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EVALUATION OF DEVELOPMENT AND GROWTH OF PERIPHERAL GIANT CELL GRANULOMA USING OSTEOCALCIN, CD68, CD34, AND KI-67 MARKERS

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ABSTRACT

Peripheral giant cell granuloma of jaws is considered as a reactive lesion. This lesion is characterized by the presence of multiple multinucleated giant cells (MNGCs) in addition to mononuclear cells. The origin of the multinucleated giant cells is controversial.

Objective: Assessment of the expression of CD34, CD68 and osteocalcin in peripheral giant cell granulomas to clarify the origin of MNGCs and to determine Ki-67 positive cells which are responsible for the growth of the lesion.

Materials and Methods: In this study, nineteen cases of peripheral giant cell granuloma presented in Oral and Maxillofacial Surgery Department and were treated by surgical excision. Five sections were prepared from a paraffin-embedded specimen of each case and stained with Haematoxylin and Eosin (H&E), CD68, CD34, Ki-67, and osteocalcin.

Results: Ki-67, showed positive expression in mononuclear cells and few multinucleated giant cells.CD34 showed negative expression in mononuclear cells and multinucleated cells, while it showed positive results in endothelial cells of the blood vessels. CD 68 showed positive expression in multinucleated cells and few stromal cells. Osteocalcin (OC); bone formation marker showed positive expression in MNGCs and bone trabeculae.

Conclusions: These results suggest that multinucleated giant cells are osteoclastic in nature and may derive from monocyte/macrophage lineage and not the endothelial cells. Additionally, we underlined the importance of mononuclear cells in the growth of these lesions while multinucleated cells showed no role in their growth and their presence is considered reactive.

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INTRODUCTION

Peripheral giant cell granuloma (PGCG) is considered as a non-neoplastic lesion. It represents a hyperplastic reaction to inflammation or injury. This reactive lesion develops only within the oral cavity[1]. The usual location of PGCG is the gingival tissue and the crest of the edentulous ridge. This lesion has never been found on oral mucosa which is not attached to the bone[2].

The exact etiology of PGCG is unknown. Though, it is believed to originate from the periosteum of the alveolar ridge or the periodontal ligament as a result of local irritation or trauma[3-5].

Clinically PGCG arises as an asymptomatic red soft tissue mass measuring about 1–1.5 cm in diameter which may cause superficial resorption of the underlying bone. Its surface is usually ulcerated. This lesion is found exclusively on the gingiva or the alveolar ridge with slight female predilection. The lower jaw is affected more than the upper [6, 7]. Regrowth of the lesion could occur on repeated trauma[8].

The PGCG has close histological features to the central giant cell granuloma, so, some pathologists consider it a soft tissue counterpart of the central giant cell granuloma[6].

Microscopically, PGCG is characterized by a non-encapsulated mass which is highly cellular and contains abundant multinucleated giant cells and inflammatory cells[7]. The mass is highly vascularized and consists mainly of small-sized, thin-walled vessels[9].

Other microscopic features are also present as the presence of interstitial hemorrhage, hemosiderin deposits, and osteoid or mature bone. Acute inflammatory cells are usually present below the ulcerated areas. Diagnosis of peripheral giant cell granuloma could be easily done using H&E stained sections[3, 7].

Treatment of PGCG consists of surgical excision with the elimination of local irritating factors. The base of the lesion should be extensively cleared to avoid relapse. Recurrence is rare[4, 7, 8].

To provide appropriate treatment, the pathogenesis of the lesions is very important [10]. However, the origin of giant cell lesions of oral cavity has been subjected to controversy for several years. Some investigators mentioned that MNGCs have osteoclast or phagocyte nature, but other studies suggested an endothelial cell origin[11, 12].

The cells which are responsible for the development and progression of these lesions; whether is it the multinucleated giant cells or the background mononuclear cells is still not clear [4].

Therefore, many studies have been shifted to mononuclear cell origin and suggested that the mononuclear cells are responsible for the biological activity of PGCG and are the proliferative components of these lesions [13].

In the current study, we aimed to assess the expression of CD34, CD68, Ki-67, and osteocalcin in PGCG in order to gain a better understanding of the origin and formation of this lesion.

CD 34 (cluster of differentiation of 34) is an endothelial cell marker [14]. Both normal and neoplastic endothelial cells of the blood vessels express CD 34 [15, 16].

CD68 (cluster of differentiation 68) is a lysosome-associated membrane protein. Cells of monocyte/macrophage lineage are the only cells that express it. Expression of CD68 confirms that the cells are of histiocytic origin [17].

Ki-67 is a protein that has a strict association with cell proliferation. This protein is found in all active phases of the cell cycle (G1, S, G2, and mitosis). It is absent in the resting cells (G0). This fact makes it a superior marker for detecting the growth of the cells [4, 18, 19].

The stromal cells can stimulate immigration of blood monocytes into the tumor tissue and promote their fusion and differentiation into osteoclasts. This action is mediated through the secretion of different cytokines and differentiation factors as monocyte chemoattractant protein-1, osteoclast differentiation factor, and Macrophage-colony stimulating factor [6, 20]. In addition, these stromal cells can differentiate along fibroblast or osteoblast lines [21].

Osteocalcin (OC) is the most abundant osteoblast-specific noncollagenous protein. OC is synthesized by mature osteoblasts [22]. Osteocalcin may assist the recruitment and differentiation of osteoclasts [3].

MATERIALS AND METHODS

Patients' characteristics:

Nineteen peripheral giant cell granuloma specimens were obtained by surgical excision in the Department of Oral and Maxillofacial Surgery, Nahda University in benisuef, Egypt during the period from May 2015 to February 2017. The patient's details regarding age, gender, size and location of lesions were recorded [Table 1].

Intraoral examination revealed bad oral hygiene with a considerable amount of plaque on the surface of related teeth.

The lesions were presented as painless pedunculated or sessile masses. Some of them showed papillomatous surface (figure 1, A).

The consistency was soft to firm. Most of the cases showed ulcerated surface. Oral function interference and bleeding were prominent in the cases that showed progressive growth. The color of the lesions ranged from pink to red.

The periapical X-rays showed no signs of bone involvement in eleven cases while in the rest of the cases a slight reduction in the interdental bone level was shown. Patients received two scaling sessions and provided with proper oral hygiene instructions.

Treatment consisted of surgical excision with an extensive clearing of the base of the lesion under field block anesthesia (figure 1, B). Six months follow up after excision denoted no signs of relapse.

Histological examination of hematoxylin and eosin stained sections confirmed the diagnosis of peripheral giant cell granuloma.

Normal mucosa samples from another 10 patients free of any systemic diseases were used as controls. Samples were taken from excised operculums (after resolution of the inflammation).

TABLE (1) Distribution of peripheral giant cell granuloma cases according to age, sex, site, and size of the lesion

Case	Age	Sex	Related teeth (Lower jaw)	Size (cm)
1	49	F	Premolars	1.5 x 1
2	57	F	Incisors	1 x 1.2
3	65	F	Incisors	0.8 x 1
4	50	M	Premolars	2 x 1.2
5	40	M	Incisors and premolars	2 x 1.5
6	45	F	Incisors	1.5 x 1.5
7	29	F	Incisors	1.2 x 1
8	39	M	Premolars	0.6 x 1.2
9	67	M	Premolars	1.8 x 1
10	19	F	Premolars	1 x 1.5
11	47	F	Incisors and canine	1.2 x 1.4
12	51	F	Premolars	1.4 x 0.5
13	39	M	Premolars	0.5 x 1
14	20	F	Incisors and canine	1.2 x 1.5
15	59	F	Premolars	1.8 x 1
16	37	M	Incisors	1 x 1.5
17	39	M	Premolars	0.5 x 1
18	33	F	Incisors and premolars	1.5 x 2
19	45	F	Premolars	0.9 x 1.5

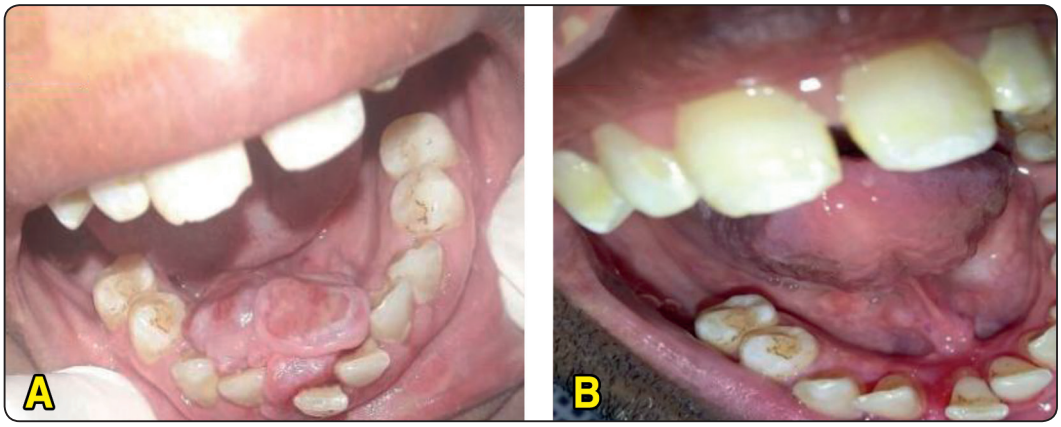

Fig. 1 (A): A Clinical view of an exophytic lesion located between lower central incisor and first premolar. (B): A postsurgical clinical view.

Tissue processing and section preparation:

All samples were fixed in formalin and embedded in paraffin.

Five sections were prepared from each formalin-fixed, paraffin-embedded tissue block at 4 µm thickness and stained with the following:

a- First section stained with hematoxylin and eosin (H& E) to verify the clinical diagnosis of peripheral giant cell granuloma.

b- Second section stained with CD68.

c- Third section stained with CD34.

d- Forth section stained with Ki-67.

e- Fifth section stained with osteocalcin.

Immunohistochemical (IHC) staining procedure

The sections were deparaffinized with xylene, and rehydrated in graded ethanol for IHC staining by CD68, CD34, Ki-67 and osteocalcin antibodies. Heat mediated antigen retrieval was done using citrate buffer PH (6.0), then the sections were immersed in hydrogen peroxide (H2O2) to block the endogenous peroxidase activity, washed in phosphate-buffered saline (PBS), and then protein blocking reagent was added and incubated for 20 minutes at 37°C within humid chamber to reduce the non-specific staining. The primary antibodies used in the present study were as follows:

- Concentrated monoclonal mouse antibody for CD 68 (code No. M 0814, at dilution 1:100, Dako, Denmark).
- Ready-to-use monoclonal mouse antibody for CD34 (code No. N 1632, Dako, Denmark).
- Concentrated monoclonal mouse antibody for Ki-67 (Code No. M 7187, at dilution 1:50, Dako, Denmark).
- Concentrated polyclonal rabbit antibody for osteocalcin (Code No. PA5-11849 at dilution 1:50, Thermo Fisher Scientific USA).

Sections were incubated with the primary antibody overnight. The bounded antibodies were detected by the streptavidin-biotin complex method, after an immunoreaction, the sections were counterstained with Mayer's Hematoxylin.

Immunohistochemical Evaluation:

Presence of brown colored reaction in the nucleus or the cytoplasm was considered a positive reaction.

In each slide, 5 microscopic fields showing the highest immunopositivity were selected and photomicrographed.

Immunoreactivity, for CD 68, CD 34, ki-67 and osteocalcin was evaluated by estimating the area percentage of positive immunostained cells in relation to the area examined in each microscopic fields using computerized image analyzer (Leice Qwin - Germany).

The image analyzer consisted of a colored video camera, colored monitor, and hard disk of *hp* personal computer connected to the microscope, and controlled by Leica Qwin 500 software. The image analyzer was calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The area and area percentage reaction were measured using a magnification x200. Mean values were then obtained for each specimen.

Statistical analysis

Values were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data) therefore one way analysis of variance (ANOVA) test was used to compare groups regarding CD68 and Ki-67 immunoexpression. This was followed by Tukey's post hoc test when the difference was found to be significant. Unpaired t-test was used to compare both groups regarding CD34 expression.

The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 18 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

RESULTS

1-Heamatoxylin and eosin stain findings

Histologically PGCG revealed a hyperplastic stratified squamous epithelium. The underlying connective tissue displayed proliferation of multinucleated giant cells. The giant cells are located within a background of collagen fibers, spindle, and ovoid mesenchymal cells. Areas of hemorrhage and acute and chronic inflammatory cells are found. A zone of dense fibrous connective separates the giant cell proliferation from the mucosal surface. Areas of reactive bone formation are seen.

For normal gingival tissue (control), the epithelium displayed a keratinized stratified squamous epithelium that covers a core of connective tissue. The epithelium revealed a normal arrangement of its layers (6–8 layers). The basement membrane was flat with no extended rete ridges. The underlying connective tissue revealed normal arrangement of its fibers, blood vessels, and few chronic inflammatory cells (Figure 2, A, B, C).

Immunohistochemical findings

CD68 immune-reactivity:

All lesions of peripheral giant cell granuloma showed cytoplasmic immunopositivity for CD68 in some mononuclear cells and most of the multinucleated giant cells. For normal gingival tissues, all specimens showed staining of few stromal cells (figure 2, D, E).

Ki67 immune-reactivity:

Nuclear Ki-67 expression was detected in all PGCG cases. The expression was mainly restricted to the mononuclear stromal cells and the basal cells of the epithelium. The Ki-67 expression was detected in all cases of normal oral epithelium (NOE) and was restricted to the basal cell layer of the epithelium (Figure 2, F, G).

CD34 immune-reactivity:

CD34 immune-reactivity was restricted to the endothelial cells of blood vessels in both PGCG and NOE. Endothelial cells showed cytoplasmic staining. Mono and multinucleated cells of PGCG revealed negative CD 34 immunostaining (Figure 2, H).

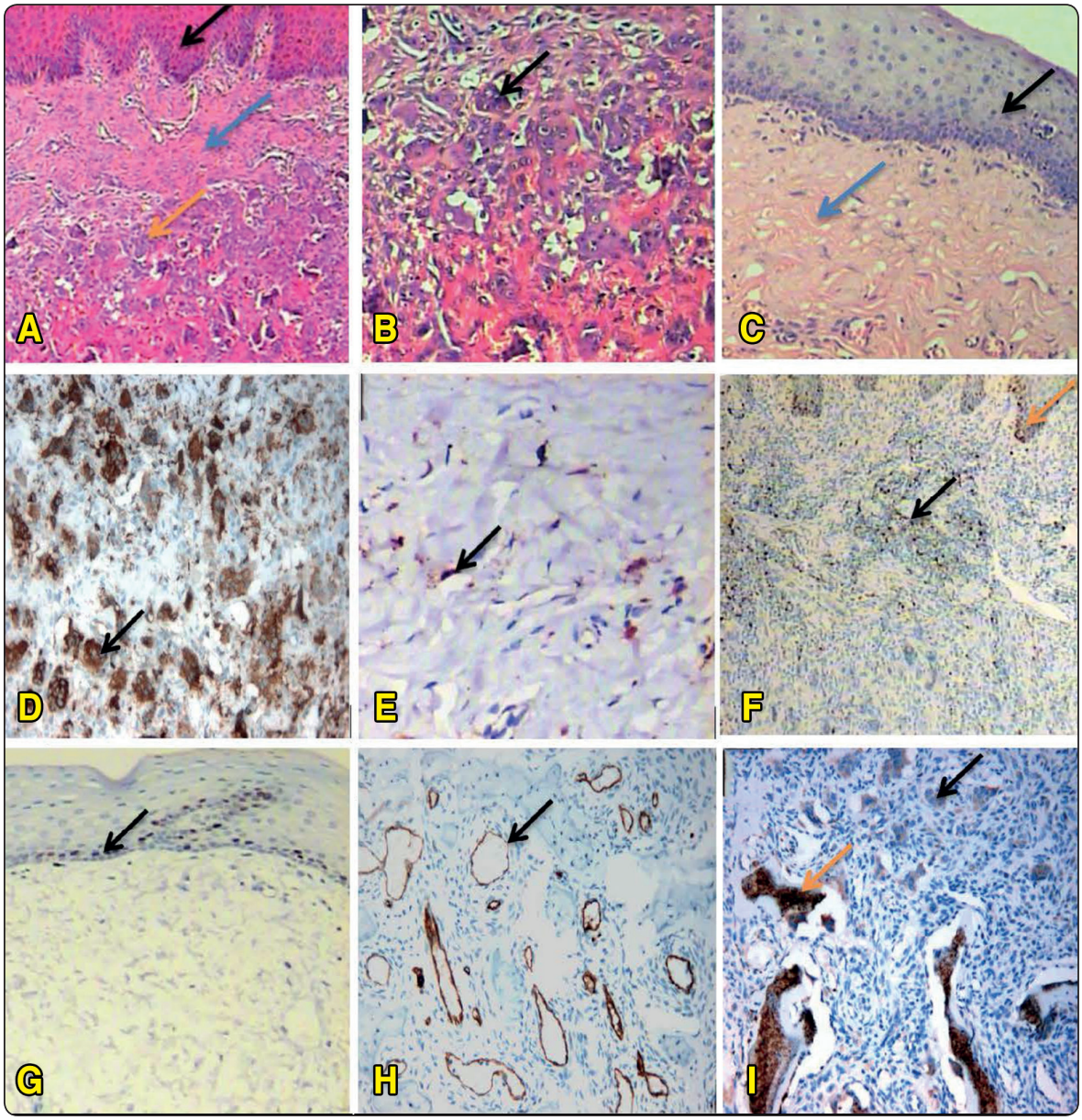

Fig. (2) (A): A photomicrograph of PGCG showing increased epithelial thickness (black arrow). The underlying connective tissue displays multinucleated giant cell proliferation (orange arrow). A zone of dense fibrous connective tissue separates giant cell proliferation from mucosal surface (blue arrow) (H&E x50). (B): A photomicrograph of PGCG showing multinucleated giant cell proliferation (black arrow) within a background of mononuclear stromal cells and collagen fibers (H&E x100). (C): A photomicrograph of normal gingival tissue (control) showing a keratinized stratified squamous epithelium covering a core of connective tissue (blue arrow). The epithelium reveals normal arrangement of its layers (black arrow) (H&E x100). (D): A photomicrograph of PGCG showing immunopositivity for CD68 in some mononuclear cells and most of the multinucleated giant cells (black arrow) (H&E x100). (E): A photomicrograph of normal gingival tissue (control) showing staining of few stromal cells with CD68 (black arrow) (H&E x200). (F): A photomicrograph of PGCG showing immunopositivity for Ki-67 in mononuclear stromal cells (black arrow) and the basal cells of the epithelium (orange arrow) (H&E x50). (G): A photomicrograph of normal oral epithelium showing Ki-67 expression in the basal cell layer of the epithelium (black arrow) (H&E x100).(H): A photomicrograph of PGCG showing immunopositivity for CD34 in the endothelial cells of the blood vessels (black arrow) (H&E x200). (I): A photomicrograph of PGCG showing immunopositivity for osteocalcin in MNGC (black arrow). It is also expressed in the bone trabeculae (orange arrow) (H&E x200).

Osteocalcin immune-reactivity:

All PGCG cases showed cytoplasmic staining in the majority of MNGC and few mononuclear cells. It was also expressed in the bone trabeculae and the osteoblast rimming the trabeculae. For normal controls, all specimens revealed negative OC immunostaining (Figure 2, I).

Results of the immunohistochemical staining:

I- CD68 immunoexpression

The greatest mean area percentage of immunoexpression was recorded in multinucleated cells (13.3±1.9). ANOVA test revealed that the difference was statistically significant (p<0.0001). Tukey's post hoc test revealed a significant difference between every 2 groups (Table 2, Figure 3)

TABLE (2) CD68 immunoexpression in multinucleated cells, mononuclear cells and normal gingival tissue

	Multinucleated cells	Mononuclear cells	Normal gingival tissue
Mean	13.297[a]	1.343[b]	0.134[c]
SD	1.921	0.504	0.009
F	246.8		
P value	<0.0001*		

Significance level p<0.05, * significant

Tukey's post hoc test: means with different superscript letters are significantly different

II- Ki-67 immunoexpression

The greatest mean area percentage of immunoexpression was recorded in mononuclear cells (25.85±5.59). ANOVA test revealed that the difference was statistically significant (p<0.0001). Tukey's post hoc test revealed a significant difference between every 2 groups (Table 3, Figure 3)

Table (3) Ki-67 immunoexpression in multinucleated cells, mononuclear cells and normal gingival tissue

	Multinucleated cells	Mononuclear cells	Normal gingival tissue
Mean	3.318[b]	25.847[a]	0.176[c]
SD	1.461	5.59	0.039
F	105.002		
P value	<0.0001*		

Significance level p<0.05, * significant

Tukey's post hoc test: means with different superscript letters are significantly different

III- CD34 immunoexpression

The greatest mean area percentage of immunoexpression was recorded in PGCG (7.019±1.6). The unpaired t-test revealed that the difference was statistically significant (p<0.0001). (Table 4, Fig, 4)

Table (4) CD34 immunoexpression in normal gingival tissue and PGCG

	Normal gingival tissue	PGCG
Mean	0.811	7.019
SD	0.303	1.625
T	9.3593	
P value	<0.0001*	

Significance level p<0.05, * significant

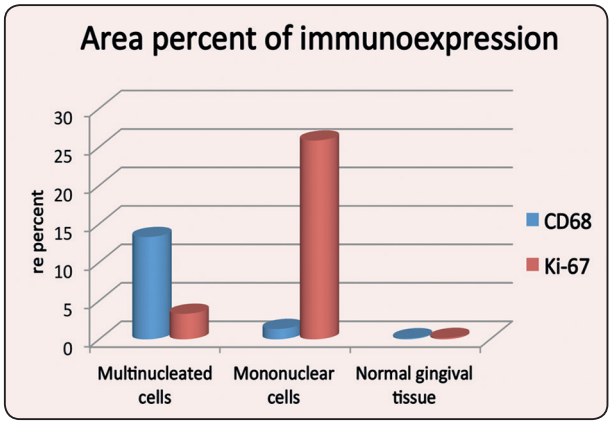

Fig. (3) Column chart showing mean area percent of CD68 and Ki-67 immunoexpression.

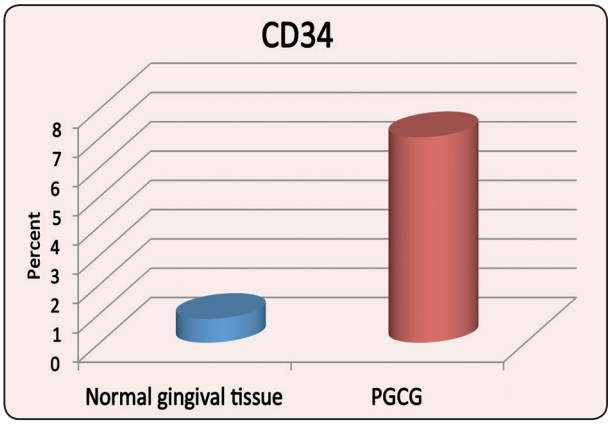

Fig. (4) Column chart showing mean area percent of CD34 immunoexpression.

DISCUSSION

The giant cell granulomas of the oral cavity can arise centrally in the bone or peripherally in the periodontal ligament and mucoperiosteum[23].

Peripheral giant cell granuloma (PGCG) is a hyperplastic lesion of the connective tissues that arises as a result of local irritation or trauma[7, 24]. Diagnosis of PGCG can be easily reached through microscopic examination of H&E stained sections [4].

Surgical excision is considered a successful method of treatment of these lesions. Recurrence is rare, especially if the local irritant factor is eliminated [4, 25, 26].

Clinically, PGCG appears as a soft exophytic red or purple nodule with a smooth or papillomatous surface. Areas of ulceration may be found [26, 27, 28, 29, 30, 31].

It can develop at any age though it arises most commonly in the third and fourth decades of life with slight female predilection. PGCG may arise in the anterior or the posterior regions of the gingiva or the alveolar ridge. The lesion has a tendency to affect the mandible more than the maxilla [23, 26]. Some lesions may become large; they may attain 2 cm in size [32].

The etiology of PGCG is not accurately defined. Local irritation or trauma may play an important role in its development such as complicated dental extractions, dental restorations in poor conditions, ill-fitting denture, plaque, and calculus [33, 34]. Formerly, the lesion was termed peripheral giant cell reparative granuloma. Though, the reparative effect of this lesion has not been proven yet as the osteoclastic activity seems doubtful [26, 28, 31, 35, 36].

The local irritating factors can induce marked proliferation of the fibroblasts of connective tissue with a consequent secretion of collagen. This secreted collagen acts as a scaffold and assists the infiltration of cells to the injured site. Furthermore, some sort of degradation occurs to provide space for angiogenesis [37].

The most characteristic feature of PGCG is the presence of numerous multinucleated giant cells that are distributed throughout the connective tissue stroma. These giant cells have no obvious damage to the adjacent structures [24].

The origin and role of the multinucleated giant cells is a debatable issue [7]. Though some investigators believe that they originate from the endothelial cells of the blood vessels due to an alteration of these endothelial cells [1].

Another hypothesis suggested that they are osteoclasts which are associated with the resorption of primary teeth. Though this theory has not received any attention as PGCG can develop in edentulous areas [7].

Many authors believe that the presence of giant cells is reactive and the mononuclear stromal cells are the proliferative component of the tumor and are responsible for the biological behavior of these lesions [4]. They suggested that giant cells are derived from monocytes via bloodstream in response to an unknown stimulus from the stroma [6]. Hence the multinucleated giant cells are formed from the fusion of monocyte/macrophage precursors [11, 21].

The osteoclastic nature of these multinucleated giant cells has been supported by their osteoclastic activity when cultured in vitro. In addition, they have membrane receptors for calcitonin which is demonstrated by immunohistochemistry [6].

The mononuclear stromal cells include a population of macrophage cells with a subset of osteoclasts precursors and proliferating spindle-shaped cells. These spindle-shaped cells can differentiate along fibroblast/osteoblast lines [11, 21].

Histologically PGCG displayed a highly cellular non-encapsulated mass. The mononuclear cells are the basic component of the PGCG. Abundant multinucleated giant cells are scattered throughout the lesion and appear to be nonfunctional as regard bone resorption and phagocytosis. Inflammation is a constant finding in this lesion. Inflammatory cells are varied in their location. The chronic inflammatory cells are scattered throughout the lesion, while the acute inflammatory cells are found in ulcer bases. Vascular proliferation especially capillaries, interstitial hemorrhage, and hemosiderin deposits are also present. Islands of metaplastic bone may be seen [38-40].

The overlying epithelium is usually hyperplastic, with ulceration in about 50% of the cases. The giant cell proliferation is separated from the epithelial surface by a clear zone of dense fibrous connective tissue [7, 23].

The X-ray picture is very important to determine the origin of the lesion; whether it is of gingival origin or it arises centrally and then spread towards the surface [8].

The clinical picture of PGCG is similar to that of pyogenic granuloma, though PGCG is more likely to produce superficial resorption of bone [40].

Traditional treatment of this lesion consists of local surgical removal down to the bone for wide clearing of its base. Removal of local irritating factors is very important. Recurrence could be related to the lack of inclusion of the periosteum or periodontal ligament in the excised specimen [23, 40]. Aggressive behavior or malignant transformation of these lesions has never been recorded [6].

To investigate the histogenic origin and formation of multinucleated giant cells in peripheral giant cell granuloma we studied the expression of CD68, CD34, and osteocalcin markers. In addition, we studied the expression of Ki-67 to clarify the proliferative components of the lesion.

CD68 is often used to investigate giant cells as it is a specific marker for monocyte-macrophage lineage. Its expression suggests macrophage origin of cellular component of lesions [3, 4].

Our results showed CD68 positive reactivity of some mononuclear cells and most of the multinucleated giant cells. Few cells of the normal gingiva were also stained. The greatest mean area percentage of immunoexpression was recorded in multinucleated cells followed by mononuclear cells. The smallest mean area percentage was recorded in the connective tissue cells of normal gingiva. There was a significant difference between every 2 groups.

This was in accordance with the study of Aragao et al., 2007 in which the expression of CD68 was

noticed in many mononuclear cells and the majority of multinucleated giant cells [17].

Also, Meng et al., 2005 described CD68 positive reaction in all multinucleated giant cells and some of the mononuclear cells [41].

Chu and Weiss 2015, and Torabinia et al., 2011 as well found that, most of the giant cells and a group of mononuclear cells of stroma expressing CD68 protein [42, 43].

These studies and ours support the hypothesis that MNGCs in giant cell granulomas arise from the fusion of the stromal macrophages. The CD68 positive stromal cells could be osteoclasts precursors [11, 21].

CD68 is located in the cytoplasmic granules of monocytes and macrophage [44, 45]. This could explain the cytoplasmic staining of CD68 in our study.

Macrophages are found in all tissues. They have many functions as endocytosis and cytotoxicity. They are also involved in inflammatory processes and angiogenesis [46]. These findings could explain the positivity of some stromal cells of the normal gingival tissue to CD68 in the present study.

The growth rate of any tissue or tumor can be determined by its proliferative activity. Ki-67 is a nuclear protein that is necessary for cellular proliferation. It is strictly associated with the proliferation of the cells [47]. This fact could explain the nuclear staining of Ki-67 in the present study.

Ki67 is an excellent marker that can be used for the estimation of the growth rate of normal and abnormal tissues. The nuclear expression of Ki-67 during a specific period of the cell cycle give it the advantage to be used as a biological marker of the mitotic activity of any tissue [48].

In the current work, the greatest mean area percentage of immunoexpression of Ki-67 was recorded in mononuclear cells as compared to multinucleated cells. The smallest mean area percentage was recorded in the epithelium of the normal gingiva. There was a significant difference between every 2 groups.

Our results are in accordance with the results of previous studies as that of Hallikeri et al., 2015 and Souza et al., 2000 in which the expression of Ki-67 was mainly restricted to mononuclear cells while few giant cells showed positive reactivity. They suggested that mononuclear cells are the proliferative components of PGCG [10, 49].

In normal stratified squamous epithelium, the proliferation is a property of stem cells of the basal cell layer [50]. Though in the present study the expression of Ki-67 is noticed in the basal cell layer of the epithelium of PGCG and normal gingival tissue.

CD34 is an endothelial cell marker and transmembranous glycoprotein and is expressed at the cell surface in the normal and neoplastic endothelial cells of blood vessels [15,51]. The immunohistochemical staining of CD34 is assessed within the endothelial cells which are positively stained. Staining is membranous and strong but is prone to background staining [52].

Our results of the expression of CD 34 was similar to the previous study of Vk et al., 2014 and revealed positive reactivity to CD 34 in PGCG and the normal gingival tissues. The expression was limited to the blood vessels while a negative reaction was noticed in mononuclear cells and MNGCs [3].

These results confirmed that the origin of MNGC is not related to endothelial cells and met the results of the study of Falaschini et al., 2007. They suggested that multinucleated giant cells do not arise from endothelial cells of the capillaries as the expression of CD34 is not evident within the multinucleate giant cells [9].

Angiogenesis is the formation of new blood vessels originating from the endothelium of existing vasculature. Angiogenesis is critical to tumor growth [53].

In the present study, the difference between CD34 immunoexpression in the normal gingival tissue and PGCG was statistically significant suggesting that increased vascularity is needed for tumor growth and related to the inflammatory process in PGCG. El-Attar and Wahba, 2016 said that the high expression of endothelial cell markers in PGCG could be due to the increased inflammatory reaction [4].

Osteocalcin (OC) protein is secreted by osteoblasts and is involved in the regulation of their functions. The high serum level of OC correlates with the increase in bone mineral density. Hence it is used as a biomarker for bone formation [54-56].

The accurate function of osteocalcin in bone metabolism has not been fully understood [57]. Previous experimental studies proved the role of osteocalcin in the recruitment of circulating monocytes and osteoclast precursors. OC has a role in their differentiation as well [58].

To our knowledge, no previous studies were done to evaluate the expression of OC in PGCG. The expression of OC in our study is observed in the majority of MNGC and few mononuclear cells. It was also expressed in the bone trabeculae and the osteoblast rimming the trabeculae. The pattern of staining is cytoplasmic.

The study of Ishida and Amano, 2004 revealed that osteocalcin can enhance the formation of osteoclasts from macrophages in the existence of macrophage colony-stimulating factor and can assist the maturation of osteoclasts [59]. These results could explain the expression of OC in MNGC in the present study. Though, our study suggests that osteocalcin may play a role in the formation of MNGCs from monocytes in PGCG and stress on the opinion that considers the MNGC seen in PGCG as osteoclasts.

The expression of OC in some mononuclear cells could be attributed to the fact that some stromal cells could differentiate into osteoblasts which secrete osteocalcin.

As bone formation may be detected in PGCG, it is not surprising that bone trabeculae and osteoblasts rimming the trabeculae were stained positive for OC. This result could be explained by the results of Zafaret et al., 2012 Toyosawa et al., 2007, and Lee et al., 2007 who said that OC is used as a biomarker for bone formation process and has a role in the regulation of osteoblast function [54-56].

CONCLUSION

The results of the present study suggest that multinucleated giant cells are osteoclastic in nature and may derive from monocyte/macrophage lineage and not the endothelial cells. Additionally, we underlined the importance of mononuclear cells in the growth of these lesions while multinucleated cells showed no role in their growth and their presence is considered reactive.

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