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RESEARCH ARTICLE

Assessment OF Ki-67 and Bcl-2 Immunohistochemical Markers in Gingival Pyogenic Granuloma: A Comparison with Peripheral Giant Cell Granuloma

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Abstract

Background and objectives: Pyogenic granuloma (PG) and peripheral giant cell granuloma (PGCG) are common focal reactive hyperplastic lesions affecting the gingiva. The aim of the present research was to study the clinical and the immunohistochemical expressions of Ki-67 and Bcl-2 for gingival pyogenic granuloma in comparison with peripheral giant cell granuloma. Materials and Methods: Formalin fixed, paraffin-embedded biopsy specimens of (48) gingival pyogenic granuloma and (39) peripheral giant cell granuloma were used in the study. Clinical and immunohistochemical analysis for Ki-67 and Bcl-2 were studied in pyogenic granuloma and peripheral giant cell granuloma. Results: The female to male ratio of pyogenic granuloma and peripheral giant cell granuloma was (1.18:1 and 1.43:1) respectively. The mean age was (32.47±19.575 and 35.03±18.22) year respectively. The pyogenic granulomas were mostly affecting the maxillary gingiva, while peripheral giant cell granulomas were mostly affecting the mandibular gingiva. Reactivity percentages for Ki-67 and Bcl-2 markers were significantly higher in peripheral giant cell granuloma compared to pyogenic granuloma (21.71±8.596% vs. 12.68±6.117 %, and 38.77±20.396% vs. 3.547±1.59%) respectively. Conclusion: PGCG showed more proliferative and anti apoptotic activity compared with PG. This can add insight to the clinical behavior and might reflect the differences in pathogenesis of these lesions.

Keywords: *Pyogenic granuloma, Peripheral giant cell granuloma, Ki-67, Bcl-2.*

Introduction

Oral PG is a common tumor-like growth, comprising 30% of all localized reactive hyperplastic lesions of the gingiva [1].Manifested as exophytic, sessile, erythematous, and painful nodule that bleeds readily [2]. Commonly seen in response to chronic irritation [3] and the streptococci and staphylococci infection may play a role [4]. Other study demonstrated that sex hormones of female promote the expression of angiogenic factor [5], or it may be result from viral oncogenes which lead to sudden and uncoordinated growth of the connective tissue papillae [6].

It should be noted that the tumor growth rate depends not only on the proliferation of tumor cells but also on the rate of cells death [7].

PGCG is a tumor-like, non-neoplastic, reactive lesion occurring only on gingiva or alveolar crest, manifested clinically as a painless, soft, nodular mass, reddish-bluish in colour, and sessile or pedunculated [8]. The etiology is not clear, it is perhaps a reactive lesion caused by local irritation or trauma, and considered as a more intense response of bone periosteum to the irritation factors [9].

In addition, marked female predilection of PGCG suggests a possible hormonal effect [10]. The overlying epithelium for PG, if present, is usually thin and atrophic. The connective tissue shows the occurrence of large numbers of vascular spaces with infiltration of polymorph nuclear leukocytes, lymphocytes, and plasma cells [11, 13].

PGCG is covered by hyperplastic parakeratinized stratified squamous epithelium. The connective tissue shows the presence of abundant proliferating fibroblasts with a numerous multinucleated giant cells and vascularity [14]. It is well known that Ki-67, a marker of cell proliferation activity and over expression is frequently seen in a variety of malignant tissues [15].

Bcl-2 is specifically considered as an important anti-apoptotic protein. A dysfunctional apoptotic system can lead to either excessive removal or prolonged survival of cells and thus dysregulated apoptosis is implicated in pathogenesis of a variety of diseases including oral pathologies [16].The pathogenesis of PG and PGCG remains to be not fully understood.

In such conditions, immunohistochemistry may provide some practical help and shed a light on the underlying pathogenesis of these lesions. The aim of the present research was to study the clinical features and immunohistochemical expressions of Ki-67 and Bcl-2 for gingival pyogenic granuloma in comparison with peripheral giant cell granuloma. In such conditions, the immunohistochemistry may provide some practical help and shed a light on the underlying pathogenesis of these lesions.

Materials and Methods

The materials used in this study were consisting of (48) formalin fixed, paraffin-embedded biopsy specimens of gingival PG and (39) formalin fixed, paraffin-embedded biopsy specimens of PGCG. They were retrieved from the archives of Rizgary Teaching Hospital, Erbil (Ministry of Health, Kurdistan region of Iraq) in the period between January/2013 and August /2016. Ten control gingival samples were obtained from clinically healthy patients undergoing orthodontic extractions in Erbil Specialized Dental Center.

The written consent to carry out biopsies which were required for the study was obtained from healthy volunteers, after the necessary instructions. Demographic data and clinical aspects which included: the patient number, laboratory serial number, patient name, age, gender, and anatomical site etc… were registered in a special form, and only patients with biopsy proven gingival PG or PGCG were included.

The pregnant and edentulous patients (epulis fissuratum) and patients with known systemic disorders such as diabetes and bleeding disorders were excluded from the study. Sample collection was authorized by Rizgary Teaching Hospital, Ministry of Health. The research project was approved by the Research Ethics Committee at College of Dentistry, Hawler Medical University under protocol. Monoclonal Mouse Anti-Human Ki-67 Antigen (Clone MIB-1, Code No .M 7240 staining system, dilution 1:300), Monoclonal Mouse Anti-Human Bcl-2 Oncoprotien (Clone 124 Code No1587, dilution 1:50), for use with Dako EnVisionTM , EnVision TM double staining and LASAB TM 2 systems, Dakocytomation, were used.

The staining procedure and instructions included with each detection system were followed. The negative control and positive control tissue specimens were run with each batch of stain. Paraffin embedded oral squamous cell carcinoma biopsies cases for Ki-67and tonsils for Bcl-2, were served as positive controls. Immunoreactivity, regardless of its intensity, was assessed in the connective tissue only. Ki-67 is expressed in the nucleus, but Bcl-2 is expressed in the cytoplasm. To ensure the objectivity of the analysis, the evaluation was carried out by two independent observers, who were unaware of the patient's age, gender and average size of the lesion. Five fields were randomly chosen for each patient.

Approximately 1000 cells from cell population were counted by two observers at a magnification of 400x using research microscope with digital camera and microcomputer image device and the percentages of Ki-67 and Bcl-2 positive cells were calculated, and the level of expression was evaluated according to the scoring system of [2, 17].

The application of this system gives a score ranging from 0 to 3 for the percentage of positively stained cells [(absent :< 1%), (mild: 1-10%), (moderate: >10-50%), and (strong: > 50%)]. After necessary data had been collected, the results were given as mean ± standard deviation. The potential difference among groups for histopathological data was evaluated using ANOVA test. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., version 19).

Statistical significance of differences between the groups was tested with the Mann Whitney-U test. P value less than or equal to 0.05 was considered statistically significant.

Result

Clinical Features

Most cases of **PG** were common among the females (26) and comprising (54.17%) and (22) cases were seen associated with the males and comprising (45.83%) with a female to male ratio equal to 1.18:1. The age of the individuals ranged between (4-76) years with a mean age of (32.47±19.575) years for the total sample, (33.90±23.186) years for the males and (31.17±16.016) years for the females. Age group (21-30) years showed the highest number of cases (11 patients), with (24.4%) prevalence, followed by the age group (11-20) years (10 patients) with (22.2%) prevalence (Table-1).

The maxilla was mostly affected (18cases, 66.7%), followed by the mandible (9 cases, 33.3%), unfortunately the location of 21 cases were unknown. The maximum diameter varied from (0.2- 7cm) with a mean of 2.05cm, 19cases (39.6%) showed a maximum diameter less than1.5cm, 25 cases (52.1%) showed a maximum diameter 1.5-3cm, and only four cases (8.3%) showed a maximum diameter more than three cm. Most cases of PGCG were common among the females (23) and comprising (58.97%), and (16) cases were seen associated with the males and comprising (41.03%), with a female to male ratio equal to 1.43:1. The age of the individuals ranged between (10-70) years with a mean age of (35.03±18.22) years for the total sample, (35.93±24.253) years for the males, and (34.4±13.156) years for the females. Age groups (21-30) years showed the highest number of cases of PGCGs (nine patients), with (26.5%) prevalence, followed by the age groups (≤10, and 31-40) years (six patients) with (17.6%) prevalence.

The age and sex distribution of 34 cases of peripheral giant cell granulomas are seen in (Table-1). The result also showed that the mandible was mostly affected (21cases, 72.4%), followed by the maxilla (eight cases, 27.6%), unfortunately the location of 10 cases were unknown. The maximum diameter varied from (0.5- 5cm) with a mean of 1.99 cm, 14cases (39.9%) showed a maximum diameter less than1.5cm, 19 cases (48.7%) showed a maximum diameter of 1.5-3cm, and only six cases (15.4%) showed a maximum diameter more than 3cm.

Table 1: The distribution of gingival pyogenic granuloma and peripheral giant cell granuloma according to the age

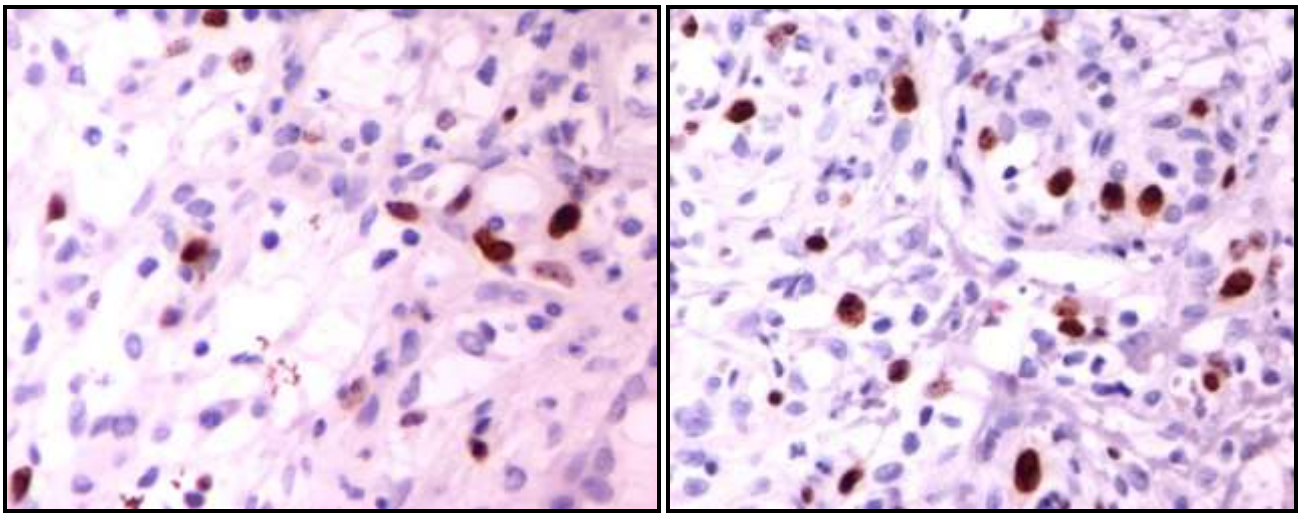
Age group	Pyogenic granuloma		Peripheral giant cell granuloma	
(year)	**Number**	Percentage %	**Number**	Percentage %
≤10	4	8.9	6	17.6
11-20	10	22.2	2	5.9
21-30	11	24.4	9	26.5
31-40	7	15.6	6	17.6
41-50	3	6.7	5	14.7
51-60	5	11.1	2	5.9
>61	5	11.1	4	11.8
Total	45	100	34	100
	Unknown age (3cases)		**Unknown age (5 cases)**	

Immunohistochemical Results

Ki-67 immunostaining Distribution

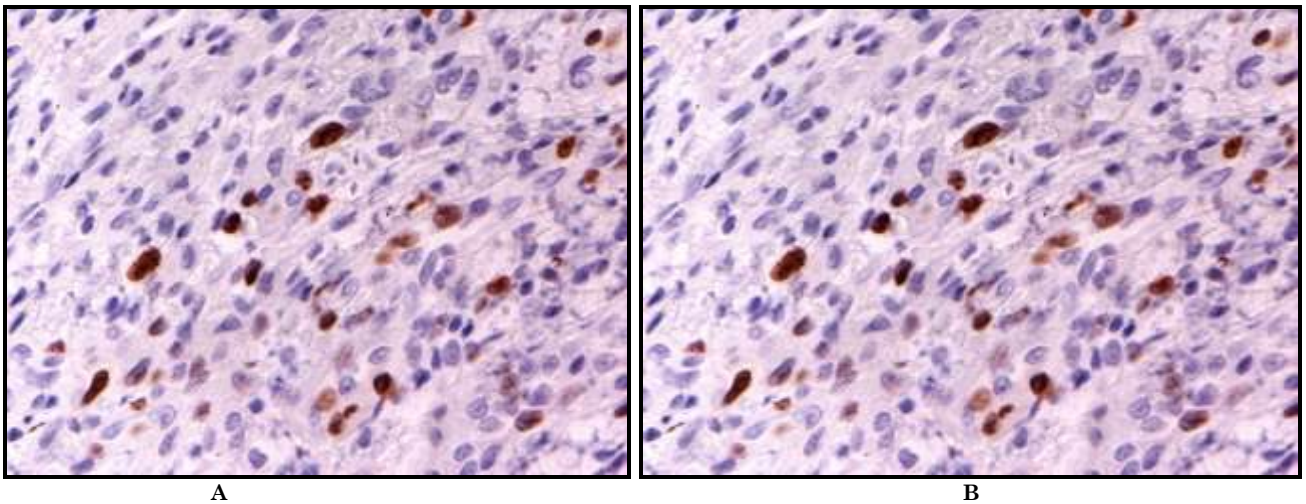
Positive expression of Ki-67 in connective tissue gave clear cut nuclear staining of brown color. All samples of normal gingiva were negative in all sections studied. All cases of **PGs** showed positive reactivity to Ki-67(Figure-1), 19 cases were mild positive, and 29 cases were moderate positive. The staining distribution was concentrated in the perivascular cells followed by endothelial cells. The highest percentages of Ki-67 moderate positive cases were seen associated with males, the age group (41-50) years, the mandible, and the maximum diameter of (>3) cm as seen in (Table-2). Regarding **PGCG**, the results showed that one case (2.6%) was appeared with Ki-67 negative expression, and 38 cases (97.4%) showed positive Ki-67 immune expression (Figure-2), six cases (17.95%) were weak positive, and 32 cases (82.05%) were moderate positive. The staining distribution was seen mainly in

mononuclear cells. Ki-67 expression was negative in multinucleated giant cells and endothelial cells. The highest percentages of Ki-67 moderate positive cases were seen associated with males (87.5%), the age groups (11-20), (41-50) and (51-60), the mandible, and the maximum diameter of (1.5-3) cm. The Ki-67 labeling index for PG and PGCG was ranging between (5.1% to 29.7%) and (5.8% to 40.5%) respectively. Statistical analysis of the mean labeling index of Ki-67 for PG showed a highly significant relation with the gender ($p<0.01$), but a non significant relations ($p>0.05$) present with the site and the diameter of the lesions. Statistical analysis of the mean labeling index of Ki-67 for PGCG showed no significant relation ($p>0.05$) with the gender, site, and diameter of the lesion (Table-3). The mean labeling indexes of Ki-67 in PG and PGCG was 12.68±6.117 % and 21.71±8.596% respectively. Statistical analysis showed a highly significant difference present between them (P=0.0001)



A B

Figure-1: Photomicrograph of the pyogenic granuloma revealed weak (A) and moderate (B) positive immunohistochemical expression of Ki-67 in the connective tissue cell nucleus (Immunohistochemistry x400)



A B

Figure-2: Photomicrograph of the peripheral giant cell granuloma revealed weak (A) and moderate (B) positive immunohistochemical expression of Ki-67 in the connective tissue cell nucleus (Immunohistochemistry x400)

Table -2: Percent distribution of the Ki-67 expression in pyogenic granuloma and peripheral giant cell granuloma in relation to different clinical features

Clinical Parameter			Total negative No. (%)	Total positive No. (%)	Mild positive No.(%)	Moderate Positive No.(%)	Total No. (%)
Gender	**Male**	**PG**	0	22(100)	4(18.18)	18(81.82)	22(100)
		PGCG	0	16(100)	2(12.5)	14(87.5)	16(100)
	Female	**PG**	0	26(100)	15(57.69)	11(42.31)	26(100)
		PGCG	1(4.35)	22(5.65)	4(18.18)	18(81.82)	23(100)
Age group (years)	**≤ 10**	**PG**	0	4(100)	2(50)	2(50)	4(100)
		PGCG	1(16.67)	5(83.33)	1(20)	4(80)	6(100)
	11-20	**PG**	0	10(100)	2(20)	8(80)	10(100)

		PGCG	0	2(100)	0	2(100)	2(100)
	21-30	**PG**	0	11(100)	6(54.55)	5(45.45)	11(100)
		PGCG	0	9(100)	2(22.22)	7(77.8)	9(100)
	31-40	**PG**	0	7(100)	6(85.71)	1(14.29)	7(100)
		PGCG	0	6(100)	1(16.67)	5(83.33)	6(100)
	41-50	**PG**	0	3(100)	0	3(100)	3(100)
		PGCG	0	5(100)	0	5(100)	5(100)
	51-60	**PG**	0	5(100)	2(40)	3(60)	5(100)
		PGCG	0	2(100)	0	2(100)	2(100)
	≥61	**PG**	0	5(100)	0	5(100)	5(100)
		PGCG	0	4(100)	1(25)	3(75)	4(100)
Site	**Maxilla**	**PG**	0	18(100)	4(22.22)	14(77.78)	18(100)
		PGCG	1(12.5)	7(87.5)	2(28.57)	5(71.43)	8(100)
	Mandible	**PG**	0	9(100)	1(11.11)	8(88.89)	9(100)
		PGCG	0	21(100)	4(19.05)	17(80.95)	21(100)
Maximum diameter (cm)	**≤ 1.5**	**PG**	0	20(100)	6(30)	14(70)	20(100)
		PGCG	1(7.14)	13(92.86)	4(30.77)	9(69.23)	14(100)
	1.5-3	**PG**	0	24(100)	12(50)	12(50)	24(100)
		PGCG	0	19(100)	1(5.26)	18(94.74)	19(100)
	>3	**PG**	0	4(100)	1(25)	3(75)	4(100)
		PGCG	0	6(100)	1(16.67)	5(83.33)	6(100)

Table-3: Distribution of the mean labeling indexes and standard deviations of Ki-67 positive nuclei in relation to the gender, location, and the maximum diameter of pyogenic granuloma and peripheral giant cell granuloma (Ki-67 immune negative cases were excluded from the analysis)

Clinical parameter		**PG/ Ki-67 X±SD**	**P-value**	**PGCG/ Ki-67 X±SD**	**P-value**
Gender	**Male**	15.16±6.492	0.008	22.56±8.054	0.41
	Female	10.57±4.983	P<0.01 HS	21.09±9.104	p>0.05 NS
Site	**Maxilla**	12.94±7.351	0.45	23.04±12.111	0.60 p>0.05
	Mandible	14.21±5.263	p>0.05 NS	20.42±7.889	NS
Maximum diameter(cm)	**<1.5**	14.05±6.887	0.15	19.28±9.013	0.10
	1.5-3	11.12±4.895	p>0.05 NS	21.326±6.930	p>0.05 NS
	>3	16±7.937		28.21±10.594	

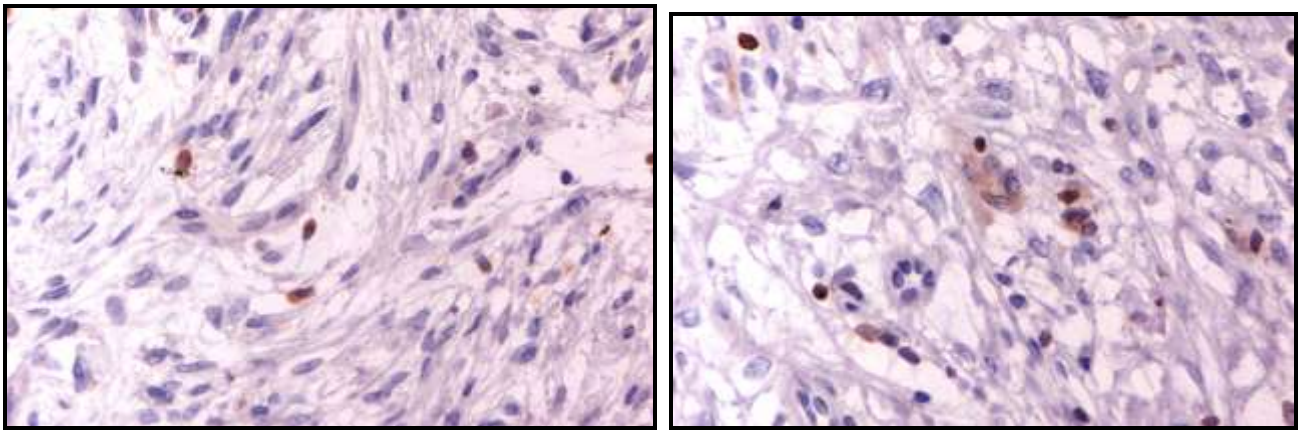
Bcl-2 immunostaining Distribution

In normal gingiva, the positive expression of the Bcl-2 protein was demonstrated in four cases (40%) samples, while other six cases (60%) showed negative expression. The labeling index of Bcl-2 in positively stained cases was (1.15± 0.07). Regarding **PG,** the results showed that 12 cases (25%) was appeared with Bcl-2 negative expression, and 36 cases (75%) showed positive Bcl-2 immune expression, all positive cases showed mild reactivity to Bcl-2.

The staining distribution was concentrated in the endothelial cell (Figure-2). The highest percentages of Bcl-2 positive cases were seen associated with males, the age group (31-40), the maxilla, and the maximum diameter of (<1.5) cm as seen in (Table-4). All cases of **PGCG** showed positive Bcl-2 immune expression, two cases (5.13%) were mild positive, 25 cases (64.10%) were moderate positive, and 12 cases (30.77%) were strong positive.

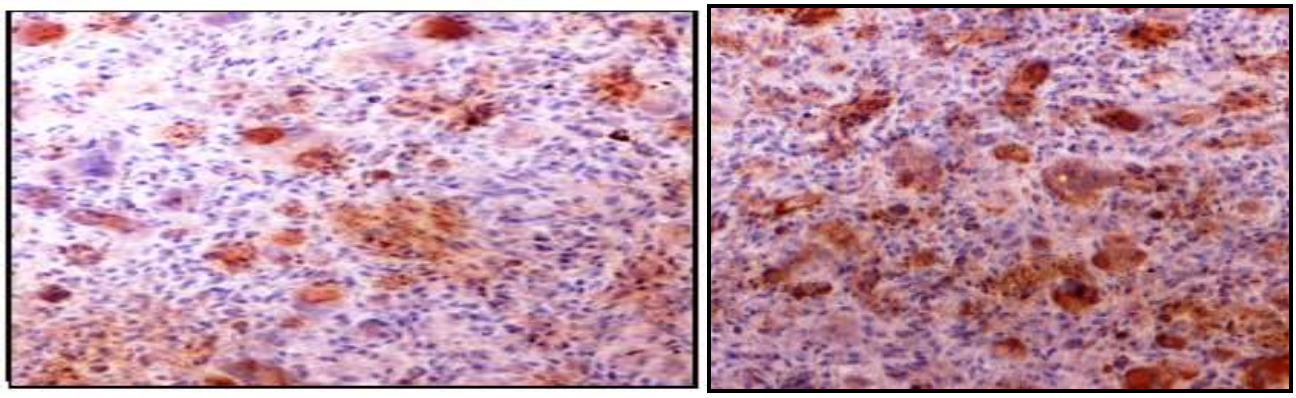
The staining distribution was seen mainly in multi nucleated giant cell (Figure -2); no positivity was found in the endothelial cell. The highest percentages of Bcl-2 strong positive cases were seen associated with females (37.5%), the age groups (11-20), the maxilla, and the maximum diameter of (>3) cm. The Bcl-2 labeling index for PG and PGCG was ranging (1.1% to 7.2%) and (8.7% to 76%) respectively. Statistical analysis of the mean labeling index of Bcl-2 for PG or PGCG showed no significant relation (p>0.05) with the gender, site and the maximum diameter of the lesions (Table-5).

The mean labeling indexes of Bcl-2 in PG, PGCG, and normal gingiva were 3.547±1.59%, 38.77±20.396%, and 1.15± 0.07%, respectively. Statistical analysis showed a highly significant difference present between PG and normal gingiva, PG and PGCG, PGCG and normal gingiva regarding the Bcl-2 immune expression (P<0.01).



A B

Figure-3: Photomicrograph of the pyogenic granuloma revealed negative (A) and week (B) positive immunohistochemical expression of Bcl-2 in the connective tissue cell cytoplasm (Immunohistochemistry x400)



A B

Figure-4: Photomicrograph of the peripheral giant cell granuloma revealed moderate (A) and strong (B) positive immunohistochemical expression of Bcl-2 in the connective tissue cell cytoplasm (Immunohistochemistry x100)

Table-4: Percent distribution of the Bcl-2 expression in pyogenic granuloma and peripheral giant cell granuloma in relation to different clinical features

Clinical Parameter			Total Negative No.(%)	Total Positive No.(%)	Mild Positive No.(%)	Moderate Positive No.(%)	Strong positive No.(%)	Total No.(%)
Gender	**Male**	**PG**	5(22.73)	17(77.27)	17(100)	0	0	22(100)
		PGCG	0	16(100)	1(6.25)	9(56.25)	6(37.5)	16(100)
	Female	**PG**	8(30.77)	18(69.23)	18(100)	0	0	26(100)
		PGCG	0	23(100)	1(4.35)	16(69.56)	6(26.09)	23(100)
Age group (years)	**≤ 10**	**PG**	2(50)	2(50)	2(100)	0	0	4(100)
		PGCG	0	6(100)	2(33.33)	4(66.67)	0	6(100)
	11-20	**PG**	3(30)	7(70)	7(100)	0	0	10(100)
		PGCG	0	2(100)	0	0	2(100)	2(100)
	21-30	**PG**	1(9.09)	10(90.91)	10(100)	0	0	11(100)
		PGCG	0	9(100)	0	4(44.44)	5(55.56)	9(100)
	31-40	**PG**	0	7(100)	7(100)	0	0	7(100)
		PGCG	0	6(100)	0	3(50)	3(50)	6(100)
	41-50	**PG**	1(33.33)	2(66.67)	2(100)	0	0	3(100)
		PGCG	0	5(100)	0	5(100)	0	5(100)
	51-60	**PG**	2(40)	3(60)	3(100)	0	0	5(100)
		PGCG	0	2(100)	0	2(100)	0	2(100)
	≥61	**PG**	2(40)	3(60)	3(100)	0	0	5(100)
		PGCG	0	4(100)	0	1(25)	3(75)	4(100)
Site	**Maxilla**	**PG**	3(16.67)	15(83.33)	15(100)	0	0	18(100)
		PGCG	0	8(100)	1(12.5)	5(62.5)	2(25)	8(100)
	Mandible	**PG**	4(44.44)	5(55.56)	5(100)	0	0	9(100)
		PGCG	0	21(100)	1(4.76)	15(71.43)	5(23.81)	21(100)
Maximum diameter (cm)	**≤ 1.5**	**PG**	4(21.05)	15 (78.95)	15(100)	0	0	19(100)
		PGCG	0	14(100)	1(7.14)	9(64.29)	4(28.57)	14(100)
	1.5-3	**PG**	7(28)	18 (72)	18(100)	0	0	25(100)
		PGCG	0	19(100)	0	13(68.42)	6(31.58)	19(100)
	>3	**PG**	1(25)	3(75)	3(100)	0	0	4(100)
		PGCG	0	6(100)	0	3(50)	3(50)	6(100)

Table-5: Distribution of the mean labeling indices and standard deviations of Bcl-2 positive cells in relation to the gender, location, and the maximum diameter of pyogenic granuloma and peripheral giant cell granuloma (Bcl-2 immune negative cases were excluded from the analysis)

Clinical parameter		PG/ Bcl-2 X ±SD	P-value	PGCG/ Bcl-2 X ±SD	P-value
Gender	Male	3.37±1.346	0.645 p>0.05 NS	39.48±22.823	0.863 p>0.05 NS
	Female	3.68±1.869		38.26±19.046	
Site	Maxilla	23.04±7.351	0.735 p>0.05 NS	37.66±20.903	0.549 p>0.05 NS
	Mandible	3.48 ± 1.985		32.60±15.878	
Maximum diameter (cm)	<1.5	3.65± 1.523	0.947 p>0.05 NS	36.74± 22.620	0.118 p>0.05 NS
	1.5-3	3.46± 1.678		36.026 ± 19.946	
	>3	3.5 ± 2.995		55.26± 11.557	

Discussion

Clinical Features

Pyogenic granuloma

The present study showed that most of PGs were common among the females. This result agrees with that of Krishnapilla et al [18], and Alwani et al studies [19]. Serum progesterone and estrogen concentrations render the gingival tissue more susceptible to chronic irritation caused by plaque and calculus may be implicated as a cause [8]. In our study, the mean age of individuals with PGs was a (32.47±19.575) year which was nearly similar to Seyedmajidi et al study [20]. The age group (21-30) years showed the highest number of cases. Our results come in agreement with Salih study [21], but disagree with Abdulai et al study [22]. Poor oral hygiene may be a precipitating factor in many PG patients [23].

PG in the present study was more common in the maxilla (66.7%). Seyedmajidi et al study [20] also found the result. These results disagree with that of Kashyap et al study [24] in which most of the cases of PGs studied (69.05%) was appeared in the mandible. The diameters of PGs varied from (0.2- 7cm) with a mean of 2.05 cm. Abdulai et al study [22] showed that the diameters of PGs studied were varied from (0.2- 8cm) with a mean of 5.8 cm.

Peripheral Giant Cell Granuloma

Regarding the gender, most of the cases of PGCGs were seen common among the females. These results agree with Merza study [25]. The finding that the majority of lesions affected female patients could reflect a greater concern and compliance in female patients towards dental care or the role of hormones. These findings disagree with that of Sarode and Sarode study [26] in which they reported a slight predilection for the male.

In our study, the mean age of individuals with PGCGs was a (35.03±18.22) year which was higher than that Khiavi study [27], but it was lesser than that of Merza study [25]. The age group (21-30) years showed the highest number of cases, this result disagree with that of Hallikeri et al study [28]. PGCG in the present study was more common in the mandible than in the maxilla, this comes in agreement with the study of Merza [25], but disagree with Sarode and Sarode study [26], they found that maxilla was more affected than mandible. In the present study, the diameters of PGCGs varied from (0.5- 5cm) with a mean of 1.99 cm. Niedzielska and Borgiel-Marek found that the diameters of PGCGs studied were varied from (1.4- 2.5cm) [29].

Immunohistochemical Results

Ki-67 immunostaining Distribution

Ki-67, a marker used to determine the proliferative condition of the cells [30].All cases of PGs showed positive Ki-67 immune expression in the connective tissues with a mean labeling index of (12.68 ± 6.117). Saghafi et al [31] found that the reactivity percentage of PG to Ki-67 was (23.00 ±12.00).

In our study we found that the staining distribution was concentrated in the perivascular cell followed by endothelial cells. These results come in agreement with other studies [30, 32]. They suggest that the cause was the luminal capillary endothelial cells are more differentiated than perivascular cells and the fact that differentiation usually has an inverse relation with proliferation; it is acceptable that luminal cells show lower proliferative activity than perivascular cells which are probably immature cells.

Regarding PGCG, the reactivity in the connective tissue to Ki-67 was found to be (21.71± 8.596); the staining distribution was restricted to the stromal mononuclear cells.

Our results disagree with Souza et al study [33]; they found the reactivity percentage of mononuclear cells was (4.31%). Merza found that the reactivity in the connective tissue to Ki-67 was found to be (65 ±33.49) [25].This difference in the results may be due to many causes like tissue fixation conditions, and the different aspects of tissue handling such as drying conditions of newly cut sections, and the heat-mediated antigen retrival techniques [34].

The present study showed that the giant cells expressed negatively for Ki-67. This finding indicates that the giant cells are not involved in the proliferative activity of the lesions, and this explains the fact that the aggressiveness of the lesion is promoted by the proliferative activity of mononuclear cells and not giant cells [35]. Our results were consistent with other previous studies like Kujan study [2].

Our results disagree with El-Attar and Wahba study [36], they studied 33 cases of peripheral giant cell granuloma and found that all cases were positively stained to Ki-67 and both mononuclear stromal cells and multinucleated giant cells were moderately stained for this stain, also they stated that these results indicates that both giant cells and mononuclear stromal cells are both actively involved in the proliferative activity of these lesions. Also the difference in Ki-67 expression could be used to differentiate aggressive from non-aggressive giant cell granuloma.

In the present study, perivascular and endothelial cells in the PG were positive while in PGCG were negative, so we suggested that the PG consisted of both endothelial and perivascular cells proliferation. The mean labeling indexes of Ki-67 in PG and PGCG was 12.68% and 21.71% respectively. Statistical analysis showed a highly significant difference present between them (P= 0.000). Our results permit to suggest that PGCG exhibit increased proliferative activity in the connective tissue compared to PG, so we could suggest that Ki-67 labeling index may reflect the behavior of the lesions. However Souza et al [33] stated that the expressions of Ki-67 in PGCG do not reflect the clinical behavior of these lesions and may only reflect the growth rate of giant cell lesions of the jaws.

Bcl-2 Immunostaining Distribution

The bcl-2 gene is a protooncogene whose protein product inhibits apoptosis. Its role is associated with keeping the cells alive, and seems to be one of the most promising members of molecular markers to evaluate tumor behavior [37]. We found that 12 out of 48 cases of PGs (25 %) investigated were completely lacked immunohistochemically detectable bcl-2 proteins.

Mild positive staining pattern of bcl-2 positive cells were found in 36 (75%) of cases, the staining distribution was mainly concentrated in the vascular structures, and the labeling index of Bcl-2 was (3.547± 1.598).Nakamura [38] found that five from 15 cases lacked the immunohistochemically detectable bcl-2 proteins. Based on these results we can conclude that Bcl-2 proteins contribute to the suppression of apoptosis at least in part in PG and this suppression of apoptosis is, to some extent, may cause the rapid growth of PG.

Regarding PGCG, all samples stained positively for bcl-2. The staining distribution was noticed mainly in giant cells, and few numbers of mononuclear cells were stained positively for bcl-2. The labeling index of Bcl-2 expression in positively stained section was (38.77± 20.396). Kuzenko et al study [39] showed that most of giant-cells were positive for Bcl-2, but the labeling index was (75.31 %) which was higher than our findings. The mean labeling indexes of Bcl-2 in PG and PGCG were 3.547% and 38.77% respectively. Statistical analysis showed a highly significant difference present between them (P= 0.000). This explains the differences in the biological behavior and pathogenesis between PG and PGCG, and may explain the frequent recurrence in PGCG.

Conclusions

The mean labeling index of Ki-67 and Bcl-2 in PGCG were significantly higher than PG (P<0.01). This can add insight to the clinical behavior and might reflect the differences in pathogenesis of these lesions.

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