

Comparison between gingival pyogenic granuloma and peripheral giant cell granuloma by immunohistochemical detection of CD34 and alpha smooth muscle actin

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(Submitted: 27 November 2018 – Revised version received: 16 December 2018 – Accepted: 12 March 2019 – Published online: 26 June 2019)

Objectives The aim of this research was to study the clinical and the immunohistochemical expressions of CD34 and alpha smooth muscle actin (α -SMA) for gingival pyogenic granuloma in comparison with peripheral giant cell granuloma.

Methods Formalin fixed, paraffin-embedded biopsy specimens of 48 gingival pyogenic granuloma and 39 peripheral giant cell granuloma were used in the study. Immunohistochemical analysis for CD34 and α -SMA were studied in pyogenic granuloma (PG), peripheral giant cell granuloma (PGCG).

Results The mean numbers of the CD34 positive microvessels in PGs and PGCGs were 32.58 ± 17.778 and 22.4 ± 11.208 , respectively. Statistical analysis showed a highly significant difference present between them ($P < 0.01$). The mean numbers of blood vessels with vascular surrounding cells non-reactive to α -SMA in PGs and PGCGs were 3.81 ± 2.228 and 10.53 ± 3.432 , respectively. Statistical analysis showed a highly significant differences present between them ($P < 0.01$).

Conclusion The mean number of CD34 positive microvessels in PGs was significantly more than that of PGCG, but the mean number of vascular surrounding cells non-reactive to α -SMA was significantly less. 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, CD34, α -SMA

Introduction

Oral mucosa is constantly exposed to external and internal stimuli and therefore manifests as a spectrum of diseases ranging from developmental, reactive, and inflammatory to neoplastic.¹ Localized gingival reactive hyperplastic lesions are classified into four sub-types like pyogenic granuloma (PG), peripheral giant cell granuloma (PGCG), focal fibrous hyperplasia, and peripheral ossifying fibroma.² Reactive lesions are non-neoplastic clinically and histologically and nearly they are clinically similar but possess distinct histopathological features.³

In general, PG is a common localized hyperplastic benign vascular lesion of the oral cavity, manifested as exophytic, sessile, erythematous, and painful nodule that is prone to bleeding and ulceration.⁴ Three quarters of all oral PGs occur on the gingiva, and most of them are in response to gingival inflammation and chronic gingival irritants.⁵⁻⁷

Peripheral giant cell granuloma is a painless, soft, reddish-bluish tumor-like reactive lesion, clinically bears resemblance to PG,⁸ however, it has significantly higher rate of recurrence than other reactive lesions and thus has to be treated with caution with complete excision and clearing of the lesion.⁹

Hematopoietic progenitor cell antigen CD34 also known as CD34 antigen is a protein that in humans is encoded by the CD34 gene. The CD34 protein is a member of a family of single-pass transmembrane sialomucin proteins that show expression on early hematopoietic and vascular-associated tissue.¹⁰ CD34 is a surface glycoprophosphoprotein expressed on developmentally early hematopoietic stem and progenitor cells, small-vessel endothelia and embryonic fibroblasts.¹⁰

Myofibroblasts are metabolically and morphologically distinctive fibroblasts expressing alpha smooth muscle actin (α -SMA), and their activation plays a key role in development of the fibrotic response.^{11,12} They are involved in morphogenesis, inflammation, fibrosis and oncogenesis in many tissues and organs. Myofibroblasts help in extracellular matrix reorganization by the production of numerous inflammatory mediators, growth factors and proteins of the extracellular matrix.¹³ Transdifferentiation of fibroblasts into myofibroblasts is an early event in tumorigenesis and it is mediated by cytokines and growth factors which are expressed by tumor cells.¹⁴

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 this research was to study the immunohistochemical expressions of CD34 and α -SMA 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 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.

NovoLink™ Polymer Detection System codes RE7140-K from Leica Microsystems (UK) which includes Monoclonal Mouse Anti-Human CD34 (Clone QBEND/10, dilution 1:100), and an anti- α -SMA Monoclonal Antibody (clone 1A4, diluted 1:100; Dako Corporation, Carpinteria, USA) were used. The staining procedure and the instructions included with each detection system were followed.

After necessary data had been collected, the results were given as mean \pm standard deviation. The potential difference among groups for histopathological data was evaluated using ANOVA test. All statistical calculations were done using computer programs 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 ≤ 0.05 was considered statistically significant.

Results

The samples used in this study consist of 48 cases of histologically proven PGs and 39 cases of histologically proven PGCGs. The age and sex distribution for both cases are seen in Fig. 1.

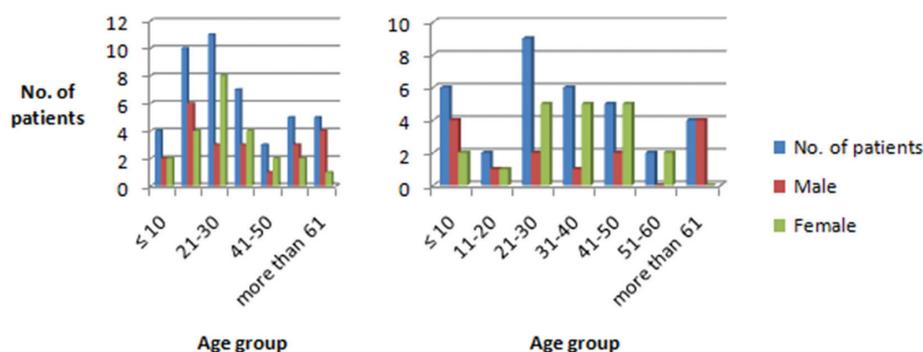


Fig. 1 Age and sex distribution of (A) pyogenic granuloma and (B) peripheral giant cell granuloma.

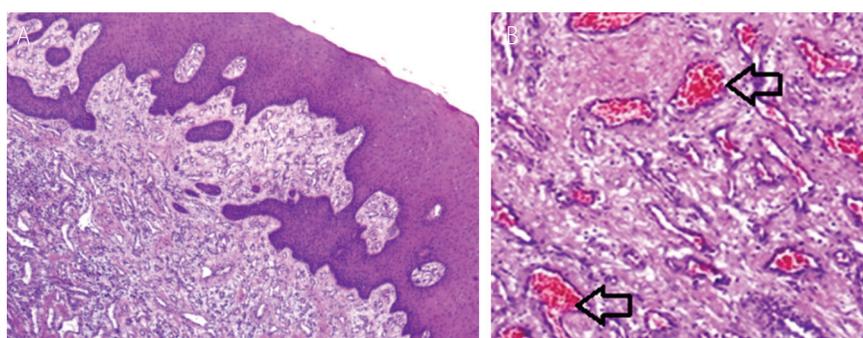


Fig. 2 Photomicrograph of pyogenic granuloma with hyperplastic epithelium that overlies a connective tissue that contains numerous inflammatory cells and blood vessels (A1: H&E 100 \times). Fibrovascular connective tissue consisting of numerous endothelium-lined vascular spaces engorged with red blood cells (arrows), and numerous fibroblasts infiltrated with inflammatory cells (A2: H&E 400 \times).

was counted as a single vessel unless there was a break in the continuity of the structure. All samples used in the study demonstrated positive reaction for CD34 (Figs. 4 and 5). The MVD for PG and PGCG was ranging (7.7–63.9) and (6.9–46.4) respectively.

Statistical analysis of the MVD for PG showed no significant relation with the gender, site and the maximum diameter of the lesions. Statistical analysis of MVD for PGCG showed significant relation ($P < 0.05$) with the gender, but a non-significant relations present with the site and the maximum diameter of the lesions ($P > 0.05$) as seen in Table 1.

The mean number of the CD34 positive microvessels in PGs, PGCGs, and normal gingiva was 32.58 ± 17.778 , 11.208 ± 22.4 , and 1.197 ± 8.21 , respectively. Statistical analysis showed a highly significant difference present between PG and normal gingiva, PG and PGCG, PGCG and normal gingiva regarding the MVD ($P < 0.01$) as seen in Table 2.

In this study, α -SMA - positive stromal cells and vascular surrounding spindle cells that showing brown cytoplasmic immunostaining, were considered to be myofibroblasts. Vascular surrounding cells and other stromal mesenchymal cells in all samples of normal gingiva revealed immune negativity to

α -SMA. Some of the vascular surrounding cells in PGs and PGCGs studied revealed immune positivity to α -SMA especially in PG, but the other stromal mesenchymal cells were negative in both PG and PGCG.

The number of blood vessels with vascular surrounding cells non-reactive to α -SMA was counted. In PG, the number of non-reactive blood vessels was ranging 1.3–9.4. While in PGCG the number of non-reactive blood vessels was ranging 4.8–18.2. Statistical analysis for PG and PGCG showed no significant relation with the gender, site and the diameter of the lesions ($P > 0.05$) as seen in Table 3.

The mean number of blood vessels with vascular surrounding cells non-reactive to α -SMA in PG was 3.81 ± 2.228 , but in PGCG, it was 10.53 ± 3.432 . Statistical analysis showed a highly significant differences present between PG and PGCG regarding the number of blood vessels with vascular surrounding cells non-reactive to α -SMA ($P < 0.01$) as seen in Table 4.

Discussion

Pyogenic granuloma and peripheral giant cell granuloma are common lesions of oral cavity. The pathogenesis remains to be

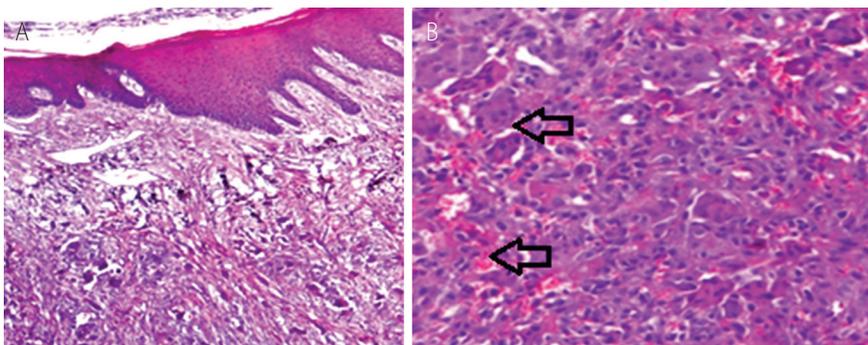


Fig. 3 Photomicrograph of peripheral giant cell granuloma, revealed hyperplastic epithelium that overlies vascular connective tissue which contain numerous multinucleated giant cells (A1: H&E 100 \times). Abundant multinucleated giant cells (upper arrow) together with inflammatory cell infiltration and areas of hemorrhage (lower arrow) are also seen (A2: H&E 400 \times).

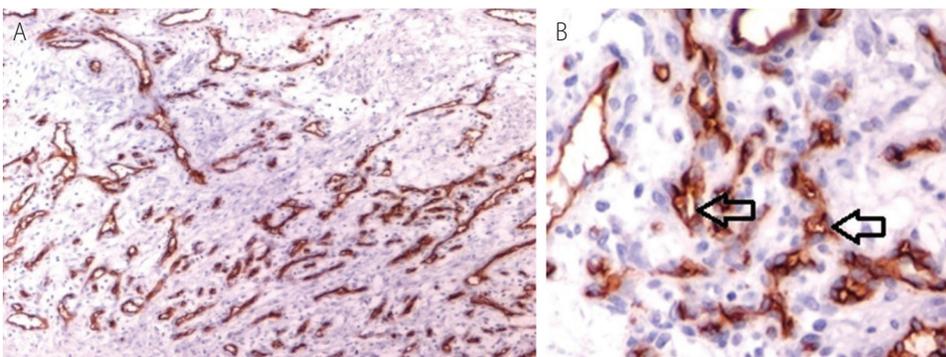


Fig. 4 Photomicrographs revealed the microvessels expressed by CD34 marker in the pyogenic granuloma (arrows) (A1: Immunohistochemistry 100 \times ; A2: Immunohistochemistry 400 \times).

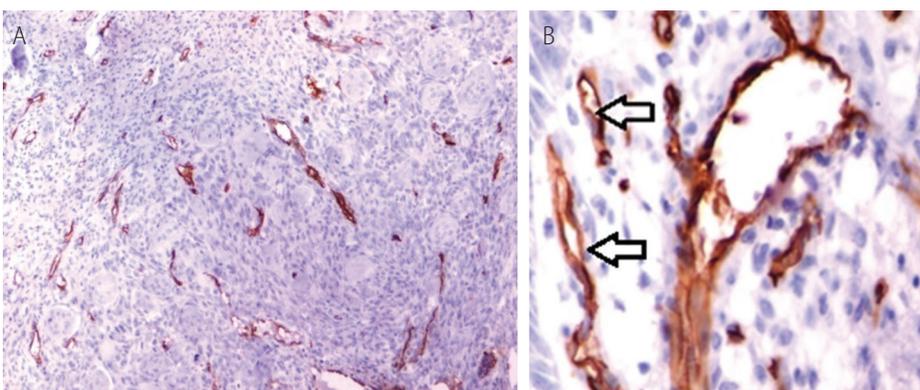


Fig. 5 Photomicrographs revealed the microvessels expressed by CD34 marker in the peripheral giant cell granuloma (arrows) (A1: Immunohistochemistry 100 \times ; A2: Immunohistochemistry 400 \times).

Table 1. Distribution of the mean and standard deviations of microvessel density in relation to the gender, location, and the maximum diameter of pyogenic granuloma and peripheral giant cell granuloma

Clinical parameter	PG		PGCG		
	X ± SD	P-value	X ± SD	P-value	
Gender	Male	26.79 ± 15.97	0.091	18.56 ± 12.047	0.03
	Female	37.06 ± 18.154	>0.05 NS	25.06 ± 9.994	<0.05 S
Site	Maxilla	30.52 ± 16.964	0.852	25.5 ± 12.074	0.379
	Mandible	31.27 ± 19.216	>0.05 NS	22.01 ± 11.806	>0.05 NS
Maximum diameter (cm)	<1.5	35.44 ± 18.363	0.591	18.23 ± 9.807	0.131
	1.5–3	30.82 ± 17.15	>0.05	24.22 ± 11.727	>0.05
	>3	27.3 ± 21.463	NS	26.35 ± 11.39	NS

PG: pyogenic granuloma, PGCG: peripheral giant cell granuloma, X: mean, SD: standard deviation, cm: centimeter, NS: non-significant, S: significant.

Table 2. The relations between the pyogenic granuloma, peripheral giant cell granuloma and normal gingiva for microvessel density

CD34 (MVD)	No. (Negative)	No. (Positive)	X ± SD	P-value
PG	0	48	32.58 ± 17.778	0.0001 * <0.01 HS
PGCG	0	39	22.4 ± 11.208	0.0001 ** <0.01 HS
Normal gingiva	0	10	8.21 ± 1.197	0.0001 *** <0.01 HS

*Comparison between PG and normal gingiva. **Comparison between PG and PGCG. ***Comparison between PGCG and normal gingiva. X: mean, SD: standard deviation, No: number, HS: highly significant.

Table 3. Distribution of the mean and standard deviations of the number of blood vessels with vascular surrounding cells non-reactive to α-SMA in relation to the gender, location, and the maximum diameter of pyogenic granuloma and peripheral giant cell granuloma.

Clinical parameter	PG		PGCG		
	X ± SD	P-value	X ± SD	P-value	
Gender	Male	3.90 ± 1.973	0.548	11.43 ± 3.614	0.241
	Female	4.73 ± 2.458	>0.05 NS	9.91 ± 3.232	>0.05 NS
Site	Maxilla	3.94 ± 2.208	0.892	10 ± 3.338	0.558
	Mandible	3.77 ± 2.538	>0.05 NS	10.85 ± 3.26	>0.05 NS

Continued

Maximum diameter (cm)	<1.5	3.73 ± 1.995	0.119	10.92 ± 3.709	0.143
	1.5–3	4.4 ± 2.415	>0.05	11.05 ± 2.837	>0.05
	>3	2 ± 0.816	NS	8 ± 3.949	NS

PG: pyogenic granuloma, PGCG: peripheral giant cell granuloma, X: mean, SD: standard deviation, cm: centimeter, NS: non-significant.

Table 4. The relations between the pyogenic granuloma and peripheral giant cell granuloma for α-SMA.

α-SMA	No. (Negative)	No. (Positive)	X ± SD	P-value
PG	0	48	3.81 ± 2.228	0.0001
PGCG	0	39	10.53 ± 3.432	* <0.0001 HS

*Comparison between PG and PGCG. X: mean, SD: standard deviation, No: number, HS: highly significant.

not fully understood. In such conditions, immunohistochemistry may provide some practical help and shed a light on the underlying pathogenesis of these lesions.

CD34 Immunostaining Distribution

In this study, the mean value of MVD of normal gingiva specimens was 8.21 ± 1.197 . Our results were nearly similar to Seyedmajidi et al.'s¹⁵ result; they found the MVD of all samples of healthy gingiva was 7.95 ± 5.56 . The mean value of MVD of samples of PGs was 32.58 ± 17.778 . Our results were less than Vasconcelos et al.'s¹⁶ result which was 48.09 ± 30.031 , but more than that of Seyedmajidi et al.'s¹⁵ which was 20.01 ± 11.88 . The increased expression of CD34 can be attributed to the increase in the number of blood vessels in pyogenic granuloma and appears to be involved in pathogenesis of oral pyogenic granuloma.¹⁵

The mean number of MVD of samples of PGCG was 22.4 ± 11.208 . Our results were less than Hallikeri et al.'s¹⁷ result which was 30.05 ± 8.006 . The higher expression of CD34 biomarkers in PG as a vessel-rich lesion compared with normal gingiva and PGCG, illustrates the role of these molecules as angiogenesis related markers. The variation of mean MVD among the groups suggests that angiogenesis may be one of the mechanisms possibly contributing to the different biological behavior, architecture or pattern of growth, and may be an important step for the study of new therapy.¹⁵

Alpha-smooth Muscle Actin Immunostaining Distribution

The most frequently used myofibroblast marker is α-SMA.¹⁸ The number of blood vessels with vascular surrounding cells non-reactive to α-SMA in PG was significantly less than that of PGCG. Most of the small, large blood vessels and abnormal blood vessels and spaces in PG had two or more outer layers of mesenchymal cells (myofibroblast) positive for α-SMA. The pattern of the distribution of these mononuclear myofibroblastic cells suggested that these cells might play a role in generating of newly formed blood vessels and spaces, since PG has more blood vessels in comparison with PGCG. Epivatianos¹⁹ and Kawachi²⁰ also found that most perivascular spindle cells in PGs studied were strongly stained to α-SMA.

The stromal mesenchymal cells between the blood vessels showed no reactivity to α -SMA. Despite the similarity of PG to granulation tissue, we did not detect any stromal myofibroblasts in all samples of PG. Our results come in agreement with Damasceno et al.,¹² they did not notice any stromal myofibroblast in PG.

Regarding the PGC, this study showed that stromal spindle cells which morphologically resemble myofibroblasts were negative for α -SMA, this result agrees with that of Damasceno et al.'s¹² study, but disagrees with Filioreanu et al.²¹ and Kujan²² studies, they found that smooth muscle specific actin was strongly stained in the spindle mononuclear cells (myofibroblasts) distributed through the lesion.

The sub-classification of blood vessels into immature, intermediate and mature is required as this is important using the combination of immunohistochemistry of CD34 and α -SMA stain to demonstrate pericyte.¹⁷ This is required to differentiate the intermediate blood vessels from the mature blood vessels. Since immature and intermediate blood vessels are considered as an indicator of the degree of angiogenic

activity, and are the main target of anti-angiogenic therapy and not the mature blood vessels, quantification of immature blood vessels may be helpful in estimation of prognosis especially for agents that do selectively target angiogenic endothelial cells, information may provide additional evidence of therapeutic anti-vascular effect for the control and prevention of the growth by inhibition of angiogenesis by anti-angiogenic therapy could be a potent therapeutic strategy.

Conclusion

Pyogenic granuloma showed more microvessel density and lesser number of blood vessels with vascular surrounding cells non-reactive to α -SMA were seen. This can add insight to the clinical behavior and might reflect the differences in pathogenesis of these lesions.

Conflicts of Interest

None. ■

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