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ASSESSMENT OF EXTRAPOLATIVE VARIABLES AND THEIR IMPENDING INTERPLAY TO DEVELOP RADICULAR CYST

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ABSTRACT

BACKGROUNG

Radicular cyst formation occurs from epithelial residues responsible for tooth development. Septic and necrotic dental pulp releases endotoxin which stimulates thedevelopment of radicular cyst by increasing chemokines and cytokines expression. LPS don't cause tissue damage directly but it stimulate several cells like macrophages, neutrophils, T and B lymphocytes, plasmocytes and fibroblasts that in turn release cytokines named interleukins, interferon, TNF-α, growth factors and prostaglandins which further stimulate bone resorption. During inflammation NO is produced by LPS stimulation and inflammatory cytokines. Xanthine oxidase produces reactive oxygen species during the process of inflammation in the final product of purine metabolism and Nitrotyrosin act as an index of $ONOO^-$ in radicular cyst. Binding of RANKL to its receptor RANK stimulates proliferation, differentiation and formation of osteoclast.

MATERIAL AND METHOD

Forty seven (n=47) samples with radicular cyst and twenty (n=20) samples of normal pulp as control from extracted teeth were substituted in the current study. Oxidative stress biomarkers (SOD, CAT, AOPPs and AGEs), Nitric oxide (NO), Lipopolysaccharides (LPS), were assessed spectrophotometrically. While the activity of inducible nitric oxide synthase (iNOS) and Matrix metalloproteinases (MMPs)were estimated with help of their respective kit methods from tissue homogenates.

RESULTS

The level (nmol/ml) of MDA was significantly (p=0.033) raised in subject as compared to healthy controls (4.11±0.24 Vs 1.37±0.03).Diseased groups had higher levels of iNOS (28.16±5.09 Vs 7.98±1.88IU/ml), Xanthine Oxide (67.8±5.99 Vs 43.98±7.98 IU/mL), Nitro tyrosine (33.09±8.56 Vs 1.78±0.012nmol/L), Lipopolysaccharides (125.25±11.26 Vs 17.25±1.26pg/ml) than control one.

CONCLUSION

Our results indicate that xanthine oxidase and Nitrotyrosin were highly expressed in several cellular types present in radicular cyst. The expression of LPS was positively correlated with disease severity therefore it play crucial role in the formation of radicular cyst. Increased level of sRANKL and decline in OPG were responsible for bone resorption.

Keywords: Matrix metalloproteinases (MMPs),Xanthine oxidase (XO), Lipopolysaccharides (LPS), Inducible nitric oxide synthase (iNOS)

INTRODUCTION

Tooth in the condition of periapical lesion shows long lasting process of infection and disparity between the severity or harmfulness and number of microorganism existing on root canal system with affection to the conflict of the host [1, 2]. In oral disease endotoxinand anaerobic gram negative bacteria play an important role to develop chronic apical periodontitis [3, 4, 5]. Lipopolysaccharide (LPS) is an important endotoxin which mediates bacteria to cause disease. In radicular cyst LPS shows strong positive correlation with disease cruelty [6]. Radicular cyst is a chronic disease caused by the action of microbes of being made impure by poisoning or polluting, death of pulp tissue and the presence or introduction of bacteria and its gradual movement just before the connective tissue that is fibrous in nature and confers the teeth with alveolar bone. The critical condition may be characterized by the inflammatory response that is meditated

along the action of antibodies under the action of excessive microbes and their spread over the infected root canal that extends towards both apical and periapical tissues that ultimately ends up at the resorption of bone and absorption into the circulation of dental cell or tissue [7].

Microorganism i.e. gram negative bacteria that are characterized with a special feature that they have different endotoxins in their outer wall which is responsible in yielding critical features and yielding toxic bi-products for both apical and periapical tissues[8]. Bacterial propagation and death leads to release of endotoxin from their outer wall and cause inflammation and bone resorption [1, 2]. Bacterial endotoxin encompassesproteins,lipids, and polysaccharides and believed to be one of the most active microbial inventor [3]. LPS did not cause tissue damage directly, it stimulate neutrophils, macrophages,T and B lymphocytes , fibroblasts, and plasmocytes to release different cytokines such as interleukins, TNF-α,interferon, various growth factors and prostaglandins [1, 9, 10]. Osteoclast local production and activation is obligatory for alveolar bone loss. Resorption of bone is mediated by various inflammatory cytokines such as TNF- α,TNF-β, several interleukins and some other factors i.e., lipopolysaccharides, endothelin, nitric oxide, prostaglandins, ROS and extracellular nucleotides [11].

In oral infestations bone resorption occurs due to both innate and adaptive immune response. During periodontal chronic inflammation PMNs, monocytes and lymphocytes are formed which upregulate the production of potent inducer to resorb bone such as cytokines (TNF-αand IL-1) which stimulate bone resorption [12]. Lymphocytes of adaptive immune response produces RANKL which is responsible for Osteoclasts formation and activation. Evidence suggests that antigen driven lymphocyte activation in adaptive immune response play central role to cause bone loss in periodontal disease [13, 14]. Nitric oxide (NO) is an inanimate fee radical. It has different physiological role in immune system such as relaxation and vasodilation of smooth muscles, regulate activation of macrophages to inhibit the proliferative reaction of lymphocyte to antigen, modulate different function of leukocyte, apoptosis, regulate inflammation and tissue damage, inhibit DNA synthesis and kill bacteria also. These outcomes suggests that NO has very vital role in cell signaling, dilation of blood vessels and in regulation of immune response. NO has very short half-life and

during oxidation reaction it voluntarily change into nitrite and nitrate.NO can be produced by different ways such as iNOS catalyzed decomposition of L-arginine into NO and L-citruline, due to stimulation by LPS during the process of inflammation and also by inflammatory cytokines. During inflammation ROS can be produced by Xanthine oxidase in the final product of purine metabolism. NO and O_2^-produced during the process of inflammation react in a combination manner and form peroxynitrate ($ONOO^-$) which is also a free radical and cause periodontal tissue injury. Nitrotyrosin act as an index of $ONOO^-$ in radicular cyst [15]. Gingival crevicular fluid (GCF) comprise not only complex array of protein component but are also discharged into the oral cavity. GCF comprisesthe molecule or cell of innate and adaptive immune response or inflammation which has greater contribution in host and bacterial interaction in apical periodontitis and cause resorption of bone [16]. Previous studies specify GCF comprises interferon-α which may have a protective role in periodontal disease because it has the capability to inhibit the activity of IL-1β and as a result no resorption of bone occurs.Osteoprotegerin (OPG), Receptor activator of nuclear factor kappa B (RANK) and Receptor activator of nuclear factor kappa B ligand (RANKL),are the three key member of TNF-α ligand and receptor family which are responsible for osteoclast activation, stimulation and disparity [11].RANKL is also called osteoclast differentiation factor. It shows their expression eitherin soluble form (sRANKL)or as membrane bound protein (mRANKL) by osteoclast or stromal cells. On the surface of osteoclast and preosteoclast cells the interaction of RANKL to its receptor RANK stimulates proliferation, differentiation and formation of osteoclast[17]. Bone marrow stromal cells and osteoblast produce OPG which is circulating protein and bind to mRANKL to inhibit the formation of osteoclast, RANKL/RANK interaction and also prevent stimulatory cell to cell interaction with preosteoclast. Other local periodontal cells such as fibrous connective tissue and gingival fibroblast play very vital role in bone metabolism i.e. bone resorption (bone loss) and ossification (New bone formation). In periodontal disease IL-1 and PGE_2 produced by infiltrate leukocytes mediate the process of inflammation and bone resorption by affecting different cells such as osteoblast, fibrous connective tissue and gingival fibroblast because they express RANKL and OPG. In apical periodontitis chronic

inflammation activated T-lymphocyte produce excessive sRANKL which mediate resorption of bone [18, 19, 20].

MATERIALS AND METHODS

SUBJECTS

The following study was carried out under the guidelines of Institutional review board (IRB) of the University of Lahore. For the evaluation of different markers aboutforty-seven (n=47) samples of radicular cyst of mean age 20-40 years, who attended the OPD (out-patient department) of University College of Dentistry Lahore were substituted in the study. Whereas, twenty (n=20)samples of normal pulp taken from extracted teeth for orthodontic treatment were substituted as control.Informed written consent was obtained from all the participants under study. None of the selected controls were on any medication (i.e., alcohol, multivitamins and smoking), before substitution of the sample they were subjected for their complete history of chronic infections,malnutrition syndrome, depression,psychosis or any type of other metabolic dysfunction (including, liver diseases, cancer or diabetes mellitus) as any of such condition could interfere with their oxidative and thyroid hormone status. Collected samples were properly stored in the containers at their optimum temperatures for their future analysis.

BIOCHEMICAL ASSAY

The used chemicals and regents were of analytical grade and were obtained from Sigma Chemical Co. (St. Louis, Mo, USA).Estimation of markers such as Catalase (CAT), Glutathione (GSH),superoxide dismutase (SOD), Glutathione peroxidase (GPx), Glutathione reductase (GRx), Malondialdehyde (MDA), Vitamins (C and E), Nitric oxide (NO), advanced oxidation protein products(AOPPs), advanced glycation end products (AGEs) andLipopolysaccharides (LPS) were carried out with their respective spectrophotometric methods. While activity of MMPs, iNOS,XO, sRANKL and NT were estimated with their respective ELISA methods.

STATISTICAL ANALYSIS

For statistical analysis SPSS version 19 was used. The data will be expressed as Mean±SD. Student (t) test was used for comparison of schizophrenics and controls. Correlation between different variables was determined by Pearsonian correlation coefficient (r). Thep-values less than 0.05 will be considered significant.

RESULTS

The level (nmol/ml) of MDA was significantly (p=0.033) raised in patients as

compared to healthy controls (4.11±0.24 Vs 1.37±0.03). The levels of antioxidants such as SOD (U/ml), GSH (µmol/L) and CAT (U/L) was significantly (p= 0.003, 0.045 and 0.004) decreased in diseased group as compared to control (0.03±0.01 Vs 0.11±0.03, 4.67±1.76 Vs 7.98±2.76 and 2.29±0.87 Vs 3.98±0.11) respectively. Current study helps illustrate the role of different cytokines and inflammatory markers that are key players in the alleviation of infestation. Levels of inflammatory cytokinesi.e.,TNF-α,IL-1 and IL-6was significantly elevated in diseased group(30.87±5.89 pg/ml,5.65±1.09 pg/ml and 7.65±2.08pg/ml) as compared to control ones (27.98±4.78 pg/ml, 3.26±1.06pg/ml and 3.99±0.011pg/ml respectively). The raised levels of 8-OHdG (pg/ml) (1.03±0.019 Vs 0.01±0.0015), AOPPs (mmol/L) (1.56±0.04 Vs 0.56±0.05), AGEs (AU) (2.31±0.55 Vs 1.66±0.10), MPP-9 (ng/ml) (205.65±7.65 Vs 45.35±8.65), NO (µmol/L) (31.78±10.78 Vs 11.67±3.76), iNOS (IU/ml) (28.16±5.09 Vs 7.98±1.88), Xanthine Oxide (XO) (IU/mL) (67.8±5.99 Vs 43.98±7.98), Nitro tyrosine (nmol/L) (33.09±8.56 Vs 1.78±0.012), Lipopolysaccharides (pg/ml) (125.25±11.26 Vs 17.25±1.26) and Neutrophils (103.99±3.88% Vs 61.89±2.78%) in diseased group were observed as compared to control.

Table 01: Levels of Ciculating Variables In Radicular Cyst

VARIABLES	CONTROL (n=20)	SUBJECT (n=47)	*P*- VALUE
MDA (nmol/ml)	1.37±0.03	4.11±0.24	0.033
8-OHdG (pg/ml)	0.01±0.0015	1.03±0.019	0.043
SOD (U/ml)	0.11±0.03	0.03±0.01	0.003
GSH (µmol/L)	7.98±2.76	4.67±1.76	0.045
CAT (U/L)	3.98±0.11	2.29±0.87	0.004
CRP (mg/dl)	1.01±0.02	1.33±0.30	0.049
IL-1 (pg/ml)	3.26±1.06	5.65±1.09	0.011
IL-6 (pg/ml)	3.99±0.011	7.65±2.08	0.000
TNF-α (pg/ml)	27.98±4.78	30.87±5.89	0.004
AOPPs (mmol/L)	0.56±0.05	1.56±0.04	0.011
AGEs (AU)	1.66±0.10	2.31±0.55	0.055
MPP-9 (ng/ml)	45.35±8.65	205.65±7.65	0.018
NO (µmol/L)	11.67±3.76	31.78±10.78	0.041
iNOS (IU/ml)	7.98±1.88	28.16±5.09	0.021
Xanthine Oxide (XO) (IU/mL)	43.98±7.98	67.8±5.99	0.016
Nitro tyrosine (nmol/L)	1.78±0.012	33.09±8.56	0.022
Lipopolysaccharides (pg/ml)	17.25±1.26	125.25±11.26	0.028
Neutrophils	61.89±2.78%	103.99±3.88%	0.001

DISCUSSION

In response to gram negative bacterial infection a succession of protective appliance is prompted by the incidence of LPS. Existence of LPS is precarious for the progression and conservation of radicular cyst and resorption of bone because it upregulate the production of inflammatory

cytokines to cause local tissue injury [21]. Host response is responsible for the extent of tissue injury initiated by LPS [3, 21]. Due to inflammation in periapical region neutrophils, monocytes, macrophages, fibroblasts,lymphocytes and plasma cells get recruited that then releases different cytokines and inflammatory markers named as Interleukins, TNF-α, MMPs, iNOS and different reactive oxygen species [1, 2, 22]. In the alleviation of infection initially immune response initiates and it identifies bacterial endotoxin (LPS) by the means of PAMPs (pathogen associated molecular patterns). During chronic inflammation hepatocyte secrete a host protein (lipoprotein binding protein) which interact with LPS and stimulate its transfer towards CD14 receptor. [10]. CD14 expression can be seen by various cells such as neutrophils,macrophages, epithelial, endothelialand fibroblasts [23]. Preliminary researches depict and state that receptors for LPS named as toll like receptor-4 (TLR4). These TLRs interact with the lipopolysaccharides with the human myeloid differentiating protein (MD2) and TLR4 [24].

LBP interact with LPS activate CD14/MD2/TLR4 complex which is crucial for the activation of intracellular signaling cascades to upregulate the formation of chemical mediators and inflammatory cytokines [25, 26, 27, 28]. Previous studies exhibited that for the increased production of inflammatory cytokines the activation of nuclear factor kappa B (NF-Kb) is very important or mandatory. LPS by interacting with TLR4 activate Myeloid Differentiation Primary Response 88 (MyD88) pathway and TIR-domain-containing adapter-inducing interferon-B (TRIF). MyD88 binds with IL-1Receptor associated kinase (IRAK) and TNF receptor associated factor 6 (TRAF6) to upregulate the activation oftransforming growth factor-β activated kinase (TAK1) complex. The activation of TAK1 stimulate IκB kinase (IKK) and NF-kappa-B essential modulator (NEMO) which phosphorylate IKB to inhibit KB and allow NF-KB to enter into the nucleus. By the activation of NF-KB pathway proinflammatory cytokines are produced. Mitogen-activated protein kinases (MAPK) is also activated by TAK1 which is required for the phosphorylation of Activator protein 1 (AP-1). NF-KB and AP-1 stimulate the production of inflammatory cytokines, TNF-α, MMPs, iNOS and osteoclast [29, 30]. Previous study shows that MMPs are involved in the pathogenesis of development and expansion of radicular cyst.

MMPs may proliferate the epithelium of cyst and play vital role in the formation of periapical cyst by stimulating the conversion of periapical granuloma to apical periodontitis. LPS upregulate the secretion of inflammatory cytokines to enhance the production of MMPs which play very vital role in bone matrix, epithelial cells and basal membrane degradation during radicular cyst expansion [31, 32]. MMP-13 is secreted by monocytes and macrophages which catalyze extracellular tissue destruction and intracellular phagocytosis [33, 34]. Studies of radicular cyst showed that in the area of inflammation xanthine oxidase and Nitrotyrosin were positive as compared to normal control [15]. Previous studies showed that size and pathological activity of radicular cyst is sustained by an increase in the production of iNOS. During inflammation NO combine with O_2 to form an intermediate molecule called peroxynitrate ($ONOO^{-}$) which is highly toxic in nature and cause mitochondrial damage by upcoming it through extra mitochondrial compartment [35]. sRANKL is osteoclast differentiation factorit binds to its receptor RANKOn the surface of osteoclast and preosteoclast cells and stimulates proliferation, differentiation and formation of osteoclast. A wide range of host and pathogenic factors stimulate immune response which induce the activation of monocyte to increase the differentiation of osteoclast and include increased RANKL expression to cause severe bone resorption and periodontal tissue damage ultimately leads to tooth loss.[11].

CONCLUSION

The most of the previous studies emphasis on the major role of superoxide ($O_2^{\cdot-}$) in the development of radicular cyst. The results of the present study reveal that xanthine oxidase and Nitrotyrosin were highly expressed in several cellular types present in radicular cyst and play a prominent role in the pathogenesis of radicular cyst. Sustained overproduction of NO via iNOS expression act as a cytotoxic molecule against microbial pathogens by releasing more and more LPS possibly leading to tissue destruction and may be responsible for the progression of radicular cyst. RANKL and OPG were also highly expressed that were responsible for bone resorption.

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