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**ORIGINAL ARTICLE**

# Up-Regulation of Interleukins (ILS) and their Potential Role in the Pathogenesis of Radicular Cyst

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## ABSTRACT

**Objective:** Study the upregulation of Interleukin levels and their potential role in the pathogenesis of radicular cysts
**Study Design:** Cross-sectional study
**Place and Duration:** Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, (9-10 Months)
**Martials and Methods:** Hundred samples were extracted from both diseased and healthy individuals and tissue homogenates were prepared. MDA and LPS were estimated with the help of spectrophotometric method. IL1, IL-6, TNF-α and MMP-9 were determined by their commercial ELISA kits.
**Results:** Malondialdehyde (MDA) was significantly (p=0.033) higher (3.99±0.99 Vs .95±0.011) in diseased group as compared to control. Higher levels of IL-1 (pg/ml) (6.59±1.56 Vs 4.56±1.23), IL-6 (pg/ml) (7.65±1.41 Vs 5.99±0.219) and TNF-α (pg/ml) (31.55±4.55 Vs 20.55±3.28) were recorded in the diseased group. In the group of patients, MMP-9 remained significantly increased when compared with the healthy controls in the case of subjects it remained (93.25±6.48 ng/ml) while in controls they were (47.22±5.29 ng/ml). Likewise in the case of LPS it was significantly increased in the group of radicular cyst (17.25±4.26 pg/ml) as compared to healthy controls (125.25±9.28 pg/ml).
**Conclusion:** Present study indicates the significant correlation of LPS with the pathogenesis of radicular cyst. Moreover, it indicates that as the oxidative stress is increased, it increases the expression of several ILs and MMP-9 that contribute in the pathogenesis of radicular cyst. Further studies may describe the role of lipopolysaccharide and other potential variables to enable better understanding about bone resorption mechanism in periapical cyst development.
**Keywords:** Interleukins, Lipopolysaccharides (LPS), Malondialdehyde (MDA), MMP-9, Radicular Cyst.

## INTRODUCTION

The tooth associated with chronic periapical lesion shows the long lasting process of infection due to a number of microorganisms existing in root canal system and depends on host response[1]. In oral disease, anaerobic gram negative bacteria and endotoxin play a vital role to cause chronic apical periodontitis[2]. Lipopolysaccharide (LPS) released from gram negative bacteria mediates the process of disease around the root apex. In periapical lesion, LPS shows a positive correlation with disease cruelty[3]. Apical periodontitis is a chronic disease initiated by the microbial action on pup tissue causing its death and the movement of bacteria towards the apical periodontal tissue that attaches teeth to the alveolar bone. This morbid condition characterizes local inflammatory response due to the presence of large amount of microorganisms and their distribution from infected root canal towards apical and periapical tissue resulting in bone resorption in periradicular area[4,24].

Microorganisms i.e. gram negative bacteria instead of having severe harmful features and yielding toxic products and bi-products for apical and periapical tissue comprise endotoxin in their outer wall[5]. Bacterial proliferation and death accelerates the release of large amount of endotoxin from their wall which ultimately leads to the inflammation and resorption of bone. Endotoxin comprises of lipids, proteins and polysaccharides and thought to be one of the most effective microbial weapon playing a key role in the development of radicular cyst[6,23]. LPS has no direct effect on tissue damage, it stimulates different cells such as macrophages, neutrophils, fibroblasts, T and B lymphocytes and plasmocytes. These inflammatory cells release various cytokines including interferon, TNF-α, interleukins, different growth factors and prostaglandins[7] which may take part in local tissue injury. Local production of bone resorbing cells and their stimulus are obligatory for alveolar bone loss around the tooth apex. Bone resorption is mediated by different factors such as interleukins (IL-1, IL-6, IL-11 and IL-17), TNF- α, TNF-β, nitric oxide, endothelin, prostaglandins, lipopolysaccharides, reactive oxygen species (ROS) and matrix metalloproteinases[8,22].

In periapical disease, resorption of bone is caused by the cooperation of both innate and adaptive immune response. Polymorphonuclear leukocytes (PMNs), macrophages and lymphocytes are formed in apical periodontal tissue during chronic inflammation and these cells are potent inducer of bone resorbing cytokines (IL-1, IL-6 and TNF-α) stimulating the bone resorption[9]. Activation and generation of osteoclasts influenced by TNF family of cytokines are produced by lymphocytes of adaptive immune response. Evidence suggests that the activation of lymphocytes by the stimulus of antigen in adaptive immune response play a major role in periapical bone loss[10]. Matrix metalloproteinases (MMPs) are zinc dependent endopeptidases that can degrade all types of extracellular matrix. Collagenases (MMP-1, MMP-8 and MMP-13) are important to break the fibrillar collagen and these small fragments of collagens are further degraded by gelatinases (MMP-2 and MMP-9). These MMPs are involved in the destruction of different periapical lesions and peri-implantitis disease[26, 27]. The aim of current study was to investigate the role of lipopolysaccharide, matrix metalloproteinase-9 and inflammatory cytokines in periapical bone resorption during the development of radicular cyst.

## MATERIALS AND METHODS

Hundred (n=100) male tissue homogenates of radicular cyst associated with root canal treated teeth (RCT) and hundred (n=100) normal healthy pulp tissue samples were taken from extracted teeth for orthodontic treatment from the de'Montmorency College of Dentistry Lahore. Inclusion and exclusion criteria of the study remain as the patients having periodontal issues especially radicular cyst were included in the following study while those having any other congenital diseases such as thyroids defects and diabetes were excluded out of the study, all of the work was approved by Research and Ethics Committee at University of Lahore. Tissue homogenates were collected in appropriate

containers and stored at required temperature for their future assay. Levels of ILs, TNF-α, MMP-9 were estimated by their ELISA whereas, MDA and LPS were estimated with spectrophotometric methods respectively[38].

Mean weight (kg), age (years), blood pressure (mmHg), BMI (kg/m$^2$), complete blood count of the controls and patients were estimated that show statistically insignificant change in weight, age, BMI, blood pressure whereas, intensity of Hb, Hct and neutrophils were significantly different in controls and patients.

**Statistical Analysis:** Statistical analysis was done by using SPSS (v.16), applied tests were Independent t-test and Pearson correlation. Results were expressed as (Mean ±S.D) taking ($p<0.05$) as significant.

## RESULTS

Results of table 1 shows the demographic distribution of the variables among the radicular cyst patients. Table shows significant differences among the variables in the patients with radicular cysts when were compared with the healthy individuals. The level (nmol/ml) of Malondialdehyde (MDA) was significantly ($p=0.033$) higher (3.99±0.99 Vs .95±0.011) in diseased group as compared to control. The role of inflammatory cytokines was recognized as medically potential analytical variable and used to establish the degree of disease severity, stage and treatment protocols. The data presented in table regarding tested inflammatory cytokines (IL-1 and TNF-α) depicted that these cytokines play a very important role in the development of a radicular cyst. Higher levels of IL-1 (pg/ml) (6.59±1.56 Vs 4.56±1.23), IL-6 (pg/ml) (7.65±1.41 Vs 5.99±0.219) and TNF-α (pg/ml) (31.55±4.55 Vs 20.55±3.28) were recorded in the diseased group as compared to normal and differed significantly from each other. In the group of patients, MMP-9 remained significantly increased when compared with the healthy controls in the case of subjects it remained (93.25±6.48 ng/ml) while in controls they were (47.22±5.29 ng/ml). Likewise, in the case of LPS it was significantly increased in the group of radicular cyst (17.25±4.26 pg/ml) as compared to healthy controls (125.25±9.28 pg/ml) as shown in table 2.

Table 1: Demographic and Hematological Profile in Patients with Radicular Cyst

| Variables | Control (n=100) | Subjects (n=100) | P-value (<0.05) |
|---|---|---|---|
| WEIGHT (Kg) | 71.33±3.26 | 70.29±6.26 | 0.589 |
| AGE (Years) | 47.21±4.55 | 48.29±4.29 | 0.581 |
| SBP (mmHg) | 120.22±1.99 | 123.26±1.88 | 0.226 |
| DBP (mmHg) | 80.26±4.26 | 81.26±2.26 | 0.4.26 |
| BMI (kg/m$^2$) | 21.26±1.56 | 22.26±4.26 | 0.421 |
| RBC (M/mcl) | 4.16±0.98 | 4.55±0.66 | 0.716 |
| WBC (k/mcl) | 7.16±0.62 | 8.16±1.14 | 0.039 |
| Hb (g/dl) | 13.26±1.55 | 12.56±2.61 | 0.253 |
| PLT (k/mcl) | 299.23±4.26 | 289.23±4.26 | 0.532 |
| Hct (%) | 39.26±2.66 | 46.23±4.55 | 0.032 |
| NEUTROPHILS (%) | 60.99±1.99 | 102.36±2.88 | 0.016 |

Table 2: Levels of Different Variables in Radicular Cyst

| Variables | Control (n=100) | Subject (n=100) | P- value |
|---|---|---|---|
| MDA (nmol/ml) | 0.95±0.011 | 3.99±0.99 | 0.004 |
| IL-1 (pg/ml) | 4.56±1.23 | 6.59±1.56 | 0.019 |
| IL-6 (pg/ml) | 5.99±0.21 | 7.65±1.41 | 0.000 |
| TNF-α (pg/ml) | 20.55±3.28 | 31.55±4.55 | 0.011 |
| MMP-9 (ng/ml) | 47.22±5.29 | 93.25±6.48 | 0.000 |
| Lipopolysaccharides (pg/ml) | 17.25±4.26 | 125.25±9.28 | 0.028 |

## DISCUSSION

In response to gram negative bacterial infection, a large amount of LPS is produced in the pulp canal and around the tooth apex. The existence of LPS may be a prerequisite for the development and progression of periapical lesion and bone resorption because it excites the inflammatory cells to release different proinflammatory cytokines which cause local tissue injury[11]. The magnitude of tissue injury originated by LPS depends on host innate immune response. In periapical area, inflammatory reaction encompasses the recruitment of neutrophils, monocytes, macrophages, lymphocytes, fibroblasts and plasma cells to secrete interleukins (IL-1 and IL-6), TNF-α, iNOS, MMP-9 and ROS[12,19]. After bacterial infection, the initial step of immune response is to recognize the bacterial pathogen i.e. LPS by using pathogen associated molecular patterns (PAMPs). The host having a protein called lipoprotein binding protein (LBP) produced by hepatocytes in the liver during chronic inflammation, binds to LPS and facilitates its transfer towards CD14 receptor[13,21]. CD14 receptors are expressed by macrophages, neutrophils, epithelial cells, fibroblasts and endothelial cells[14,20]. Studies show that the definite receptor of LPS is toll like receptor-4 (TLR4) and CD14 binding with LPS presents it to human myeloid differentiating protein (MD2) and ultimately to TLR4[15,25].

LBP binding with LPS activates CD14/MD2/TLR4 complex which is critical for sensitivity and activation of intracellular signalling pathways to stimulate the production of inflammatory cytokines and chemical mediators[16]. Previous studies show that the activation of nuclear factor kappa B (NFκβ) is crucial for the increased expression of pro-inflammatory cytokines, receptor activator of nuclear factor kappa-B ligand (RANKL) and matrix metalloproteinases. The increased production of proinflammatory cytokines, RANKL and MMPs by LPS is crucial for degradation of the bone and periradicular tissue during cyst expansion[17,18]. Martinho et al. showed that when the size of periapical lesion was compared with the production of IL-1 and IL-6, it was concluded that large sized periapical lesions had increased production of these cytokines[28]. Current study suggests that these cytokines are associated with increased tissue injury at the site of inflammation. Furthermore, IL-1 and IL-6 may directly cause the activation and production of osteoclasts by stimulating the transcription factor NFκB[29]. Wan et al. concluded that TNF-α, IL-1 and IL-6 may stimulate the differentiation of osteoclasts in a synergic way and they may interact among each other to enhance the periapical bone resorption[30, 31].

TNF-α and IL-1 are found to stimulate the increased expression of RANKL on the surface of osteoblasts which is a key factor for competitive binding to RANK on the surface of osteoclast replacing osteoprotegerin (TNF-α vs RANKL, r= 0.644** and IL-1 vs RANKL, r=0.765**) . This binding of RANKL which is a ligand for its receptor RANK initiates the activation of preosteoclasts to mature osteoclasts [32].These activated osteoclasts attach with bone and release acid which demineralize the inorganic part of bone and organic matrix of bone is degraded by matrix metalloproteinases[33]. The oral bacteria present in infected root canal triggers the production of MMPs by macrophages and neutrophils in the periapical area. The aim of root canal treatment is to eliminate bacteria, toxins, virulent factors and chronic inflammation in the peri-radicular area. As the root canal is free from bacteria and its toxins the amount of cytokines, MMPs and inflammatory cells in the periradicular area is also decreased[34,35]. Hence, IL-1, IL-6 and TNF- α dependent periapical bone loss and MMP-9 dependent tissue injury is also decreased. IL-1 secreted by epithelial cells of periapical cyst and inflammatory cells not only upregulates the pro MMP-9 secretion but also activates MMP-9 by autocrine and paracrine signals[36,37]. It suggests that IL-1 dependent upregulatory mechanism of MMP-9 expression degrades the collagen fragments and extracellular matrix. The bone resorption by IL-1, IL-6, TNF- α and MMP-9 favours the expansion of periapical cyst [27]. The present study also represents positive correlation among IL-6, TNF-α and MMP-9 (IL-6 vs MMP-9, r=0.514* and TNF-α vs MMP-9, r= 0.768**). The results of present study support the view that lipopolysaccharide may play a prominent role in the pathogenesis of radicular cyst. It triggers the production of proinflammatory cytokines (IL-1, IL-6 and TNF- α) and MMP-9 from macrophages and other inflammatory cells at the site of inflammation and

stimulates the bone resorption by activation of osteoclastic activity (LPS vs IL-1, r= 0.661** and IL-1 vs MMP-9, r= 0.715**). Further investigation may explore the complex mechanism involved in the development of radicular cyst and periapical bone resorption.

## CONCLUSION

Present study indicates the significant correlation of LPS with the pathogenesis of radicular cyst. Moreover, it indicates that due to increased bacterial toxins in the root canal system the expression of several interleukins and MMP-9 are also overexpressed that contribute the periapical bone resorption and hence take part in the pathogenesis of radicular cyst.

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**Authors Contribution**

**AQ, AS, KA** writing the article and reviewing the sources

**NT, SA, NS** Resourcing materials, practical performance.

**SZ, SW, AM** Conceptualization, performing statistics, reviewing and editing the article.

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