics and results of Kruskal-Wallis test for comparison of median area fractions of CD163 immunohistochemical expression and yellow-green fibers in different groups.

	Normal mucosa (n = 10)	Peripheral giant cell granuloma (n = 10)	Non-aggressive central giant cell granuloma (n = 10)	Aggressive central giant cell granuloma (n = 10)	*P-value*	Effect size (Eta squared)
Median (Range) of CD163 %	0.47 (0.98-4.72) ^{C}	2.82 (0.96-6.78) ^{B}	3.04 (1.54-6.31) ^{B}	16.76 (10.98-20.75) ^{A}	<0.001*	0.907
Median (Range) of yellow-green fibers %	1.67 (0.32-2.852) ^{C}	3.18 (0.31-5.35) ^{B}	3.72 (1.34-5.87) ^{B}	17.57 (11.53-21.81) ^{A}		0.912

*: Significant at $P \leq 0.05$, Different superscripts indicate statistically significant difference between groups

DISCUSSION

Giant cell granulomas of the jaws represent a group of heterogeneous lesions whose origin and etiology are not fully elucidated. In the jaws, two entities of giant cell granulomas have been identified: peripheral giant cell granuloma and central giant cell granuloma. Central giant cell granuloma can be categorized into non-aggressive and aggressive central giant cell granuloma based on the clinical and radiographic criteria. Although the three lesions have similar histological components; multinucleated giant cells and mononuclear mesenchymal cells, however they have different clinical behavior[26,27]. Due to the significant growth of aggressive central giant cell granuloma, it can be accompanied by various complications such as pain, destruction of cortical plates and tooth mobility and therefore, it need more aggressive treatment[28]. Mysteries are still shadowing the pathogenesis of the three lesions, and it is still unclear why some of these lesions act aggressively, similar to the pattern of giant cell tumor of long bones. Various questions regarding clinical behavior of these lesions have not been clarified entirely[29].

CD163 is a characteristic marker of TAMs. Previous studies have shown that its expression is higher in tumor microenvironment as compared to the healthy tissue. Moreover, CD163 expression has shown association with poor response to radiotherapy and poor prognosis of head and neck squamous cell carcinoma[30,31]. CD163 expression is upregulated in several diseases including inflammatory and chronic diseases. This up-regulation was detected in CD163-expressing macrophages at the site of inflammation. The expression of CD163 appears to be associated with the increased aggressive behavior of the lesion[32].

In this study, we investigated the presence of TAMs by measurement of immunohistochemical expression of CD163 in peripheral giant cell granuloma and aggressive and non-aggressive central giant cell granulomas in an attempt to correlate this expression to aggressiveness of these lesions and the possible effect on clinical behavior. Our results have shown a statistically significant higher existence of TAMs in aggressive central giant cell granuloma than in the other non-aggressive entities. Although peripheral giant cell granuloma and non-aggressive central giant cell granuloma showed statistically non-significant difference in CD163 immuno-expression, however they still showed significantly higher levels of TAMs when compared to normal oral mucosa. This was in accordance with Mansor and Al-drobie[33] who reported a statistically non-significant difference in the immune-histochemical expression of CD163 in peripheral giant cell granuloma and central giant cell granuloma. Detection of CD163 positive cells in the connective tissue of normal oral mucosa in this study was in accordance with many previous studies who reported similar minor expression[34,35] which could be explained by its expression in resident tissue macrophages[36].

The abundance of TAMs in aggressive central giant cell granuloma in this study was in line with other studies which reported the association of TAMs with aggressiveness of different lesions[8,30,31,37]. This association could be explained through different pathways. TAMs are key cells controlling angiogenesis in several lesions. They recognize hypoxia in avascular parts of the lesion and respond by formation of angiogenic factors, including vascular endothelial growth factor -A which accelerates chemotaxis of endothelial cells and macrophages. Besides, TAMs release a significant number of pro-angiogenic factors, including tumor necrosis factor α, semaphorin 4D, basic fibroblast growth factor, urokinase-type plasminogen activator, thymidine phosphorylase, and adrenomedullin[38]. In addition, conditioned medium derived from TAMs can induce angiogenesis in various in *vivo* model systems as reported by Hitoe *et al*.[39].

Various efforts have been made to realize the biological importance of angiogenesis and the factors impacting it in peripheral and central giant cell granulomas, and to reach histopathological parameters as reliable indicators of clinical behavior[40,41]. Peacock *et al*, studied giant cell lesions and found that the vascularity and level of angiogenesis in aggressive giant cell lesions were greater than those in non-aggressive lesions[42]. Such findings propose that TAMs-induced angiogenesis may have a part in clinical behavior of these lesions, that is why we hypothesized that TAMs posses a vital role in the aggressiveness and proliferation of giant cell granulomas.

Then again, TAMs can modify matrix production in the lesion to favor its growth[43]. In addition, TAMs can produce MMPs, cathepsins, and secreted protein acidic and rich in cysteine (SPARC), all of which can degrade and remodel the extracellular matrix[44,45]. So, the other hypothesis in this study was that TAMs affect collagen quality in terms of density, packing and type in the stroma

of giant cell granuloma thus affecting the clinical behavior of these lesions.

Many studies suggest that the higher the ratio between collagen type III to collagen type I, the poorer the matrix organization will be[46,47]. Thus, the present study relay on color birefringence of collagen fibers in the stroma of giant cell granulomas to reflect the collagen quality.

The results of PSR stained sections in the current study revealed statistically significant lowest collagen quality in aggressive central giant cell granuloma by predominance of yellow-green birefringence of collagen fibers in comparison to other groups. Samples of peripheral giant cell granuloma and non-aggressive central giant cell granuloma showed equivalent low percentages of yellow-green birefringence of collagen fibers with statistical non-significant difference between them. The collagen density and packing in these two groups was higher than aggressive central giant cell granuloma as denoted by predominance of red-orange birefringence of collagen fibers. Normal oral mucosa on the other hand showed the statistically significant lowest yellow-green birefringence of collagen fibers among all groups and hence the highest collagen quality and density.

The correlation between the aggressiveness of the lesion and low collagen density has been reported in many previous studies[48–50] which agreed with our study. The link between low collagen quality and lesion aggression could be explained by easier denaturing of this structurally unstable collagen which usually consists of pro-collagen, intermediate, or pathologic collagens with disorganized pattern by the action of proteolytic enzymes of the stromal tissue[51].

The responsibility of myofibroblasts for immaturity of collagen and its low quality has been elucidated in many studies[52,53]. Surprisingly, Peaccok *et al*.[42] and O'Mally *et al*.[54] revealed myofibroblastic differentiation of many fibroblasts in central and peripheral giant cell granulomas. At the same time, Kujan *et al*.[29] suggested that the presence of macrophages and myofibroblasts were responsible for the behavior of central giant cell granuloma. Also, Maiz *et al*.[55] reported a positive significant association between myofibroblast detection and root resorption as well as cortical destruction in central giant cell lesions.

Interestingly, TAMs can drive the differentiation of myofibroblasts in many lesions through different pathway as CCL18-driven signaling cascade[56,57], secretion of transforming growth factor-β[58], insulin-like growth factor-1, which encourages the proliferation and endurance of myofibroblasts[59].

In line with abovementioned data, we found a direct statistically significant correlation between the area fraction of yellow-green birefringence of collagen fibers and immunohistochemical expression of the CD163 in the studied giant cell granuloma samples. Samples with high CD163 expression (higher TAMs content) have higher yellow-green birefringence of collagen fibers (low collagen quality) and vice versa. Accordingly, an influencing role of CD163 positive TAMs in determination of collagen quality in giant cell granulomas of the jaw is possible and could explain the aggressive behavior of some entities of this category of lesions. Thus, treatment strategies targeting TAMs in aggressive central giant cell granuloma could help in controlling the aggressive clinical course of this entity.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Gupta S, Narwal A, Kamboj M, Devi A, Hooda A. Giant Cell Granulomas of Jaws: a Clinicopathologic Study. J Oral Maxillofac Res. 2019;10(2):1–10. DOI: 10.5037/jomr.2019.10205

2. Dimitakopoulos I, Lazaridis N, Sakellariou P, Asimaki A. Giant-cell granuloma in the temporal bone: A case report and review of the literature. J Oral Maxillofac Surg. 2006;64(3):531–6. DOI: 10.1016/j.joms.2005.11.006

3. Motamedi MHK, Eshghyar N, Jafari SM, Lassemi E, Navi F, Abbas FM, *et al*. Peripheral and central giant cell granulomas of the jaws: A demographic study. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007;103(6). DOI: 10.1016/j.tripleo.2006.12.022

4. Kruse-Lösler B, Diallo R, Gaertner C, Mischke KL, Joos U, Kleinheinz J. Central giant cell granuloma of the jaws: A clinical, radiologic, and histopathologic study of 26 cases. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2006;101(3):346–54. DOI: 10.1016/j.tripleo.2005.02.060

5. De Lange J, van den Akker HP, van den Berg H. Central giant cell granuloma of the jaw: a review of the literature with emphasis on therapy options. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007;104(5):603–15. DOI: 10.1016/j.tripleo.2007.04.003

6. Falaschini S, Ciavarella D, Mazzanti R, M DC, Turco M, Escudero N, *et al*. Peripheral giant cell granuloma : immunohistochemical analysis of different markers. Study of three cases. Av Odontoestomatol. 2007;23(4):189–96. <<chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://scielo.isciii.es/pdf/odonto/v23n4/en_original2.pdf>>

7. Vasconcelos RG, Vasconcelos MG, Queiroz LMG. Peripheral and central giant cell lesions: Etiology, origin of giant cells, diagnosis and treatment. J Bras Patol e Med Lab. 2013;49(6):446–52. DOI: 10.1590/S1676-24442013000600011

8. Genin M, Clement F, Fattaccioli A, Raes M, Michiels C. M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide. BMC Cancer. 2015;15:1–14. DOI:10.1186/s12885-015-1546-9

9. DeNardo DG, Ruffell B. Macrophages as regulators of tumor immunity and immunotherapy. Nat Rev Immunol. 6th ed. 2019;19(6):369–82. DOI: 10.1038/s41577-019-0127-6

10. Pan Y, Yu Y, Wang X, Zhang T. Tumor-Associated Macrophages in Tumor Immunity. Front Immunol. 2020;11(December). DOI: 10.3389/fimmu.2020.583084

11. Bernsmeier C, Merwe S Van Der, Périanin A. Innate immune cells in cirrhosis. J Hepatol. 2020;73(1):186–201. DOI: 10.1016/j.jhep.2020.03.027

12. Brassart-pasco S, Brézillon S, Brassart B, Ramont L. Tumor Microenvironment : Extracellular Matrix Alterations Influence Tumor Progression. Front Oncol. 2020;10(April):1–13. DOI: 10.3389/fonc.2020.00397

13. Zhou J, Tang Z, Gao S, Li C, Feng Y, Zhou X. Tumor-Associated Macrophages : Recent Insights and Therapies. Front Oncol. 2020;10(February):1–13. DOI: 10.3389/fonc.2020.00188

14. Brett EA, Sauter MA, Machens H, Duscher D. Tumor-associated collagen signatures : pushing tumor boundaries. Cancer Metab. 2020;8(14):1–5. DOI: 10.1186/s40170-020-00221-w

15. Ouellette JN, Drifka CR, Pointer KB, Liu Y, Lieberthal TJ, Kao WJ, *et al*. Navigating the Collagen Jungle : The Biomedical Potential of Fiber Organization in Cancer. Bioengineering. 2021;8(17). DOI: 10.3390/bioengineering8020017

16. Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ. Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC Med. 2006;16:1–15. DOI: 10.1186/1741-7015-4-38

17. Segnani C, Ippolito C, Antonioli L, Pellegrini C. Histochemical Detection of Collagen Fibers by Sirius Red / Fast Green Is More Sensitive than van Gieson or Sirius Red Alone in Normal and Inflamed Rat Colon. PLoS One. 2015;1–10. DOI: 10.1371/journal.pone.0144630

18. G. S. MontesL. C. U. Junqueira. The use of picrosirius-polarization method for the study of the biopathology of collagen. Inst Oswaldo Cruz. 1991;86(3):1–11. DOI: 10.1590/s0074-02761991000700002

19. Brown SR, Melman L, Jenkins E, Deeken C, Frisella MM, Brunt LM, *et al*. Collagen type I : III ratio of the gastroesophageal junction in patients with paraesophageal hernias. Surg Endosc. 2011;25:1390–4. DOI: 10.1007/s00464-010-1373-7

20. Gopinathan PA, Kokila G, Siddeeqh S, Prakash R, Pradeep L. Reexploring picrosirius red : A review. 2020;7(2):196–203. DOI:10.18231/j.ijpo.2020.038

21. Arseni L, Lombardi A, Orioli D. From Structure to Phenotype : Impact of Collagen Alterations on Human Health. Int J Mol Sci. 2018;19(1407):1–36. DOI: 10.3390/ijms19051407

22. Larue MM, Parker S. Metabolic reprogramming of tumor-associated macrophages by collagen turnover promotes fi brosis in pancreatic cancer. Cell Biol. 2022;119(16):1–10. DOI: 10.1073/pnas.2119168119

23. Shi Q. , Jiakun L. , Kun J. , Lu Y. QWW. Tumor associated macrophages promote bladder tumor growth through PI3k/AKT signal induced by collagen. Eur Urol Suppl. 2019;18(1). DOI: 10.1111/cas.14078

24. Kalogirou EM, Tosios KI, Christopoulos PF. The Role of Macrophages in Oral Squamous Cell Carcinoma. Front Immunol. 2021;11(March):1–8. DOI: 10.3389/fonc.2021.611115

25. Chuong R, Kaban LB, Kozakewich H, Perez-atayde A. Central Giant Cell Lesions of the Jaws : A Clinicopa thologic Study. J Oral Maxillofac Surg. 1986;44:708–13. DOI: 10.1016/0278-2391(86)90040-6

26. Boşca AB, Sovrea AS, Miclăuş V, Ruxanda F, *et al*. Diagnostic and therapeutic approaches in oral cavity granulomas based on new data concerning their origin and pathogenesis. Rom J Morphol Embryol. 2018;59(3):679–90. https://www.researchgate.net/publication/329876990_Diagnostic_and_therapeutic_approaches_in_oral_cavity_granulomas_based_on_new_data_concerning_their_origin_and_pathogenesis

27. Baskaran P, Gopal M, Rastogi V, Misra SR. Case Report Aggressive central giant cell granuloma of the mandible , a diagnostic dilemma. J oral Maxillofac Radiol. 2015;3(3):88–91. DOI:10.4103/2321-3841.170614

28. Ramesh V. “Central giant cell granuloma” – An update. J oral Maxillofac Pathol. 2020;24:413–5. DOI:10.4103/jomfp.jomfp_487_20

29. Kujan O, Al-Shawaf AZZ Azzeghaiby S, AlManadille A, Sar KA. Immunohistochemical Comparison of p53, Ki-67, CD68, Vimentin, α-smooth Muscle Actin and Alpha-1-Antichymotry- psin in Oral Peripheral and Central Giant Cell Granuloma. J Contemp Dent Pract. 2015;16(1):20–4. DOI: 10.5005/jp-journals-10024-1629

30. Kwiecień I, Polubiec-kownacka M, Dziedzic D, Wołosz D, Rzepecki P, Domagała-kulawik J. CD163 and CCR7 as markers for macrophage polarization in lung cancer microenvironment. Clin Immunol. 2019;44(4):395–402. DOI: 10.5114/ceji.2019.92795

31. Hu JM, Liu K, Liu JH, Jiang XL, Wang XL, Chen YZ. CD163 as a marker of M2 macrophage , contribute to predict aggressiveness and prognosis of Kazakh esophageal squamous cell carcinoma. Oncotarget. 2017;8(13):21526–38. DOI: 10.18632/oncotarget.15630

32. Skytthe MK, Graversen JH, Moestrup SK. Targeting of cd163+ macrophages in inflammatory and malignant diseases. Int J Mol Sci. 2020;21(15):1–31. DOI: 10.3390/ijms21155497

33. Mansor SM, Al-drobie BF. Clinicopathological and Immunohistochemical Comparison of Peripheral and Central Giant Cell Granuloma of the Jaws Using CD68 and CD 163. J Res Med Dent Sci. 2022;10(6):213–8. << chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.jrmds.in/articles/clinicopathological-and-immunohistochemical-comparison-of-peripheral-and-central-giant-cell-granuloma-of-the-jaws-using-.pdf>>

34. He K, Zhang L, Huang C, Ma S, Wang Y, Wang W, *et al*. CD163+ Tumor-Associated Macrophages Correlated with Poor Prognosis and Cancer Stem Cells in Oral Squamous Cell Carcinoma. Biomed Res Int. 2014;2014. DOI: 10.1155/2014/838632

35. Mori K, Haraguchi S, Hiori M, Shimada J, Ohmori Y. Tumor-associated macrophages in oral premalignant lesions coexpress CD163 and STAT1 in a Th1-dominated microenvironment. 2015;1–11. DOI: 10.1186/s12885-015-1587-0

36. Polfliet MMJ, Fabriek BO, Danie WP, Dijkstra CD, Berg TK Van Den. The rat macrophage scavenger receptor CD163 : Expression , regulation and role in inflammatory mediator production. Immunobiology. 2006;211:419–25. DOI: 10.1016/j.imbio.2006.05.015

37. Lu C, Huang C, Tjiu J, Chiang C. Infiltrating macrophage count: a significant predictor for the progression and prognosis of oral squamous cell carcinomas in taiwan. Head neck. 2010;(january):18–25. DOI: 10.1002/hed.21138

38. Riabov V, Gudima A, Wang N, Mickley A, Orekhov A, Red G, *et al*. Role of tumor associated macrophages in tumor angiogenesis and lymphangiogenesis. Front Physiol. 2014;5(March):1–13. DOI: 10.3389/fphys.2014.00075

39. Torisu H, Ono M, Kiryu H, Furue M, Ohmoto Y, Nakayama J, *et al*. Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant melanoma : possible involvement of tnf alpha and IL-1alpha. Int J Cancer. 2000;188(May 1999):182–8. << https://pubmed.ncbi.nlm.nih.gov/10629075/>>

40. Kumar VV, Krishanappa SJ, Prakash SG, Channabasaviah GH. Quantification and Correlation of Angiogenesis with Macrophages by Histomorphometric Method in Central and Peripheral Giant Cell Granuloma : An Immunohistochemical Analysis. J Clin diagnostic Res. 2016;10(3):1–5. DOI: 10.7860/JCDR/2016/15657.7349

41. Miguel CC, Galva C, Matos FR, Nonaka CFW, Souza B De, Freitas RDA. Immunoexpression of MMP-9 , VEGF , and vWF in central and peripheral giant cell lesions of the jaws. J Oral Pathol Med. 2011;40:338–44. DOI: 10.1111/j.1600-0714.2010.00993.x

42. Peacock ZS, Jordan RCK, Schmidt BL. Giant Cell Lesions of the Jaws : Does the Level of Vascularity and Angiogenesis correlate With Behavior ? J Oral Maxillofac Surg. 2012;70(8):1860–6. DOI: 10.1016/j.joms.2011.08.020

43. Deligne C, Midwood KS. Macrophages and Extracellular Matrix in Breast Cancer : Partners in Crime or Protective Allies ? Front Oncol. 2021;11(February):1–12. DOI: 10.3389/fonc.2021.620773

44. Bergers G, Brekken R, Mcmahon G, Vu TH, Itoh T, Tamaki K, *et al*. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol. 2000;2(October):737–44. DOI: 10.1038/35036374

45. Gocheva V, Zeng W, Ke D, Klimstra D, Reinheckel T, Peters C, *et al*. Distinct roles for cysteine cathepsin genes in multistage tumorigenesis. Genes Dev. 2006;20:543–56. DOI: 10.1101/gad.1407406

46. Thankam FG, Evan DK, Agrawal DK, Dilisio MF. Collagen type III content of the long head of the biceps tendon as an indicator of glenohumeral arthritis. Mol Cell Biochem. 2019;454(1–2):25–31. DOI: 10.1007/s11010-018-3449-y

47. Lui PPY, Chan LS, Lee YW, Fu SC, Chan KM. Sustained expression of proteoglycans and collagen type III/type I ratio in a calcified tendinopathy model. Rheumatology. 2010;49(2):231–9. DOI: 10.1093/rheumatology/kep384

48. Udompatanakorn C, Rungsiyanont S. The Collagen Fibers Analysis of the Odontogenic Cysts: A Study with Picrosirius Red Staining Under Polarizing Microscopy. Chiang Mai Dent J. 2021;43(1). DOI 10.14456/cmdj.2022.4

49. Afifi NS, Shebl IM. Role of myofibroblasts and collagen quality in elaboration of debatable nature of odontogenic keratocyst. Egypt J Histol. 2021;44(3):779–86. DOI: 10.21608/EJH.2020.45572.1370

50. Xu S, Xu H, Wang W, Li S, Li H, Li T. The role of collagen in cancer : from bench to bedside. J Transl Med. 2019;17:1–22. DOI: 10.1186/s12967-019-2058-1

51. Raj Y, Sekhar MSM, Shylaja S, Bhavani SN, Ramanand OV, Patha S, *et al*. Evaluation of the nature of collagen fibers in KCOT, dentigerous cyst and ameloblastoma using picrosirius red stain–A comparative study. J Clin Diagnostic Res. 2015;9(11):ZC01–4. DOI: 10.7860/JCDR/2015/14154.6708

52. Ehrlich HP, Hunt TK. Collagen Organization Critical Role in Wound Contraction. Adv Wound Care. 2012;1(1):3–9. DOI: 10.1089/wound.2011.0311

53. Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. J Pathol. 2003;200(4):500–3. DOI: 10.1002/path.1427

54. O'Malley M, Pogrel MA, Stewart JCB, Silva RG, Regezi JA. Central giant cell granulomas of the jaws: Phenotype and proliferation-associated markers. J Oral Pathol Med. 1997;26(4):159–63. DOI: 10.1111/j.1600-0714.1997.tb00451.x

55. Noya Maiz N, de la Rosa-García E, Irigoyen Camacho ME. Immunohistochemical expression of alpha-smooth muscle actin and glucocorticoid and calcitonin receptors in central giant-cell lesions. J Oral Pathol Med. 2016;45(4):289–94. DOI: 10.1111/jop.12377

56. Chen J, Yao Y, Gong C, Yu F, Su S, Chen J, *et al*. CCL18 from Tumor-Associated Macrophages Promotes Breast Cancer Metastasis via PITPNM3. Cancer Cell. 2011;19(4):541–55. DOI: 10.1016/j.ccr.2011.02.006

57. Nie Y, Chen J, Huang D, Yao Y, Chen J, Ding L, *et al*. Tumor-associated macrophages promote malignant progression of breast phyllodes tumors by inducing myofibroblast differentiation. Cancer Res. 2017;77(13):3605–18. DOI: 10.1158/0008-5472.CAN-16-2709

58. Wynn TA, Barron L. Macrophages: Master regulators of inflammation and fibrosis. Semin Liver Dis. 2010;30(3):245–57. DOI: 10.1055/s-0030-1255354

59. Wynes MW, Riches DWH. Induction of macrophage insulin-like growth factor-I expression by the Th2 cytokines IL-4 and IL-13. J Immunol. 2003;171(7):3550–9. DOI: 10.4049/jimmunol.171.7.3550

الملخص العربى

الخلايا البلعمية المرتبطة بالأورام وعلاقتها بجودة الكولاجين ودورها في عدوانية الأورام الحبيبية العملاقة في الفكين

نيرمين سامي عفيفي[١،٢]، إيناس حلوة[٢]، راندا حسن مختار[٢]، إسماعيل محمد شبل[٣]

[١]قسم علم أمراض الفم، كلية طب الأسنان، جامعة عين شمس وجامعة مصر الدولية، القاهرة، مصر

[٢]قسم أمراض الفم، كلية طب الفم والأسنان، جامعة مصر الدولية، القاهرة، مصر

مقدمه: لا يزال هناك غموض بشأن التفاوت في العدوانية السريرية للأورام الحبيبية العملاقة، مما يثير التساؤل حول العلاقة المحتملة بين سلوكها ومكوناتها النسيجية.

هدف البحث: هدفت هذه الدراسة إلى التحقيق في العلاقة المحتملة بين الكشف المناعي الكيميائي عن البلاعم المرتبطة بالأورام (TAMs) باستخدام CD١٦٣ وكثافة وترتيب ونوع الكولاجين في الأورام الحبيبية العملاقة للفكين، ودور هذه العلاقة في التفاوت في العدوانية السريرية لهذه الآفات.

مواد وأساليب العلاج: قمنا بقياس التعبير المناعي الكيميائي لـ CD١٦٣ وجودة ألياف الكولاجين باستخدام صبغة بيكروسيرياس الحمراء في عينات من الغشاء المخاطي الفموي الطبيعي، والأورام الحبيبية العملاقة المحيطية، والأورام الحبيبية العملاقة المركزية غير العدوانية والعدوانية (١٠ عينات لكل مجموعة).

النتائج: أشارت نتائجنا إلى أن التعبير عن CD١٦٣ أظهر زيادة ذات دلالة إحصائية في الأورام الحبيبية العملاقة المركزية العدوانية مقارنة بالعينات غير العدوانية. علاوة على ذلك، أظهرت العينات من الآفات العدوانية انكساراً ضوئياً باللون الأخضر-الأصفر بشكل رئيسي، مما يشير إلى زيادة التعبير عن الكولاجين من النوع الثالث في هذه الآفات.

الاستنتاج: أشارت هذه النتائج إلى دور محتمل مؤثر للبلاعم المرتبطة بالأورام وزيادة ترسب الكولاجين من النوع الثالث في تطور وسلوك الأورام الحبيبية العملاقة العدوانية في الفكين.