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Research Article

INTER-RELATIONSHIP OF PRO-INFLAMMATORY TNF- α , IL-6, C-REACTIVE PROTEIN AND NITRIC OXIDE WITH THE FORMATION OF RADICULAR CYSTSahar Javed¹, Arif Malik², Hira Sohail¹, Ambreen Tauseef³, Aamenah Malik¹,
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Abstract:

Background: Granulomas and radicular cysts are taken as the stages of development of inflammation. Increased level of TNF- α is observed in inflammatory exudates from radicular cyst and plays an important role in pathogenesis.

Objectives: Study was designed to evaluate the inter-relationship of pro-inflammatory IL-6, TNF- α , C-reactive protein and nitric oxide with the formation of radicular cyst.

Methods: Seventy patients with age range 20-40 years who visited Department of Dentistry of University of Lahore during the period of September -November. These patients were included in the study. Subject under study were comprised as group A and group B: Group A was included 20 individuals with healthy attached gingival with probing depth of less than 3mm. Group B was included 50 patients with radicular cysts selected with the criteria of clinical attachment loss of greater than 3mm.

Results: Levels of biochemical variables were evaluated in suffering with radicular cyst. It is observed that the level of IL-6, TNF- α and of Nitric oxide was significantly increased in subjects as compared to the values of these variables of control or normal subjects. However the level of CRP was slightly increased in patients as compared to controls.

Conclusion: Pro-inflammatory markers including serum IL-6, TNF- α and nitric oxide may have a role in the formation of radicular cyst. However, there was no role of serum C-reactive protein in the formation of radicular cyst.

Key Words: Radicular cyst, Pro-inflammatory markers, granulomas

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INTRODUCTION:

Apical periodontitis (AP) consist of inflammatory response of tissue of host in response of infection of the root canal to prevent the infection of bone. Bacteria cause the damage of periradicular tissues by releasing exotoxin and enzymes¹. A radicular cyst is an inflammatory odontogenic cyst of area of tooth². The occurrence of radicular cyst was 19.81%. The cyst may be formed at any stage but mostly observe in middle age³.

Granulomas and radicular cysts are taken as the stages of process of inflammation notable by infiltration of leukocytes. These leukocytes are the regulators of destruction and turnover of extracellular matrix and the chief source of mediators of bone resorption including ROS or reactive oxygen species and metallo matrix proteins⁴.

In periradicular part, the process of resorption of bone is modulated by cytokines, which may act as pro-inflammatory agents like interleukins, interferons and tissue necrosis factor- α . Many cytokines are up-regulated due to bacteria. However, this regulation is failed to prevent the destruction of tissue. It is thought that a co-existence of protective and destruction mechanism in the inflammatory periarticular response^{5,6}.

Cytokine interleukin-6 is synthesized by the epithelial lining cells of radicular cyst and this may activate nitric oxide synthetase level of the epithelial cells in autocrine manner⁷. It is observed that this radicular cyst may take part in resorption of bone and enlargement of cyst due to increased synthesis of nitric oxide⁸.

TNF- α is mainly secreted from monocytes and macrophages of fibrous capsule of cyst. Increased level of TNF- α is observed in inflammatory exudates from radicular cyst and plays an important role in pathogenesis. This increased level block cell division in epithelial lining of radicular cyst. The TNF- α also induced apoptosis by it receptors, thus alter the homeostasis of cell in many pathologies involving inflammation^{9,10}.

Nitric oxide is an important mediator of transduction pathway in many diseases including inflammation. Production of nitric oxide in cyst may take part in

the process of resorption of bone and enlargement cyst, due to the activation of matrix metalloproteases by nitric oxide¹¹.

In persistent and recurrent forms of inflammatory reactions, the presence of acute phase C reactive protein (CRP) aggravates the infection. There is a direct relationship of CRP with periodontitis in patients with inflamed periodontium. Increased level of CRP was observed in patients with localized periodontitis but its levels were less compared to patients with aggressive periodontitis¹².

The study was designed to evaluate the inter-relationship of pro-inflammatory TNF- α , IL-6 and C-reactive protein oxide and nitric oxide with the formation of radicular cyst.

MATERIAL AND METHODS:

Seventy patients with age range 20-40 years visited Department of Dentistry of University of Lahore during the period of September–November. Patients were comprised as group A and group B.

Group A was included 20 individuals with healthy gingival tissue, no signs of periodontal disease, absence of bleeding on probing, absence of clinical attachment loss and probing depth of less than 3mm. Group B consisted of 50 patients with periapical granulomas and radicular cysts selected with the criteria of clinical attachment loss of greater than 3mm, Probing depth of > 5mm bleeding on probing, Radiographic evidence of periapical round or ovoid well defined radiolucencies.

Patients with history of antibiotic/PDL therapy, history of any disease that may affect periodontal status like hypertension, diabetes etc, other conditions like smoking, pregnancy was excluded from the study. Blood of patients was drawn for the estimation of level of TNF α , nitric oxide and IL-4 was estimated by standard kits of ELISA.

Letter of consent was taken from each participant. Study was approved by Ethical committee of Institute.

Statistical Analysis: Data was analyzed by SPSS 20. Variables were expressed as mean \pm SD. Pro-inflammatory variables of control subject and patients were compared by using student 't' test. Variables were taken as significant at $p < 0.05$.

Table: Levels of circulating biochemical variables in patients suffering from Periapical cyst

Variables	Controls (n=20)	Patients (n=50)	p- values
CRP	1.01±0.02	1.33±0.30	0.049
IL-6	4.98±0.55	7.78±1.98*	0.041
TNF- α	27.98±4.78	30.87±5.89*	0.004
NO	22.67±3.76	61.78±10.78*	0.041

IL-6: interleukin-6; CRP:C-reactive protein; TNF- α : tumor necrosis factor- α ; NO: nitric oxide

RESULTS:

Levels of biochemical variables were evaluated in association with radicular cyst. It is observed that the levels of IL-6, TNF- α and Nitric oxide were significantly increased ($P < 0.05$) in subjects as compared to healthy subjects. However, the level of CRP was slightly increased in patients as compared to controls (Table 1).

DISCUSSION:

A balance between the expression of pro-inflammatory and anti-inflammatory cytokines was associated with the chronic periradicular inflammatory process. These cytokines are co-existed in apical periodontitis lesions¹³.

Our assessment of the CRP levels in patients versus controls did not lead to considerable results as there were no marked changes in the serum levels of CRP between the two groups under study. A reasonable explanation for no marked changes between the groups can be the acute phase response conservatively disappearing within a period of few days and levels of C-reactive protein thus normalize. A study developed a directly proportional relationship between serum CRP values and degree of inflammatory degeneration of periodontium. Their study stated the levels of CRP were mounting progressively in the inflamed odontogenic tissue¹³.

Our study is in-line with studies who observed an increased level of IL-6 levels in the patients with radicular cyst^{14,15}. It was observed that the production of IL-6 by epithelial cells as a first line of defense in response to different types of stimulation and has an ability to induce bone resorption and play an important role in the pathogenesis and progression of radicular cyst¹⁶.

Our results coincide positively with researches, who observed an increased level of TNF- α in radicular cyst^{17,18}. It is reported that production of TNF- α from osteoclast rationalize their function as bone resorbing mediator in expansion of radicular cyst¹⁹ which in turn involve in bone degeneration, vascularization and clustering of many cells in cyst. Increased level of TNF- α can be related with the degree of disease progression²⁰.

Our study coincides with studies who observed an increased level of nitric oxide in patients with radicular cyst as compared to controls. It is demonstrated that production of nitric oxide by inflamed gingival fibroblasts may also stop the mechanism of tissue repair involve in healing of periapical lesion^{8,21}.

Nitric oxide act as a relaxator of vascular smooth cells, a suppressor of platelet aggregation, a chemical messenger as well as damage the DNA, proteins / lipids and may play a role in inflammatory odontogenic diseases²². A study found an increased expression of nitric oxide synthetase in inflammatory cell of most of the radicular cells and justified the role of nitric oxide in inflammation zone as modulator²¹.

CONCLUSION:

Pro-inflammatory markers including serum IL-6, TNF- α and nitric oxide may have a role in the formation of radicular cyst. However, there was no role of serum C-reactive protein in the formation of radicular cyst.

Conflict of interest:

The authors confirmed no conflict of interest with respect to authorship, research and publication of this article.

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