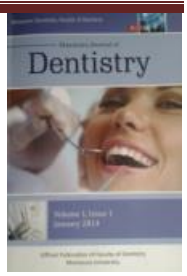
Comparing proliferative activity of Giant Cell Granuloma Lesions with Osteoclastic and Histiocytic derivation using PCNA,TRAP and CD163



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Abstract:
Background: Giant cell granulomas of the jaws are lesions that arise either peripherally in periodontal ligament and mucoperiosteum or centrally in the bone. The aim of this study was to evaluate expression of tartrate-resistant acid phosphatase (TRAP) and CD163 proteins in multinucleated giant cells and mononuclear cells and compare proliferative activity between central giant cell granuloma (CGCG) and peripheral giant cell granuloma (PGCG) by immunohistochemistry of PCNA.
Methods: The study was conducted on 40 formalin fixed paraffin embedded tissue blocks (20 CGCG ,20 PGCG). Five micron thick sections were cut from the paraffin blocks; two for hematoxylin and eosin to confirm diagnosis, while three sections were prepared for the immunohistochemical detection of TRAP, CD163 and PCNA by using *Avidin-Biotin complex according to the manufacturer's instructures.*
Results: There was statistically significant difference regarding CD163 between CGCG and PGCG, while there was no statistically significant differences of TRAP and PCNA between CGCG and PGCG. Also, there was high negative correlation between immunoexpression of TRAP and CD163 in CGCG and PGCG. There was no correlation between TRAP and PCNA and between CD163 and PCNA.
Conclusions: From the findings of the present study, it can be concluded that the histogenesis of MNGs of CGCG and PGCG was suggested to be of the osteoclastic origin, while there was no differences between proliferative activity between these two lesions.

Introduction

The giant cell granuloma of oral cavity and jaws are lesions that occur peripherally from periodontal ligament and periosteum (PGCG) or centrally in the bone(CGCG). Histologically, both peripheral and central giant cell granulomas are characterized by presence of few to many multinucleated giant cells in a fibrocellular connective tissue stroma[(1)], although multi-nuclear giant cells are the hallmark of these lesions, the histogenesis of the giant cells has not been specified yet [(2)].

Tartrate-resistant acid phosphatase (TRAP) is an iron-containing enzyme that is found in humans. It occurs in diverse tissues including bone and cartilage .TRAP is highly expressed in osteoclasts as well as chondroclasts therefore, used as a specific histochemical marker for these cells. TRAP promots the dephosphorylation of bone matrix phosphoproteins like osteopontin and bone sialoprotein[(3)], so it's related to bone resorption[(1)].

Cluster of differentiation163 (CD163) antigen is a member of the scavenger receptor cysteine-rich (SRCR) super family class B, it's expressed by cells of macrophages lineage. The function of CD163 is a homeostatic one and related to the binding of Hemoglobin-Haptoglobin complexes[(4)].

Proliferating cell nuclear antigen (PCNA) was originally characterised as a DNA sliding clamp for replicative DNA polymerases and used as a marker of cell proliferation[(5)].

Therefore, the present study was carried out to compare the histogenesis of MNGCs in CGCG and PGCG using immunohistochemical identification of TRAP and CD163. And to compare proliferative activity between these lesions by using of immunohistochemistry of PCNA.

Material and Methods

The present study was conducted on 40 formalin fixed paraffin embedded tissue blocks (20 CGCG and 20 PGCG). Five micron thick sections were cut from each paraffin block; two were stained with hematoxylin and eosin to confirm diagnosis, while three sections were prepared for the immunohistochemical detection of TRAP, CD163 and PCNA by using Avidin-Biotin complex according to the manufacturer's instructures. The immunoreactivity of TRAP, CD163 and PCNA was evaluated by computer assisted digital image analysis (Digital morphometric study) .

Results

In CGCG, giant cells reacted for TRAP marker with a mean number of positive cells (88.86±SD6.023) and were negative to CD163 marker. In mononuclear cells, expression of TRAP with a mean number of positive cells was (14.63±SD 2.45) and for CD163 was (13.37±SD3.43) (table1,2) (figure1,3), while the mean number of positive cells to PCNA was (78.75±SD23.34) (table3) (figure5).

In PGCG, giant cells reacted for TRAP marker with a mean number of positive cells (86.81±SD 8.41) and were negative to CD163 marker. In mononuclear cells, expression of TRAP with a mean number of positive cells was (14.05±SD 3.001) and for CD163 was (9.76±SD2.34). (table1,2) (figure2,4), while the mean number of positive cells to PCNA was (73.58±SD18.98)(table3) (figure6).

Table(1):Comparison of TRAP expression in CGCG and PGCG groups .

		CGCG	PGCG	P	
TRAP (%)	Giant cells	Mean	88.86	86.81	0.44
		±SD	6.023	8.411	
	Mononuclear cells	Mean	14.63	14.05	0.57
		±SD	2.450	3.001	

Table(2):Comparison of CD163 expression in CGCG and PGCG groups.

			CGCG	PGCG	P
CD163(%)	**Giant cells**	**Mean**	**0**	**0**	-
		±SD	**0**	**0**	
	Mononuclear cells	**Mean**	**13.37**	**9.767**	**0.002***
		±SD	**3.432**	**2.337**	

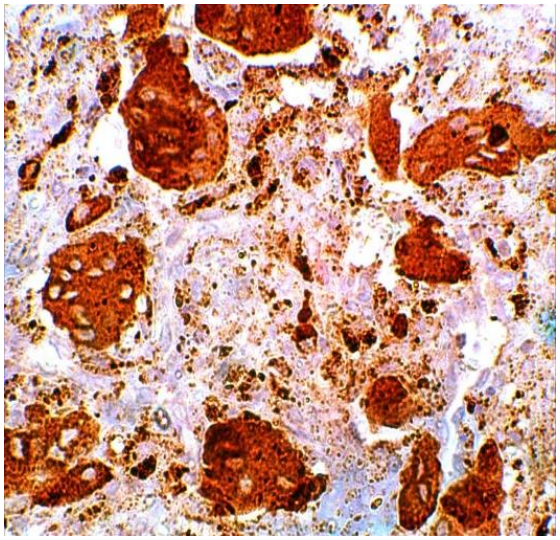
***:significance <0.05**
Test used: Student' t-test

Table(3):Comparison of PCNA average area between CGCG & PGCG groups .

		CGCG	PGCG	P
PCNA(average area)	**Mean**	**2607**	**2498**	**0.7**
	±SD	**677.3**	**833.6**	

***:significance <0.05**

Figure 1: A case of CGCG with diffuse brown granular reaction in the cytoplasm of the



multinucleated giant cells
(red arrow) and mononuclear
cells (green arrow) (TRAP X 400).

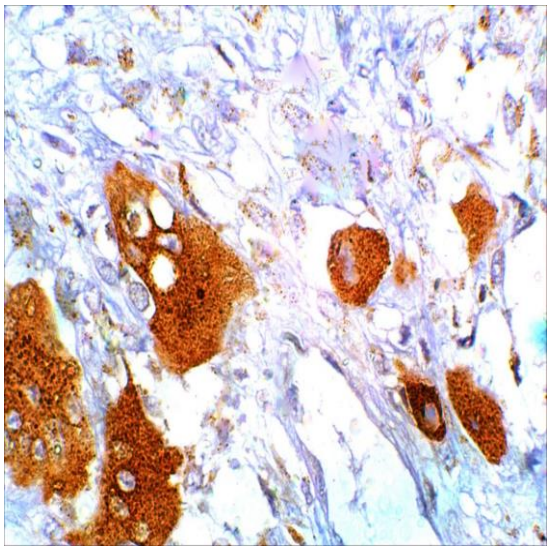
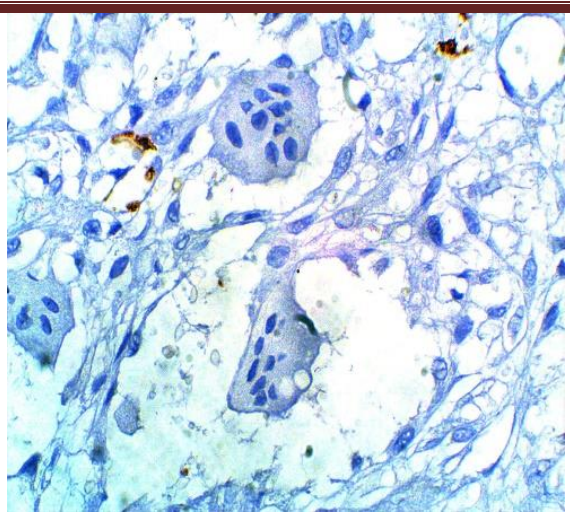
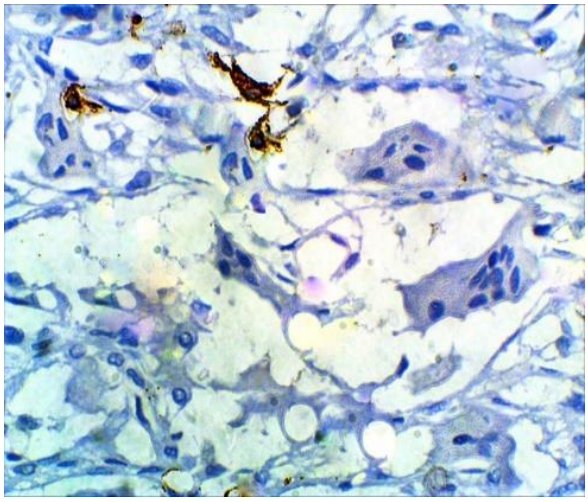


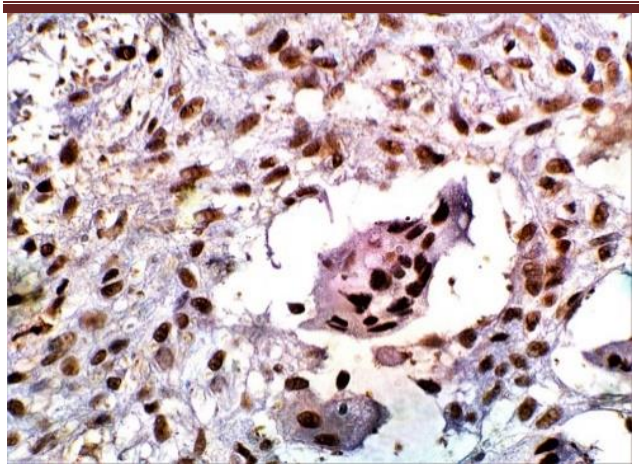
Figure 2: A case of PGCG with diffuse brown granular reaction in the
cytoplasm of the multinucleated giant cells (red arrow) and mononuclear cells (green arrow) (TRAP X 400).



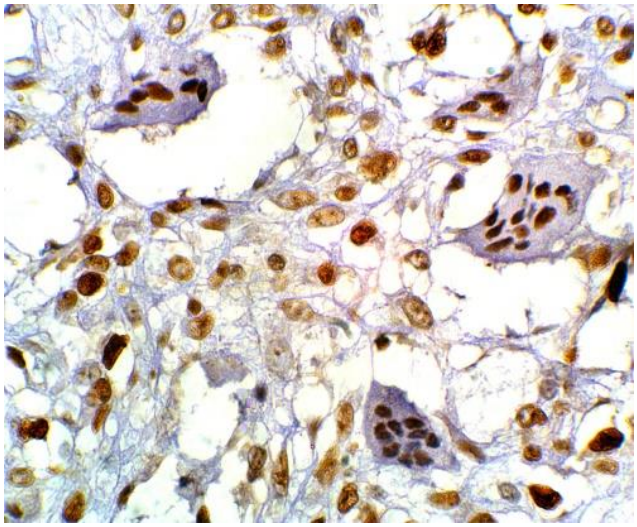
Fig(3):Acase of CGCG showing negative immunoreaction in the MNGCs (CD163X400).



Fig(4):Acase of PGCG showing negative immunoreaction in the MNGCs and positive mononuclear cells (CD163X400).



Fig(5):A case of CGCG showing prominent nuclear reaction to anti-PCNA in MNGC and fibroblasts (PCNAX400).



Fig(6):A case of PGCG showing prominent nuclear reaction to anti-PCNA in MNGC and fibroblasts (PCNAX400).

Discussion

Microscopic examination of central and peripheral giant cell granuloma consists of multinuclear giant cells in a background of ovoid, spindle-shaped mesenchymal cells and foci of hemorrhage, although multi-nuclear giant cells are the hallmark of these lesions, the histogenesis of the giant cells has not been specified yet [2].Some investigators believe that the giant cells show the immunohistochemical characteristics of osteoclasts[6] while others have suggested the macrophages as an origin for these cells, it was also suggested that the stromal mononuclear cells play an important role in the evolution of giant cells.[7]

The present study was carried out to examine TRAP, CD163 and PCNA immunohistochemical expression in CGCG and PGCG to investigate the histogenesis of multinucleated giant cells and compare proliferative activity between central and peripheral giant cell granuloma lesions.

In the present study, the immunohistochemical expression of TRAP in CGCG and PGCG appeared in most of multinucleated giant with no statistical significant differences which was in accordance with Itonaga I. et al[8], Flanagan AM [9]and Liu B[10] who suggested the osteoclastic phenotype of MNGCs, while Torabinia N et al [1] found high percentage of TRAP expression in 99% of CGCG and PGCG cases.

Similar to the findings of the present study, Liu B and co-workers[11] supposed that MNGCs in CGCG and PGCG of the jaws showed some similarities to the osteoclasts, such as the presence of acid phosphatase (TRAP) and osteoclast specific cellular antigens. But, their relation to the osteoclasts has never been fully established because the previous enzymes and antibodies were not unique or sufficient to identify the osteoclasts. Especially these antibodies did not confirm whether they had the potential to resorb the bone. In addition, previous studies only focused on the CGCG and PGCG, while MNGCs in other types of the giant cell containing lesions, such as cherubism and aneurysmal bone cyst, have rarely been investigated, although these lesions share considerable similarities in histomorphology.

Meanwhile, the findings of the present study showed that a group of mononuclear cells of stroma of CGCG and PGCG expressed osteoclastic TRAP marker with no statistical significant differences which was in accordance with Torabinia N et al [1] and Adkins et al[12] who suggested that the mononuclear cells considered as a progenitor of giant cells which were formed by fusion and adhesion of stromal mononuclear cells, but the underlying mechanism remains unknown.

On the contrary to the findings of the present study, N Mohtasham et al[13], Kumar et al[14] ,HJ Sherlin et al[15] and Hallikeri K et al[16] supposed a macrophage origin for MNGCs of CGCG and PGCG lesions .

In the present study, the immunohistochemical expression of CD163 in CGCG and PGCG appeared in most of mononuclear macrophages while negative in multinucleated giant cells which was in accordance to Kahn and co-workers[17] who stated that expression of CD163 is restricted to cells of macrophages lineage predominant in perivascular location due to metabolism of Hb is a main function of tissue macrophages because of their ability to engulf senescent erythrocytes (extravasated hemolysis) or take up Hb released from ruptured erythrocytes (intravascular hemolysis). Giant cells closely located to these extravasation areas[18].

The findings of the current study showed a statistical significant differences in macrophages CD163 expression between CGCG (13.37±SD3.43) and PGCG (9.76±SD2.33) which was in accordance with Kumar et al [14] who stated that the number of macrophages were significantly higher in CGCG than PGCG.

In another study carried out by Leek et al., on breast carcinomas showed increased number of macrophages as a predictor of poor prognosis indicating that it plays a role in belligerent behaviour of tumour[19]. Similarly Lu CF et al., correlated poor prognosis with a high frequency of macrophages in oral squamous cell carcinoma[20]. Similar to these findings, the present study revealed that macrophages were higher in CGCG than PGCG which suggest that it may

be considered as a predictor of poor prognosis signifying its aggressive behaviour.

On the contrary to the findings of the present study Pedreira and co-workers[21]revealed that MNGCs expressed CD163 (2.7 ±SD 0.7) in oral paracoccidioidomycosis granulomas which explained by CD163 positive macrophages represent the main inflammatory cells in the immune response against fungus Paracoccidioides brasiliensis (Pb).

In the present study, the immunohistochemical expression of PCNA appeared in a fraction of the spindle shaped mononuclear cells and MNGCs with no statistical significant differences were reported between percentage of positive PCNA cells in central and peripheral giant cell granuloma which was in accordance with Bo Liu and co-workers[11], Itonaga et al[8] and Houpis et al[22] who suggested that these cells were the proliferating tumor cells responsible for the biologic activity of the lesions.

On the contrary to the findings of the present study, Souza *et al*.[23] concluded in their study that PCNA positive cells were more in PGCG. Thus, according to Souza *et al*. although CGCG is more aggressive, however, PGCG is more proliferative than CGCG.

The findings of the present work showed that MNGCs were strongly positive to anti-TRAP (osteoclastic marker) and negative to anti-CD163 (macrophage marker), this proved osteoclastic origin of MNGCs in central and peripheral giant cell granulomas. Meanwhile there was no statistical significant differences of the proliferative activity of central and peripheral giant cell granuloma regarding PCNA expression.

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