Immunohistochemical detection of the expression of the cell adhesion molecules ICAM-1 & VCAM-1 in Pyogenic granuloma

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Abstract

Pyogenic granuloma is a relatively common benign vascular lesion of the oral mucosa & skin whose exact cause is unknown. The study of the expression of cell adhesion molecules (ICAM-1 & VCAM-1) in oral pyogenic granuloma was performed.

Fifteen formalin- fixed, paraffin-embedded oral pyogenic granuloma were studied using LSAB/HRP immunohistochemical technique.

Mild to moderate expressions of cell adhesion molecules was demonstrated among different cell types of pyogenic granuloma suggesting that inflammatory response of gingival tissue against oral antigens (gram negative bacteria) may play a major role in inflammatory neovasculairization & hence pathogenesis of pyogenic granuloma .

Keywords: Cell adhesion molecules, ICAM-1, VCAM-1, Pyogenic granuloma

Introduction

Pyogenic granuloma is a relatively common benign vascular lesion of the oral mucosa & skin whose exact cause is unknown. The misnamed entity is neither infectious nor granulomatous [1].

Pyogenic granuloma is now thought to represent exuberant tissue response to local irritation or trauma [2]. The lesion usually occurs in children & young adults as a solitary glistening red papule or nodule that is prone to bleeding & ulceration [3].

Prominent capillary growth in hyper plastic granulation tissue is characteristic histopathologically in pyogenic granuloma [4].

Cell adhesion molecules were simply the glue of life, the stuff that served to hold cells, ligaments & everything else together. They play role in every aspect of human biology from the embryo where they are crucial for tissue & organ development, to the adult where they act as traffic signals to direct the actions of immune-system cells in wound healing, inflammation, cancer & even AIDS [5].

ICAM-1(intercellular adhesion molecules) is a membrane-bound molecules belonging to the Ig-superfamily that are involved in immune reaction [6]. ICAM-1 is expressed or induced by inflammatory mediators on many cell types, including endothelial cells, epithelial cells, keratinocytes, synovial cells, lymphocytes & monocytes [7,8]. The soluble form of ICAM-1 is regarded as a useful parameter in the diagnosis & monitoring of various inflammatory, neoplastic & immune disorders [9].

VCAM-1(vascular cell adhesion molecules) are a transmembrane glycoprotein belonging to the Ig-superfamily & are expressed on the

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surface of mononuclear cells (monocytes, T-cells & eosinophils) [10,11,12] .Its expression on endothelial cells has been shown to increase adhesiveness & migration of activated mononuclear cell [13].

ICAM-1 & VCAM-1 had been shown to play an important role in pathogenesis & development of periodontal diseases [14] , odontogenic keratocyst & ameloblastoma [15], but no one up to our knowledge had studied their expression & role in other common oral lesions like pyogenic granuloma.

The aim of this paper is to study the expression of ICAM-1 & VCAM-1 in pyogenic granuloma & to investigate their relationship with major cell components of oral pyogenic granuloma.

Materials and Method

Fifteen cases of oral pyogenic granuloma routinely fixed & embedded in paraffin samples were retrieved from the files of the department of oral diagnosis/college of Dentistry/Baghdad University. Two cases were not included in the study due to its bad processing . After rehydration step, antigen retrieval of 5 µm sections of formalin-fixed, paraffin-embedded tissue includes the placement of the sections in autoclave for 3 minutes at 121ºC.was carried out.

The labeled streptavidin-biotin (LSAB) method was performed according to the manufacture's instructions. The HRP (horse raddish peroxidase) detection system was applied using primary monoclonal antibody against ICAM-1 & VCAM-1 (mouse monoclonal anti-human clone 6.5Bs & 1.3C3 Dakocytomation,USA) for 30 minutes after quenching of endogenous peroxidase by peroxidase blocking agent, followed by the application of secondary biotinylated antibodies (biotin labeled goat anti-rabbit & goat anti-mouse immunoglobulin in phosphate buffer saline "PBS") which were incubated for 30 minutes, as well as diaminobenzidien (DAB) for 30 minutes as chromogen. Finally, the sections were washed in distilled water & counterstained with Meyer's Hematoxylin. Sections incubated in phosphate buffer saline instead of primary antibody were used as negative controls & normal tonsillar tissue were used as positive control. The staining intensity was evaluated according to the immunoreactivity-staining score, first introduced by Remmele & co-workers [16], based on the semi quantitative scoring of cell staining as follows: grade (-) , no staining; grade 1 (+), mild but definite staining; grade 2 (++), moderate staining ; grade 3 (+++), strong staining.

Statistical analysis was performed using a computer software package, SPSS version 10 (SPSS Inc., Chicago,IL) $p \leq 0.05$ was considered statistically significant.

Results

The expression of ICAM-1 & VCAM-1 in pyogenic granuloma specimens was detected by immunohistochemistry. The percentage of stained cells for ICAM-1 & VCAM-1 in epithelial cells, inflammatory cells & endothelial cells are shown in table (1).

ICAM-1:

The majority of epithelial cells expressed mild to moderate ICAM-1 activity (41-47%), while inflammatory cells (mononuclear cells) expressed moderate to strong ICAM-1 activity (38.4%), on the other hand , endothelial cells expressed moderate ICAM-1 activity (53.8%).(figure 1)

VCAM-1:

Epithelial cells showed fluctuation in staining pattern between the scores for VCAM-1, while inflammatory cells expressed mild to moderate VCAM-1 activity (38.46%), unexpectedly, majority of endothelial cells exhibits mild VCAM-1 expression (69.2%).(figure 2).

Total ICAM-1 expression on epithelial cells, inflammatory cells & endothelial cells showed a significant difference among cell types, whereas VCAM-1 expression did not show any significant differences among different cell types (table 2).

Chi square test was applied on each cell to measure the staining difference between ICAM-1 & VCAM-1, table (3) showed a non significant statistical difference between them in epithelial cells & inflammatory cells, whereas a highly significant difference was observed between the two markers in endothelial cells.

Discussion

The development of an inflammatory response involves coordinated & sequential adhesive interaction between leukocytes & endothelial cells that are manifested as leukocyte rolling, adherence & migration [17]. ICAM-1 & VCAM-1, both are members of immunoglobulin gene super family; appear to mediate the firm adherence & emigration of leukocytes across endothelial cell monolayer [18].These findings have led investigators to invoke the role of ICAM-1 & VCAM-1 in pathogenesis of acute & chronic inflammatory diseases. Studies performed on monolayers of cultured endothelial cells have revealed that ICAM-1 & to a lesser extent VCAM-1 are constitutively expressed on these cells [19,20]. Our results revealed that ICAM-1 was expressed strongly on inflammatory cells & to a lesser extent in endothelial cells & the least expression on epithelial cells of pyogenic granuloma. The weak ICAM-1 expression in epithelial cells may be due to small population of responsive cells (antigen presenting cells (keratinocytes) that responds to oral antigens to induce inflammatory response.

Regarding VCAM-1, it was shown that marked (6fold) increase in endothelial VCAM-1 expression is constitutively associated with active inflammation [20 , 21]. Frenette & Wagner [22], Crooke et al [23] all demonstrated that VCAM-1 is not a constitutive adhesion molecule of human umbilical vein endothelial cells but is inducible by TNF-α & IL-1. TNF is produced by macrophages as a consequence of lipopolysaccharide release from gram negative bacteria since (LPS) is a major component of their cell walls [24,25].TNF production up-regulate VCAM-1 expression which play an important role in regulation the movement of leukocytes from the blood to the foci of inflammation. Our study revealed a weak expression in endothelial cells of majority of pyogenic granuloma cases suggesting that this lesion is toward involution where acute inflammation is subsided and inflammatory mediators (like IL-1 & TNF) were vanished.

From all the abovementioned observations, one can conclude that inflammatory response of gingival tissue against oral antigens (gram negative bacteria) may play a major role in pathogenesis of pyogenic granuloma. Furthermore ICAM-1& VCAM-1 expression can be used as reliable markers for inflammatory neovasculairization that can be used as a marker to measure micro -vessel density due to inflammatory reasons & as a marker to differentiate

inflammatory pyogenic granuloma from other developmental vascular lesions.

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Table (1): Percentage of ICAM-1& VCAM-1 expression in different cells of pyogenic granuloma

ICAM-1	Mild (grade 1)	Moderate(grade2)	Severe (grade3)
Epithelial cells	8 (47%)	7 (41%)	2 (12%)
Inflammatory cells	3 (23.07%)	5 (38.46%)	5 (38.46%)
Endothelial cells	4 (30.76%)	7 (53.8%)	2 (15.5%)
VCAM-1	Mild (grade 1)	Moderate(grade2)	Severe (grade3)
Epithelial cells	6 (46.15%)	2 (15.3%)	5 (38.46%)
Inflammatory cells	5 (38.46%)	5 (38.46%)	3 (23.7%)
Endothelial cells	9 (69.2%)	1 (7.6%)	3 (23.7%)

Table (2): Total ICAM-1 & VCAM-1 expression among different cells of pyogenic granuloma

ICAM-1	Mild (grade 1)	Moderate(grade2)	Severe (grade3)
Epithelial cells	8	7	2
Inflammatory cells	3	5	5
Endothelial cells	4	7	2
X^2	9.11		
DF	4	P<0.05	
VCAM-1	Mild (grade 1)	Moderate(grade2)	Severe (grade3)
Epithelial cells	6	2	5 (
Inflammatory cells	5	5	3
Endothelial cells	9	1	3
X^2	5.245		
DF	4	p>0.05	

Table (3): Staining differences among different cells of pyogenic granuloma

Epithelial cells	Mild (grade 1)	Moderate(grade2)	Severe (grade3)
ICAM-1	8	7	2
VCAM-1	6	2	5
X^2	4.17		
DF	2	P>0.05	
Inflammatory cells	Mild (grade 1)	Moderate(grade2)	Severe (grade3)
ICAM-1	3	5	5
VCAM-1	5	5	3
X^2	1		
DF	2	P>0.05	
Endothelial cells	Mild (grade 1)	Moderate(grade2)	Severe (grade3)
ICAM-1	4	7	2
VCAM-1	9	1	3
X^2	6.62		
DF	2	P<0.02	

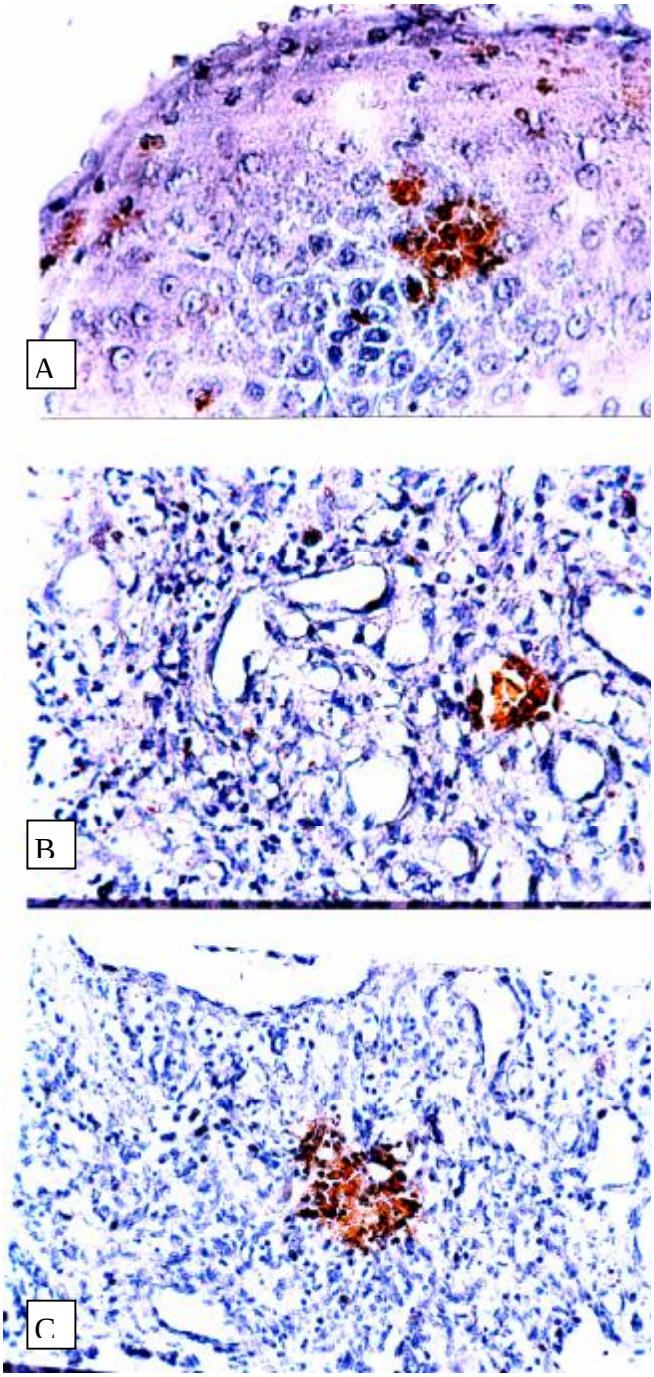

Figure (1): ICAM-1 expression on A-Epithelial cells, B- Inflammatory cells , C- Endothelial cells cells

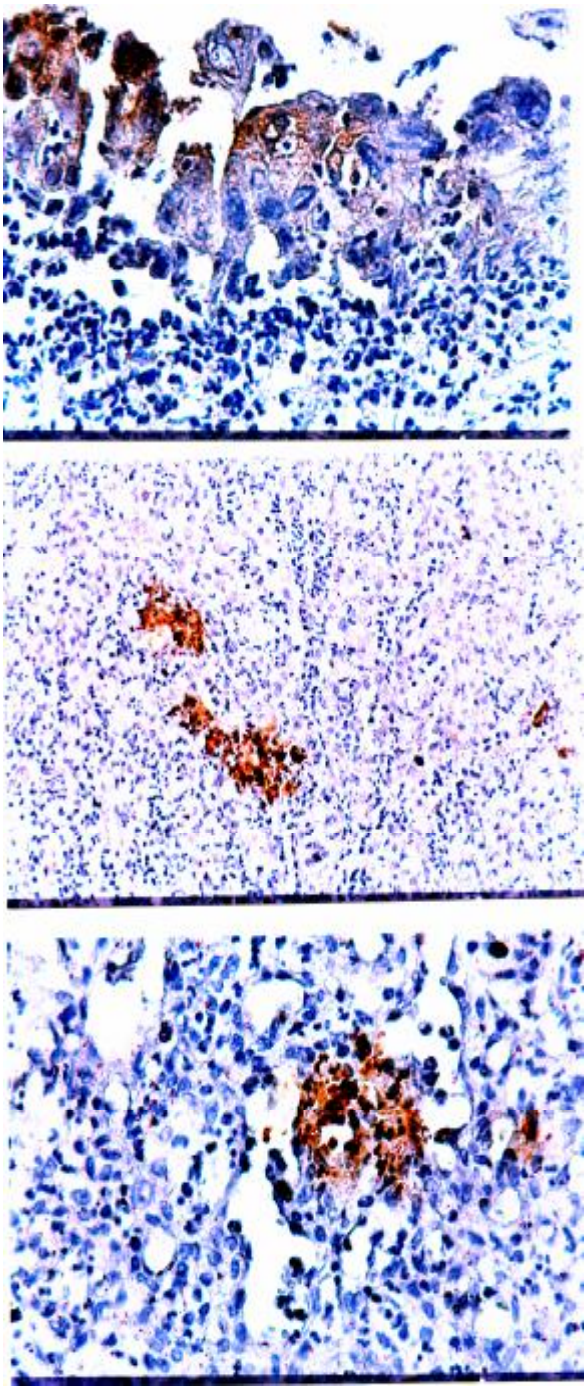

Figure (2): VCAM-1 expression on A-Epithelial Cells, B- Inflammatory cells , C- Endothelial