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## Immunohistochemical localization of intercellular cell adhesion molecule (ICAM-1) & vascular cell adhesion molecule (VCAM-1) in Radicular cyst

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### Abstract

**Objectives:** Odontogenic cysts are one of the commonest bone destroying lesions of the maxillofacial skeleton, with the inflammatory radicular cyst being the most common jaw cyst.

Cell adhesion molecules are known to be essential mediators of white blood cell adhesion & extravasations during inflammatory & immune reaction.

**Materials & Methods:** Twelve formalin-fixed, paraffin-embedded radicular cysts were studied using LSAB/HRP immunohistochemical technique.

**Results:** ICAM-1 expression was significantly high in radicular cysts with high expression in inflammatory cells infiltrate & connective tissue cells in cyst wall in comparison to VCAM-1 expression.

**Conclusion:** In conclusion, the identification of the nature of adhesion molecules & inflammatory mediators involved not only expanded our knowledge of the molecular mechanisms but also provided the basis for a new understanding of the inflammatory response & its role in tissue homeostasis.

**Keywords:** Cell adhesion molecules;ICAM-1; VCAM-1; Radicular cyst

### Introduction

Odontogenic cysts are one of the commonest bone destroying lesions of the maxillofacial skeleton, with the inflammatory radicular cyst being the most common jaw cyst <sup>(1)</sup>. Radicular cyst is a sequel of endodontic infection and manifests itself as the host defense response to microbial challenge emanating from the root canal system <sup>(2)</sup>. It is viewed as a dynamic encounter between infected radicular pulp and periodontal ligament that results in local inflammation, resorption of hard

tissue & destruction of other periapical tissues with eventual formation of various Histopathological categories of apical periodontitis referred to as periapical lesion <sup>(3)</sup>. The stimulus for the formation of radicular cyst is thought to be endotoxins released from the infected necrotic tooth pulp <sup>(4)</sup>. Bacterial Lipopolysaccharides (LPS) or (Endotoxins) can signal the endothelial cells to express leukocyte adhesion molecules that initiate extravasations of leukocytes into the area of the infection. Moreover, LPS can also activate macrophages to

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produce several molecular mediators, such as TNF- $\alpha$  & Interleukins <sup>(5)</sup>. Several classes of body cells participate in periapical defense; a majority of them are PMNLs, lymphocytes, plasma cells & monocytes/macrophages. Structural cells like fibroblasts, osteoblasts and epithelial cell rest of Mallassez also play a significant role <sup>(2)</sup>. In order for circulating leukocytes to enter inflamed tissue, the cells must adhere to and pass between the endothelial cells lining the wall of blood vessels, a process called extravasation <sup>(6)</sup>. Endothelial cells express leukocyte – specific cell adhesion molecules (CAMs). Some of these membrane proteins are expressed constitutively; others are expressed only in response to local concentration of cytokines produced during an inflammatory response <sup>(7)</sup>. Intercellular cell adhesion molecule [ICAM-1 (CD54)] is a membrane glycoprotein belonging to the Ig-superfamily that are involved in immunological reaction <sup>(8)</sup>. ICAM-1 is expressed or induced by inflammatory mediators on many cell types including endothelial cells, epithelial cells, keratinocytes, synovial cells, lymphocytes & monocytes <sup>(9)</sup>. It is normally found on the surface of endothelial cells, but its expression can be significantly increased upon endothelial activation with cytokines or endotoxins <sup>(10)</sup>. Vascular cell adhesion molecule [VCAM-1 (CD106)] is a transmembrane- glycoprotein, belonging to the Ig-superfamily which interacts with its integrin ligand VLA-4 receptor expressed on the surface of activated mononuclear cells (Monocytes, T-cells & eosinophils) <sup>(11)</sup>. These molecules are known to be essential mediators of white blood cells adhesion & extravasation during inflammatory & immune reaction <sup>(12)</sup>.

A limited number of researches have focused on the role of these two

cell adhesion molecules in odontogenic lesions <sup>(13&14)</sup>, but no one up to our knowledge had studied their expression & role in other common oral lesions like radicular cyst. The aim of this paper is to study the expressivity of ICAM-1 & VCAM-1 in different cell types of Radicular cyst.

## Materials and method

Fifteen routinely fixed paraffin-embedded blocks of histologically confirmed radicular cysts were retrieved from the archives of Oral Pathology Dept./ College of Dentistry/ University of Baghdad. Three cases were not included in the study due to its bad processing. Four micrometer thick sections were obtained from each case using positive charged slides. Biotin-Streptavidin immuno-assay was used to perform immunoperoxidase activity. The HRP (Horse Raddish Peroxidase) detection system was applied using diluted (1:50) anti ICAM-1 & antiVCAM-1 monoclonal primary antibodies ( mouse monoclonal antihuman clone 6.5 Bs & 1.3 C3 Dakocytomation,USA) for 30 minutes after quenching of endogenous peroxidase by peroxidase blocking agent. Secondary biotinylated antibodies (Biotin-labeled goat anti-rabbit & goat anti-mouse Immunoglobulins in phosphate buffer saline "PBS" was then applied & incubated for 30 minutes with diaminobezidine "DAB" as chromogen. The sections were eventually washed & counterstained with Meyer's Hemotoxylin. The analysis of distribution of the antibody-stained cells was performed under optical microscopy at 40X by one examiner for all slides. The staining intensity was evaluated according to the immuno-reactivity staining score <sup>(15)</sup> into: (0), absence of staining, (1), light staining, (2), moderate staining &

(3), strong staining. In order to determine inter-rater reliability of this scale, two independent Oral Histopathologists scored a series of 10 separate fields on slides from 5 cases. The degree of concordance was then assessed by using Chi square test which yielded a value of ( $X^2= 4.85$ ,  $df= 9$ ), indicating a high degree of agreement between the histopathologists. Statistical analysis was performed using computer software package, SPSS ver. 10 &  $p < 0.05$  was considered statistically significant.

## Results

Immunohistochemical localization of ICAM-1 & VCAM-1 in radicular cyst specimens was demonstrated in term of percentages of stained cells in lining epithelia, inflammatory cells, endothelial cells as well as connective tissue cells present in the cyst as shown in table (1).

Lining epithelial cells expressed a strong (score3) ICAM-1 activity in 66.66% of cases, whereas VCAM-1 expressed itself as mild staining reactivity (score 1) in 58.33% of lining epithelial cells.

Connective tissue cells of the cyst wall expressed a strong ICAM-1 activity in 83.33% of cases. At the same time, VCAM-1 was of mild expression on these cells in 50% of cases, the rest of cases ranged from moderate to strong staining. The inflammatory cells in radicular cysts were of mixed acute & chronic types. These cells have had strong ICAM-1 staining reactivity in 83.33% of cases. However, VCAM-1 expressed mild staining reaction in 58.33% of the cases examined. Endothelial cells have had interesting variation of ICAM-1 & VCAM-1 expressivity with negative expression of limited number of cases.

Total ICAM-1 expression among different cell components of the cyst (lining epithelia, inflammatory cells, endothelial cells & connective tissue cells) showed a significant differences ( $p < 0.05$ ), whereas, VCAM-1 expression did not showed any significant differences among different cell types (table 2). Chi square test was applied on each cell type to measure the staining differences between ICAM-1 & VCAM-1. Table (3) showed significant statistical differences between the connective tissue cells & non-significant differences between them in epithelial & endothelial cells.

## Discussion

In the literatures, sufficient attention has not been paid to the precise subcellular localization of immunohistochemical signals, the knowledge of which is essential for proper interpretation of immunostains. Aberrant localization of the molecules, when present, can provide important insight into disease process & aid in their diagnosis<sup>(16)</sup>. Several authors have studied the expression of several molecules in radicular cysts in an attempt to find their roles in pathogenesis of radicular cyst<sup>(17- 20)</sup>. 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<sup>(21)</sup>. These findings have led investigators to invoke the role of ICAM-1 & VCAM-1 in pathogenesis of acute & chronic inflammatory diseases<sup>(22)</sup>. The strong ICAM-1 expression in all cell types of radicular cyst found in this study usually depends on constant stimulation by cytokines like IL-1 & TNF- $\alpha$ , since ICAM-1 was described as a cytokine-inducible cell adhesion molecule<sup>(6)</sup>. IL-1 & TNF- $\alpha$

are produced by macrophages as a consequence of endotoxins released from gram negative bacteria comes from necrotic tooth pulp<sup>(4)</sup>. Bacterial LPS (endotoxins) can signal endothelial cells to express leukocyte adhesion molecules that initiate extravasation of leukocytes into the area of the infection<sup>(5-23)</sup>. The presence of mixed (acute & chronic) inflammatory cells in the cyst wall in the present study support the hypothesis of Mims<sup>(24)</sup>, who suggests that the antigenicity of LPS can occur in several forms that include mitogenic stimulation of B & T- lymphocytes which are of importance in apical periodontitis. The high expression of ICAM-1 in inflammatory cells is due to the fact that all leukocytes expressed LFA-1 molecules which exert its function primarily by binding ICAM-1 that is upregulated on the inflamed endothelium. Thus ICAM-1 serves as the major ligand that mediates firm adhesion of neutrophils to inflamed endothelial cells & therefore plays a central role in neutrophils recruitment to site of inflammation<sup>(6)</sup>. Furthermore, tissue cells in inflamed areas are known to up regulate ICAM-1, which allows adhesive interaction between tissue cells & emigrated neutrophils. Torabinejad<sup>(25)</sup>, however, believes that most periapical cysts have a discontinuous epithelial lining. These findings led us to suppose that the distribution of chronic inflammatory cells are directly associated with the presence & concentration of antigens, since the discontinuity of the epithelia allows the contact of antigens with the immune cells of the lesion in cystic fluid.

Regarding VCAM-1, our study revealed a mild expression (score 1) in all cells for most cases (table 1). The same finding was demonstrated in other oral lesion<sup>(26)</sup>, suggesting that VCAM-1 in radicular cyst is not a

constitutive adhesion molecule but is inducible by TNF- $\alpha$  & IL-1<sup>(27)</sup>.

The variation in adhesion molecule cellular distribution may be due to their level of expression, posttranslational modification of the molecules, differential splicing, constitutive and/or inducible expression following cellular activation & so forth<sup>(6)</sup>.

In conclusion, the identification of the nature of adhesion molecules & inflammatory mediators involved not only expanded our knowledge of the molecular mechanisms but also provided the basis for a new understanding of the inflammatory response & its role in tissue homeostasis.

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Table (1): Percentage of ICAM-1& VCAM-1 expression in different cells of Radicular cyst

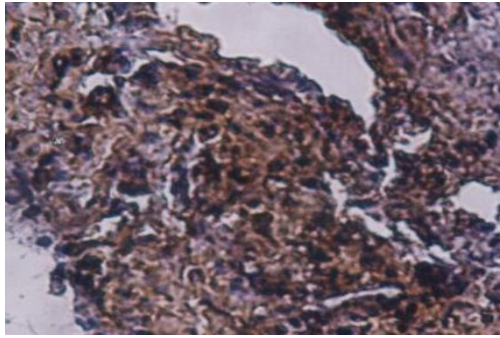
ICAM-1	Negative	Mild (grade 1)	Moderate(grade2)	
Epithelial cells	0	1 (8.33%)	3 (25%)	8 (66.66%)
Inflammatory cells	0	0	2 (16.66%)	10 (83.33%)
Endothelial cells	2 (16.66%)	4 (33.33%)	2 (16.66%)	4 (33.33%)
Connective tissue cells	0	0	2 (16.66%)	10 (83.33%)
VCAM-1	Negative	Mild (grade 1)	Moderate(grade2)	Strong (grade3)
Epithelial cells	0	7 (58.33%)	2 (16.66%)	3 (25%)
Inflammatory cells	0	7 (58.33%)	2 (16.66%)	3 (25%)
Endothelial cells	1 (8.33%)	8 (66.66%)	2 (16.66%)	1 (8.33%)
Connective tissue cells	0	6 (50%)	2 (16.66%)	4 (33.33%)

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

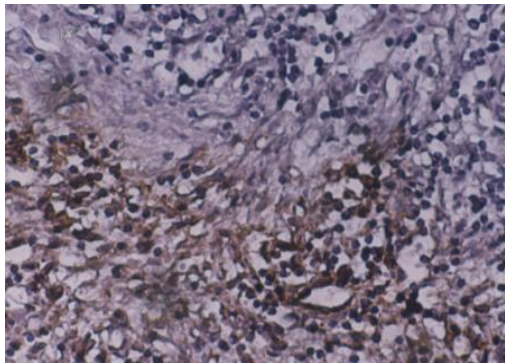
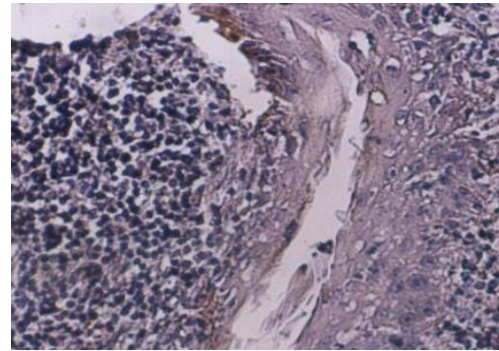
ICAM-1	Negative	Mild (grade 1)	Moderate(grade2)	Strong (grade3)
Epithelial cells	0	1	3	8
Inflammatory cells	0	0	2	10
Endothelial cells	2	4	2	4
Connective tissue cells	0	0	2	10
X2	19.11			<b>P&lt;0.05</b>
DF	9			
VCAM-1	Negative	Mild (grade 1)	Moderate(grade2)	Strong (grade3)
Epithelial cells	0	7	2	3
Inflammatory cells	0	7	2	3
Endothelial cells	1	8	2	1
Connective tissue cells	0	6	2	4
X2	4.99			<b>p&gt;0.05</b>
DF	9			

Table (3): Staining differences among different cells of Radicular cyst

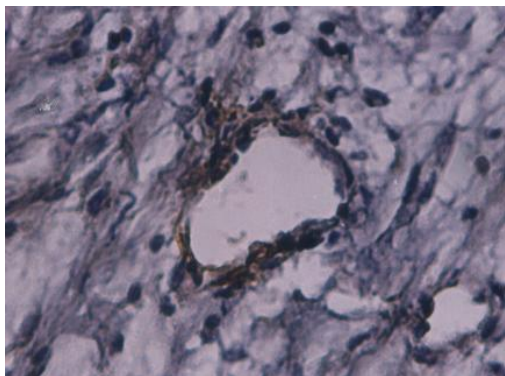
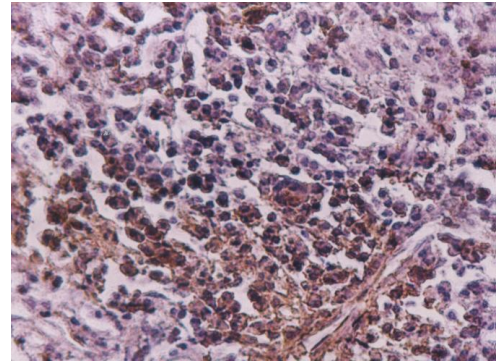
Epithelial cells	Negative	Mild (grade 1)	Moderate(grade2)	Strong (grade3)
ICAM-1	0	1	3	8
VCAM-1	0	7	2	3
X2	6.88			<b>p&gt;0.05</b>
DF	3			
Inflammatory cells	Negative	Mild (grade 1)	Moderate(grade2)	Strong (grade3)
ICAM-1	0	0	2	10
VCAM-1	0	7	2	3
X2	10.76			<b>P&lt;0.05</b>
DF	3			
Endothelial cells	Negative	Mild (grade 1)	Moderate(grade2)	Strong (grade3)
ICAM-1	2	4	2	4
VCAM-1	1	8	2	1
X2	3.34			<b>p&gt;0.05</b>
DF	3			
Connective tissue cells	Negative	Mild (grade 1)	Moderate(grade2)	Strong (grade3)
ICAM-1	0	0	2	10
VCAM-1	6	2	4	
X2	8.56			<b>P&lt;0.05</b>
DF	3			



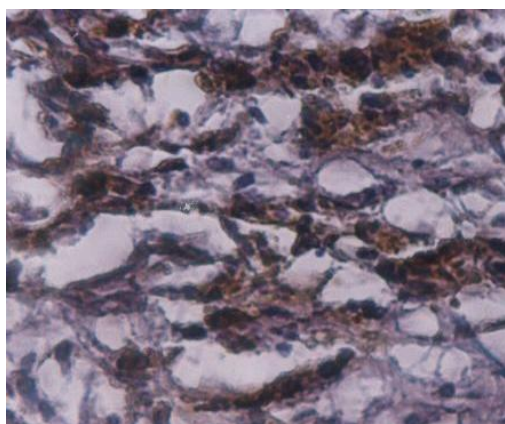
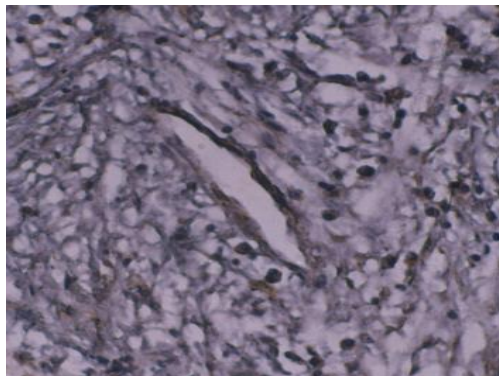
A



B



C



D

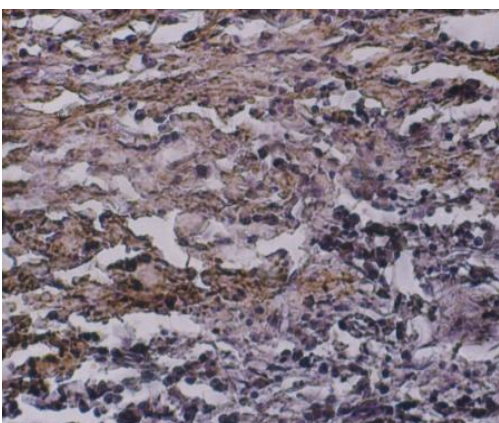


Figure (1): ICAM-1 expression  
 A-Epithelial cells  
 B- Inflammatory cells ,  
 C- Endothelial cells  
 D- Connective tissue cells

Figure (2): VCAM-1 expression