



CODEN [USA]: IAJ PBB

ISSN: 2349-7750

INDO AMERICAN JOURNAL OF  
**PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.3648283>Available online at: <http://www.iajps.com>

Research Article

**TO EVALUATE THE INTER-RELATIONSHIP OF PRO-INFLAMMATORY TNF-A, IL-6 AND C-REACTIVE PROTEIN AND NITRIC OXIDE WITH THE FORMATION OF RADICULAR CYST**Sahar Javed<sup>1</sup>, Arif Malik<sup>2</sup>, Hira Sohail<sup>1</sup>, Ambreen Tauseef<sup>3</sup>, Aamenah Malik<sup>1</sup>,  
Masooma Talib<sup>4</sup>, Brig (R) Dr. Haroon Rashid, SI (M)<sup>5</sup><sup>1</sup> Department of Biochemistry, CMH Lahore Medical College & IOD, Lahore<sup>2</sup> Institute of Molecular Biology and Biotechnology, University of Lahore<sup>3</sup> Department of Physiology, CMH Lahore Medical College & IOD, Lahore<sup>4</sup> Assistant Professor Biochemistry Department, Watim Medical College<sup>5</sup> Vice Principal CMH Lahore Medical College & IOD, Lahore

Article Received: December 2019 Accepted: January 2020 Published: February 2020

**Abstract:**

**Background:** Granulomas and radicular cysts are taken as the stages of development of inflammatory. Increased level of TNF- $\alpha$  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- $\alpha$ , 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 2014 -November 2014. 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- $\alpha$  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- $\alpha$  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

**Corresponding author:****Dr Sahar Javed,**

Department of Biochemistry,

CMH Lahore Medical College &amp; IOD, Lahore

E-mail: zara.asif777@gmail.com

QR code



Please cite this article in press Sahar Javed *et al.*, To Evaluate The Inter-Relationship Of Pro-Inflammatory TNF-A, IL-6 And C-Reactive Protein And Nitric Oxide With The Formation Of Radicular Cyst., *Indo Am. J. P. Sci.*, 2020; 07(02).

**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 enzymes<sup>1</sup>. A radicular cyst is an inflammatory odontogenic cysts of area of tooth<sup>2</sup>. The occurrence of radicular cyst was 19.81%. The cyst may be formed at any stage but mostly observe in middle age<sup>3</sup>.

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 proteins<sup>4</sup>.

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- $\alpha$ . 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<sup>5,6</sup>.

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 manner<sup>7</sup>. It is observed that this radicular cyst may take part in resorption of bone and enlargement of cyst due to increased synthesis of nitric oxide<sup>8</sup>.

TNF- $\alpha$  is mainly secreted from monocytes and macrophages of fibrous capsule of cyst. Increased level of TNF- $\alpha$  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- $\alpha$  also induced apoptosis by it receptors, thus alter the homeostasis of cell in many pathologies involving inflammation<sup>9,10</sup>.

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 oxide<sup>11</sup>.

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 periodontitis<sup>12</sup>.

The study was designed to evaluate the inter-relationship of pro-inflammatory TNF- $\alpha$ , 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 2014 –November 2014. Patients were comprised as group A and group B.

Group A was included 20 individuals with healthy gingival, no signs of periodontal disease, absence of bleeding on probing, Absence of Clinical attachment loss, 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 $\alpha$ , nitric oxide and IL-4 was estimated by standard kits using the technique 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- $\alpha$	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- $\alpha$ : tumor necrosis factor- $\alpha$ ; NO: nitric oxide

### RESULTS:

Levels of biochemical variables were evaluated in suffering with radicular cyst. It is observed that the level of IL-6, TNF- $\alpha$  and of Nitric oxide was significantly increased ( $P < 0.05$ ) 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 (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 lesions<sup>13</sup>.

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 tissue<sup>13</sup>.

Our study is in-line with studies who observed an increased level of IL-6 levels in the patients with radicular cyst<sup>14,15</sup>. It is reported 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 cyst<sup>16</sup>.

Our results coincide positively with researches, who observed an increased level of TNF- $\alpha$  in radicular cyst<sup>17,18</sup>. It is reported that production of TNF- $\alpha$  from osteoclast rationalize their function as bone resorbing mediator in expansion of radicular cyst<sup>19</sup> which in turn involve in bone degeneration, vascularization and clustering of many cells in cyst. Increased level of TNF- $\alpha$  can be related with the

degree of disease progression<sup>20</sup>.

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<sup>8,21</sup>.

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 diseases<sup>22</sup>. A study found an increased expression of nitric oxide synthetase in inflammatory cell of most of the radicular cells and justifies the role of nitric oxide in inflammation zone as modulator<sup>21</sup>.

### CONCLUSION:

Pro-inflammatory markers including serum IL-6, TNF- $\alpha$  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 probable conflict of interest with respect to authorship, research and publication of this article.

### REFERENCES:

1. Siqueira JF Jr, Antunes HS, Rôças IN, Rachid CT, Alves FR. Microbiome in the apical root canal system of teeth with post-treatment apical periodontitis. *PLoS One*. 2016;11(9):e0162887.
2. Bava FA, Umar D, Bahseer B, Baroudi K. Bilateral radicular cyst in mandible: an unusual case report. [J HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/25859111" HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/25859111" HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/25859111" Int HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/25859111" HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/25859111"](https://www.ncbi.nlm.nih.gov/pubmed/25859111)

- 111" [HYPERLINK](#)  
["https://www.ncbi.nlm.nih.gov/pubmed/25859111"](https://www.ncbi.nlm.nih.gov/pubmed/25859111) *Oral Health*. 2015 Feb;7(2):61-3.
3. Yang N, Zhou Y, Zhou H, Liu X, Sun Z, Shang J. Increased interleukin 1 $\alpha$  and interleukin 1 $\beta$  expression is involved in the progression of periapical lesions in primary teeth. *BMC Oral Health* 2018; 124: 1-6
  4. Belmar MJ, Pabst C, Martinez B, Hernandez M: Gelatinolytic activity in gingival crevicular fluid from teeth with periapical lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2008, 105: 801-806.
  5. Bracks IV, Armada L, Goncalves LS, Pires FR. Distribution of mast cells and macrophages and expression of interleukin-6 in periapical cysts. *J Endod*. 2014;40(1):63-8
  6. Jakovljevic A, Knezevic A, Karalic D, Soldatovic I, Popovic B, Milasin J, et al. Pro-inflammatory cytokine levels in human apical periodontitis: correlation with clinical and histological findings. *Aust Endod J*. 2015;41(2):72-7.
  7. Poomsawat S, Punyasingh J, Vejchapipat P. Immuno-histochemical expression of p53 protein and iNOS in odontogenic cysts. *J Med Assoc Thai*. 2009;92:952-60
  8. Akshatha BK, Karupiah K, Manjunath GS, Kumarswamy J, Papaiah L, Rao J. Immunohistochemical evaluation of inducible nitric oxide synthase in the epithelial lining of odontogenic cysts: A qualitative and quantitative analysis. *J Oral Maxillofac Pathol*. 2017;21(3):375-381.
  9. Waqar-ur-Rahman Q, M Idris and SA Khan. Role of tumor necrosis factor in pathogenesis of radicular cyst. *JAMCA*. 2011;23(2):87-89.
  10. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol*. 2016;12(1):49-62.
  11. Rodini CO, Batista AC, Dionísio TJ, Santos CF, Cunha FQ, Lara VS. Morphologic evaluation and expression of matrix metalloproteinases-2 and 9 and nitric oxide during experimental periodontal disease in rat. *J Mol Histol*. 2008;39:275-82.
  12. Trang NS, BT Overstreet, JD Rogers, JV Califano, AM Best and HA Shenkein (2006). C-reactive protein levels in patients with aggressive periodontitis. *Journal Of Periodontology*. 77(6):933-939.
  13. Neto D, Porpino N, Maneschy MT, Antunes A, dos Santos H, Costa Val RR et al. Pro-inflammatory and anti-inflammatory cytokine expression in post-treatment apical periodontitis. *J of App Oral Sci* 2018; 26, e20170455
  14. Bansal T, Pandey A, D D, Asthana AK. C- Reactive Protein (CRP) and its Association with Periodontal Disease: A Brief Review. *J Clin Diagn Res*. 2014;8(7):ZE21-ZE24.
  15. Gervasio AM, Silva DAO, Taketomi EA, Souza CJA, Sung SSJ and Loyola AM. Levels of GM-CSF, IL-3 and IL-6 in fluid and tissue from human radicular cysts. *JDR*. 2002;81(1):64-68.
  16. Ulyana LH. Characteristic of immunological changes in odontogenic cysts. *The Pharma Innovation Journal*. 2013; 2(5):91-94.
  17. Graunaite I, Lodiene G, Maciulskiene V. Pathogenesis of apical periodontitis: a literature review. *J Oral Maxillofac Res*. 2012;2(4):e1.
  18. Mehtap M, Komerik N, Bulut E, Yarim GF, Celebi N and Sumer M. Cytokine and chemokine levels in radicular and residual cyst fluids. *J Oral Pathol Med*. 2008; 37:185-189.
  19. Safoura S, Mehdizadeh M, Maliji G, Korsavi ZS and Nosrati K. Comparison of TNF- $\alpha$  and TGF $\beta$ 1 level in radicular cyst and odontogenic keratocyst fluid and its association with histopathological findings. *Research in molecular medicine*. 2013; 1(2):39-43.
  20. [Coli](#) [HYPERLINK](#)  
["https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor\\_uid=18510067"](https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor_uid=18510067) [HYPERLINK](#)  
["https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor\\_uid=18510067"](https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor_uid=18510067) [HYPERLINK](#)  
["https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor\\_uid=18510067"](https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor_uid=18510067) [HYPERLINK](#)  
["https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor\\_uid=18510067"](https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor_uid=18510067) [HYPERLINK](#)  
["https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor\\_uid=18510067"](https://www.ncbi.nlm.nih.gov/pubmed/?term=Coli%20C4%20S5BAuthor%5D&cauthor=true&cauthor_uid=18510067) [HYPERLINK](#)  
["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) [HYPERLINK](#)

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067)Chir HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067)Iugosl HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

["https://www.ncbi.nlm.nih.gov/pubmed/18510067"](https://www.ncbi.nlm.nih.gov/pubmed/18510067) HYPERLINK

2008;55(1):87-92.

21. Gazivoda D, Dzopalic T, Bozic B, Tatomirovic Z, Brkic Z, Colic M. Production of proinflammatory and immunoregulatory cytokines by inflammatory cells from periapical lesions in culture. *J Oral Pathol Med.* 2009;38(7):605-11.
22. Swetha P, Ramesh K, Madhavan N, Veeravarmal V, Sameera A. Expression of inducible nitric oxide synthase in the epithelial linings of odontogenic keratocyst, dentigerous cyst and radicular cyst: a pathological insight. *Ann Med Health Sci Res.* 2014;4(4):583–589.
23. Jin RC, Loscalzo J. Vascular Nitric Oxide: Formation and Function. *J Blood Med.* 2010;2010(1):147–162.

