Expression of Platelet-Endothelial Cell Adhesion Molecule PECAM -1 in Gingival Tissue of Patients with Chronic Periodontitis

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Abstract: Background: Periodontitis is a response of highly vascularized tissues to the adjacent microflora of dental plaque. Platelet-endothelial cell adhesion molecule PECAM-1(CD31) is a key regulator of blood vessel endothelium. Tissue levels of this angiogenesis marker are not well known in human gingival tissue, and its role in the pathogenesis of periodontal disease is not yet fully investigated. **Objective:** the aim of this study was to evaluate PECAM-1 expression in healthy versus periodontally diseased patients and in moderate versus severe chronic periodontitis. **Material and Methods:** Thirty subjects were participated in this study. They were divided equally into three groups: group I none periodontally affected subjects (healthy control) (CAL = 0); group II patients with moderate chronic periodontitis (CAL = 3 to 4 mm); and group III patients with severe chronic periodontitis (CAL =<5 mm). All participants were evaluated clinically and histologically by the use of monoclonal antibodies (anti-PECAM -1) for immunohistochemical expression of this marker in gingival tissues. **Results:** Histologically, both experimental groups (group II and group III) expressed increased level of PECAM -1 when compared to control. Moreover, the expression of PECAM -1 was higher in group III than in group II. However, this increase was not statistically significant. **Conclusion:** PECAM -1 overexpression in patients with periodontitis highlighted the role of this marker in pathogenesis of periodontal disease which may be useful in the early diagnosis of periodontal diseases.

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Key Words: platelet-endothelial cell adhesion molecule; PECAM-1; CD31; angiogenesis**;** chronic periodontitis.

1. Introduction:

Periodontitis is a chronic inflammatory disease affecting the well-vascularized connective tissues of the periodontium (1). It induces destruction of periodontal connective tissue matrix, loss of fibrous attachment, alveolar bone resorption and an impaired formation of new bone (2). Bacterial and viral infections represent the main etiological factor in the development of chronic periodontitis, but the progress and intensity of the disease are strictly related to the immune reaction of the body to foreign antigens (3, 4). Microbial challenge at the gingival sulcus leads to an inflammatory response in the adjacent soft tissues that is characterized by migration of leukocytes from postcapillary venules into the extravascular tissue (5).

Characteristic endothelial cell adhesion molecule expression related to leukocyte infiltration of human gingiva has been demonstrated (6, 7). Platelet-endothelial cell adhesion molecule- 1 (PECAM-1, CD31) represents one of the adhesion molecules which manifests the highest expression in endothelial cells. Studies revealed that PECAM-1 playing a key role in adhesion between endothelial cells and in interactions of the cells with leukocytes (8, 9). Moreover the transmigration of lymphocytes through the endothelial wall takes place under the effect of PECAM-1.(9) The expression of PECAM-1 has been demonstrated mainly on lymphocytes and endothelial cells in patients with gingivitis and periodontitis (10). The role of angiogenesis stimulating factors in chronic periodontitis is poorly recognized. Studies pointed to the potential role of PECAM-1 as selective marker of blood vessels in gingiva in pathogenesis of chronic periodontitis (11).

The current study investigated the expression of PECAM-1 (CD31) in the gingiva of patients with chronic periodontitis by immunohistochemistry to determine the prognostic value of the angiogenesis rate in chronic periodontitis.

2. Subjects and Methods:

Study Population

Thirty subjects were participated in this study under an informed protocol (# EA/30/2013) approved by the Ethical Committee of Qassim University. Research objectives were explained to the patients, and all patients provided written informed consent before being included in the study. Participants were divided equally into three groups: group I none periodontally affected subjects (healthy control) (CAL = 0); group II patients with moderate chronic

periodontitis (CAL = 3 to 4 mm); and group III patients with severe chronic periodontitis (CAL =< 5 mm)(12).

Determination of Periodontal Status

All participants were evaluated clinically. The following clinical and periodontal parameters were assessed by the same examiner: plaque index [PI]; gingival index [GI](13) ; probing depth [PD]; and clinical attachment loss [CAL]. The PD and CAL were assessed at six sites around each tooth for the whole mouth excluding third molars.

Gingival Biopsies

A marginal gingival biopsy of the size ranging from 1-1.5 mm maintaining the scalloped contour was obtained from experimental and control sites providing tissue for histological examination. Gingival samples from experimental sites were isolated using a scalpel from the vicinity of pockets of various maxillary and mandibular teeth in the course of gingival curettage. Tissue material from control patients was obtained in the course of the extraction of teeth for orthodontic purposes, or in the course of the procedure of clinical crown lengthening.

Immunohistochemistry

Samples were fixed in formalin, and then placed in suitable labeled cassettes. Dehydration of the samples was carried out by immersing them in a series of alcohol solutions of increasing concentration. Samples clearing followed using Xylene and then embedding by thorough infiltration with paraffin wax, which formed into blocks and sectioned to 6um thickness. PECAM -1 immuno-staining was evaluated in epithelial cells and endothelial cells of subepithelial connective tissue vessels using Image optical density (IOD) of immuno-staining. The image of each slide of tissue in both control and experimental groups were captured using a 40 X objective (Bar = 50) with numerical aperture of a high resolution of 16-bit digital camera (2048 X1536 pixel). Images were viewed and recorded using Olympus microscope – equipped with Spot digital camera, using computer program MATLAB software (image J). Image optical density (IOD) of immuno-staining of PECAM -1 was evaluated by the maximum, minimum and integrity of intensity color based on Gray-level acquisition, analysis of the data, were carried out by reading 10 fixed areas in one image (10 images for each case). The mean values of each reaction were based on the mean of pixel number.

Statistical Analyses

All collected data were analyzed using the Statistical program for Social Science (SPSS) Version 20. Independent- T test was used to compare statistics of different groups with Significance set at P <0.05.

3. Results:

In this study, the data obtained was subjected to appropriate statistical analysis. Regarding the clinical parameters of periodontal disease, there was a statistically significant difference between the two experimental groups and control one [p>0.05] (table-1).

When comparing GI and PI between the two experimental groups (group II &group III) versus control group (group I) there was a statistically significant difference. However, the difference between the two experimental groups regarding the same indices was not significant.

Regarding PD and CAL there was significant difference between the two experimental groups versus control one. Furthermore a statistically significant difference was observed between the two experimental groups regarding CAL but not for PD.

Table (1) clinical parameters of periodontal disease in control versus experimental groups

	Group I	Group II	Group III	**(p)value** between group I &II
PI	0.30±0.34	0.60±0.38*	0.70±0.26	0.005
GI	0.50±0.47	0.98±0.40*	1.50±0.40	0.005
PD	2.60 ± 0.42	5.20±0.41*	5.50±0.51	0.002
CAL	0.0 ± 0.0	3.80±0.82*	5.89±0.77	0.001

Immunohistochemical results revealed that**,** the mean of gingival PECAM-1 stained cells was (150.4575) in group I and was (154.5353) in group II while was (164.5222) in group III. Despite there was an increase in the level of PECAM-1 from control to both periodontitis groups but this increase was not statistically significant (p>0.05). PECAM-1-positive cells were also increased in the gingival tissues of patients with severe periodontitis (162.7222 for group III) versus those with moderate periodontitis (154.5353 for group II) also, this difference was not statistically significant (p>0.05).

Image optical density (IOD) of immuno-staining revealed that the gingival expression of PECAM-1 in endothelium and connective tissues (CT) was strong at sites of sever periodontitis (group III) (Figure 2- A, B), while moderate reaction was observed in moderate periodontitis (group II) (Figure 3- A, B) versus weak reaction in healthy control (group I) (Figure 4- A, B).

Tables (2, 3, and 4) illustrate the comparison between the mean values of the IOD/ pixel in the three studied groups

Table (2) illustrate the comparison between the mean value of the IOD/ pixel between Control Group (group I) & moderate Periodontitis (group II)				
Groups	Mean	Std.Deviation	T	P
Control	150.45	14.948	-0.3858	0.7086
Moderate Perodontitis	154.53	17.742		

Table (3) illustrate the comparison between the mean value of the IOD/ pixel between Control Group (group I) & severe Periodontitis (group III)				
Groups	Mean	Std. Deviation	T	P
Control	150.4575	14.948	-1.240	0.2548
Severe Periodontitis	164.52	18.228		

Table (4) illustrate the comparison between the mean value of the IOD/ pixel between moderate Periodontitis (group II)&severe Periodontitis (group III)				
Groups	Mean	Std. Deviation	t	P
Moderate Perodontitis	154.5351	17.74242	-0.9506	0.364209
Severe Periodontitis	164.52	18.22808		

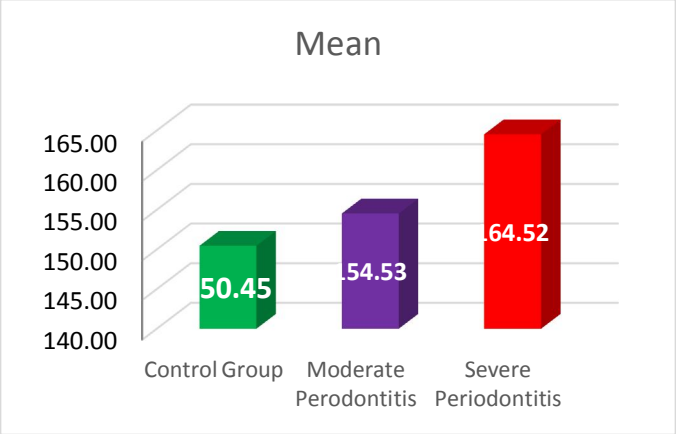

Figure 1: The mean density of PECAM-1 in all groups (the highest activity of the PECAM-1 was shown in severe periodontitis (group III) about 164 pixel and the least activity was shown in control (group I) about 150 pixel)

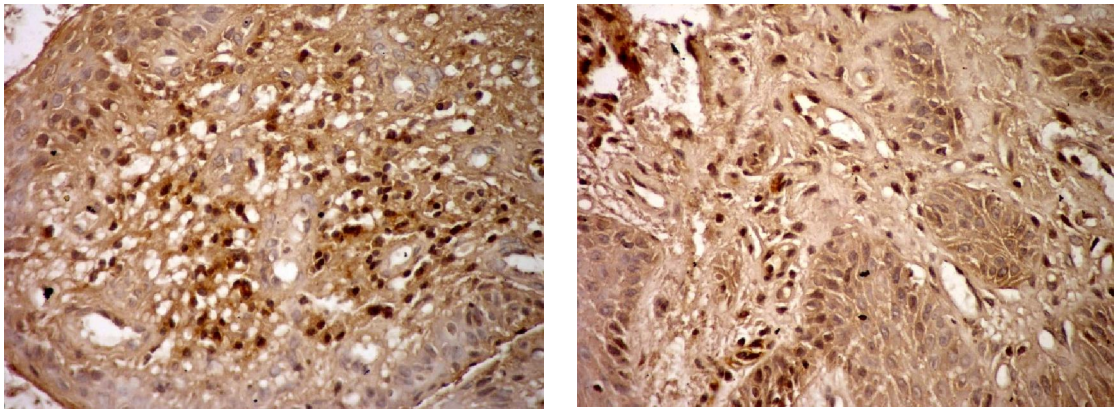

Figure 2 (A, B): Photomicrographs of gingival tissues from severe periodontitis (group III) showing intense brown staining of PECAM-1 in the endothelial cells of blood capillaries and connective tissue.

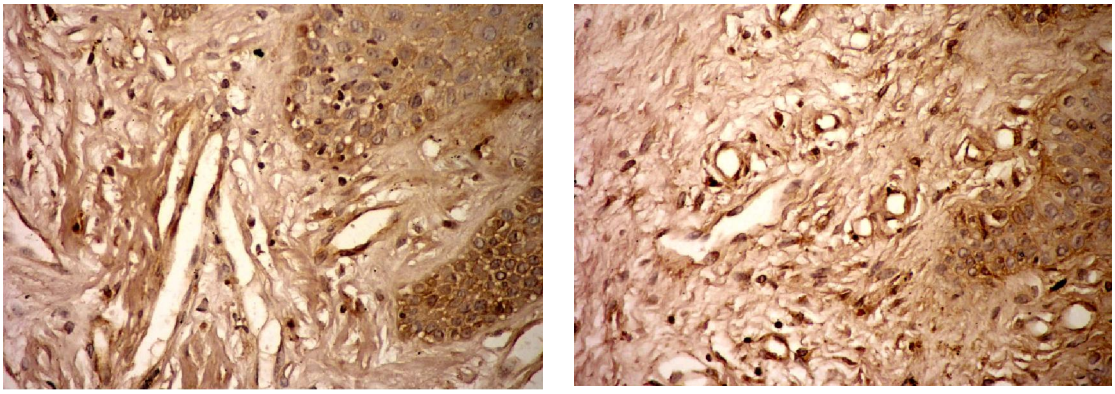

Figure 3 (A, B): Photomicrographs of gingival tissues from moderate periodontitis (group II) showing moderate brown staining of PECAM-1 in the endothelial cells of blood capillaries and connective tissue.

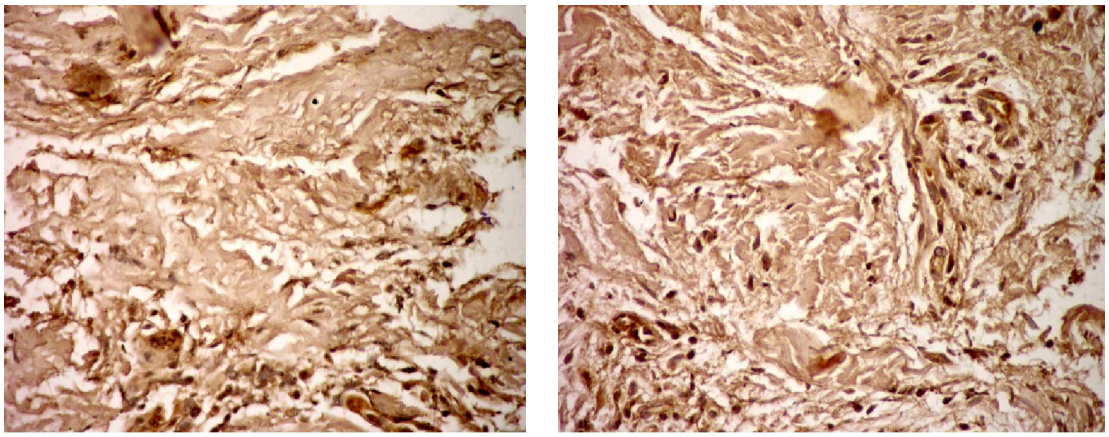

Figure 4 (A, B): Photomicrographs of gingival tissues from healthy control (group I) showing weak brown staining of PECAM-1 in the endothelial cells of blood capillaries and connective tissue.

4. Discussion:

The role of angiogenesis stimulating factors in chronic periodontitis is poorly recognized. Earlier immunocytochemical studies pointed to their potential role as selective markers of blood vessels in gingiva in pathogenesis of chronic periodontitis (11). In this study, we investigated the expression of platelet endothelial cell adhesion molecule-1(PECAM-1) in the gingivae of patients with chronic periodontitis by immunohistochemistry. The aim of the present study was to determine the prognostic value of the angiogenesis rate in chronic periodontitis.

Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) is a 130-kD vascular cell adhesion and signaling molecule of the immunoglobulin (Ig) superfamily that is expressed on the surface of circulating platelets, monocytes, neutrophils, and selected T-cell subsets. It is also a major constituent of the endothelial cell intercellular junction and plays a role in neutrophil recruitment at inflammatory sites. There is good evidence to suggest that PECAM-1 is a key participant in the adhesion cascade leading to extravasation of leukocytes during the inflammatory process (14).

Periodontitis is accompanied by the proliferation of small blood vessels in the gingival lamina propria(1). Recent observations suggest that chronic periodontitis is an independent risk factor for systemic vascular disease and may result in stimulation of the synthesis of acute phase protein by cytokines released by periodontal high endothelial cells(15). However, tissue expression of adhesion molecules has not been substantially evaluated in the gingiva of patients with chronic periodontitis. This is significant in relation to potential therapy targeting expression of the adhesion molecules.

Results of the present study revealed that PECAM-1expression in the gingival tissues of chronic periodontitis was higher than that of healthy control, furthermore the expression of PECAM-1was increased with the severity of the disease from moderate to severe periodontitis, however this increase was not statistically significant (tables 2, 3, 4).

These results are in consistent with Chapple *et al* (2000) who reported that the density of vascular profiles in the connective tissue increases significantly subjacent to the altered epithelial lining of the periodontal pocket. They concluded that density of blood vessels increases with increasing pocket depth (5).

Furthermore, these findings were -to some extent- in accordance with a recent study conducted by Kasprzak *et al* in (2013) on the expression of surface adhesion molecules of high endothelial cells [(CD34, platelet endothelial cell adhesion molecule 1 (PECAM-1), endoglin and intercellular adhesion molecule 1 (ICAM-1)]. The authors concluded that surface adhesion molecules are involved in the key steps of the adhesion cascade in periodontal diseases, and consequently, in the development and progression of these diseases (15).

Chen *et al* reported that PECAM-1 was noted as a component of a mechanosensory complex that mediates endothelial cell responses to shear stress (16). Others confirmed that inflamed periodontal tissues may lead to mechanical stress, which can also increase vessel volume or remodeling thus sharing in the progression of periodontal disease (17).

The findings of the current study revealed that PECAM -1 over expression in patients with chronic periodontitis may depict the role of this marker in the pathogenesis and severity of periodontal disease. However further studies on large sample population still needed to confirm this issue and to benefit from these results in the early diagnosis of periodontal diseases.

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