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RESEARCH ARTICLE

OXIDATIVE STRESS IN PERIODONTITIS IN HARYANA

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease associated with gram-negative bacteria characterized by connective tissue and alveolar bone destruction. Though many studies have revealed the alterations in the salivary NO and MDA concentrations, desperately only few studies have reported simultaneous estimation of the serum NO and MDA levels. Therefore present study was planned to estimate the level of NO and MDA in patients of periodontitis in population of Haryana.

Methods: A total of 50 subjects were included in the study. Out of these, 25 were healthy controls and 25 were periodontitis patients. The NO level (measured as nitrite-plus-nitrate (NO(x)) concentration) was estimated by Griess reagent method. Serum MDA level was estimated by thiobarbituric acid (TBA) reaction.

Results: Patients in periodontitis group showed a significant increase in serum MDA levels and NO levels when compared to controls ($p < 0.05$ and < 0.001 respectively). As per Pearson's correlation coefficient, serum NO levels were found to be positively correlated with serum MDA levels in periodontitis patients but the correlation was not significant statistically.

Conclusion: Patients with periodontitis show higher systemic oxidative stress and inflammation as compared to healthy controls. NO and MDA can be considered as a biological marker, the presence of which in serum can partially determine the extent of periodontitis. Finally the use of NOS inhibitors and antioxidants can prove beneficial in limiting the progress of disease.

INTRODUCTION

Periodontitis is a chronic inflammatory disease associated with gram-negative bacteria characterized by connective tissue and alveolar bone destruction. These pathogens produce lipopolysaccharides (LPS). LPS activates different cells, including epithelial cells, fibroblasts, neutrophils and macrophages, by activating Toll-like receptor-4 (TLR4) signaling pathways,[1, 2] promoting phagocytosis, production of reactive oxygen species (ROS), cytokines, and release of antimicrobial peptides from azurophil granules.[3] The production of ROS is an essential protective mechanism against diseases associated with phagocytic infiltration. However, overproduced ROS oxidizes DNA, lipids and proteins that contribute to tissue damage.[4] Nitric oxide (NO) and malondialdehyde (MDA), both are parameters of oxidative stress.

NO is a ubiquitous intercellular messenger molecule. It is synthesized by endothelial cells from L-arginine and oxygen. Nitric oxide synthase (NOS) catalyzes the conversion of L-arginine to citrulline and NO in an NADPH dependent manner. There are three isoforms of NOS: the endothelial form (eNOS), the neuronal form (nNOS) and the inducible form (iNOS). eNOS, the calcium-dependent form of the enzyme, is in many cellular types and it is responsible for NO production in healthy blood vessels. nNOS is a special type of NOS, expressed in the central nervous system. Both of them are constitutive and they release small amounts of NO for a short period following the stimulation of their receptors. In contrast, iNOS is expressed in response to proinflammatory stimuli and it produces large amounts of NO for sustained time periods. iNOS, is induced by immunological stimuli and expressed in the myocytes, macrophages and in the endothelial cells.[5]

NO imbalances have been noted in a variety of chronic infectious and inflammatory conditions including periodontal disease. It has been demonstrated by human in vitro studies that iNOS expression and activity is induced in gingival fibroblasts and neutrophils following stimulation by periodontal pathogens, cytokines, and other inflammatory mediators.[6-8] Levels of L-arginine and L-citrulline, the precursors and by-products of NO synthesis respectively, are increased in the gingival tissues of patients with periodontitis. iNOS up-regulation and subsequent peroxynitrite formation

are detrimental to periodontal health via induction of DNA damage and poly ADP ribose polymerase (PARP) activation.[9-11] Also, several studies have demonstrated a protective function of NO, pointing to its role in bacterial killing and clearance of pathogenic organisms, as well as its possible anti-apoptic effects on polymorphonuclear leucocytes (PMNs).[12, 13]

Though many studies have revealed the alterations in the salivary NO and MDA concentrations, desperately only few studies have reported simultaneous estimation of the serum NO and MDA levels. Therefore present study was planned to estimate the level of NO and MDA in patients of periodontitis in population of Haryana.

MATERIAL AND METHODS

The present study was conducted on 50 subjects who attended the outpatient department of periodontics, Government Dental College associated with PGIMS, Rohtak. Out of them 25 were healthy controls (probing depth <3 mm, without bleeding on probing, with no detectable radiographic alveolar bone loss on radiography) and 25 were periodontitis patients (pocket depth ≥ 4 mm and clinical attachment loss of ≥ 4 mm). Patients with history of smoking, alcoholism and chronic diseases such as endocrinal abnormalities, hypertension, malignancy, kidney and liver disease and on any drugs etc were excluded from the study.

After obtaining informed consent from the subjects venous blood was collected from median cubital vein aseptically. Serum was separated and analyzed the same day. The NO level (measured as nitrite-plus-nitrate (NO(x)) concentration) was estimated by Griess reagent method. In this method nitrite reacts under acidic conditions with sulfanilic acid ($HO_3SC_6H_4NH_2$) to form a diazonium cation ($HO_3SC_6H_4–N{\equiv}N^+$) which subsequently couples to the aromatic amine 1-naphthylamine ($C_{10}H_7NH_2$) to produce a red–violet coloured ($\lambda_{max} \approx 540$ nm), water-soluble azo dye ($HO_3SC_6H_4–N{=}N–C_{10}H_6NH_2$).[14] Serum MDA level was estimated by thiobarbituric acid (TBA) reaction.[15]

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20 for windows. Values shown in the text, tables and figures are mean ±SD. Student t test were applied for comparison of means of study groups. p value < 0.05 were considered significant. Correlations between groups were analyzed using Pearson correlation coefficient (r) formula.

RESULTS

The mean age of the patients in periodontitis group was 31.92 ± 12.27 years (14-60) while in healthy controls the mean age was 35.06 ± 11.8 years (18-57). Out of 25 patients 14 were females and 11 were males while there were 12 females and 13 males in control group. Table 1 shows the levels of plasma MDA and serum NO in periodontitis and healthy controls. Patients in periodontitis group showed a significant increase in serum MDA levels (2.060 ± 1.267 *μmol/L*) and NO levels (77.60 ± 17.14 *μmol/L*) when compared to controls (p < 0.05 and < 0.001 respectively). As per Pearson's correlation coefficient, serum NO levels were found to be positively correlated with serum MDA levels in periodontitis patients (p > 0.05, r = 0.027) but the correlation was not significant statistically.

DISCUSSION

Our results revealed increased levels of NO and MDA in periodontitis as compared to healthy controls. In accordance to our findings, studies have revealed increased expression of iNOS[16, 17] and MDA[18-20] in periodontal disease biopsy samples and gingival fibroblast cell culture. Lipid peroxidation, a free radical induced mechanism has been implicated in the pathogenesis of several disorders including periodontal disease. NO has half life of few seconds. It decays into equal amounts of nirite and nitrate, which can be used as indices of nitric oxide synthesis in vitro.[21] In the present study, NO production was measured indirectly using the level of nitrite in serum.

The increased NO production could be due to stimulation of iNOS by LPS of gram negative bacteria of periodontal lesion as periodontitis is a chronic inflammatory disease associated with gram-negative bacteria, including *Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, Bacteroides forsythus* and *Actinobacillus actinomycetemcomitans*. These bacteria produce special enzymes and proteins which induce host inflammation leading to increased concentration of PMNs which are the first and predominant defence cells produced in response to host inflammation. These pathogens along with their products produce oxidative stress and generate free radicals via respiratory burst leading to increased NO levels. ROS cause damage to carbohydrates, lipids, proteins and nucleotides in periodontal tissues which produces structural and chemical alteration in cell membrane[22] and produce lipid peroxidation

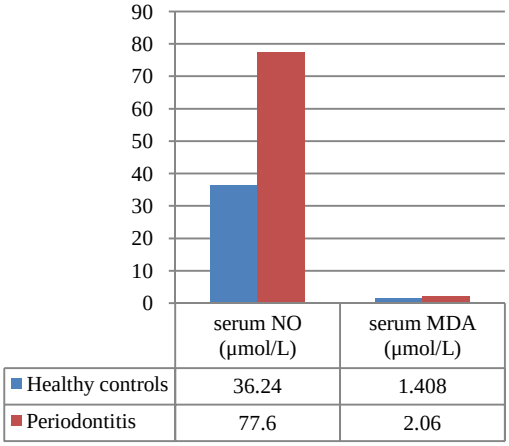

Figure 1 Figure showing levels of NO and MDA in periodontitis patients and healthy controls.

Table 1 Levels of plasma MDA and serum NO in periodontitis and healthy controls

Parameter	Healthy controls (n = 25)	Periodontitis (n = 25)	p value
Serum NO (*μmol/L*)	36.24 ± 7.61	77.60 ± 17.14	<0.001**
Serum MDA (*μmol/L*)	1.408 ± 0.653	2.060 ± 1.267	< 0.05*

*Significant result, ** Highly significant result; all values are in mean ± SD.

products such as MDA. It is a stable end product of peroxidation of lipids by ROS.[23]

Signs and symptoms of periodontitis can also be explained because of increased NO concentration. NO has vasodilatory action so increased concentration leads to increased vascularity, gingival redness and swelling. Bleeding on gentle probing is also due to increased NO as it inhibits platelet aggregation. NO stimulates the activity of osteoclasts which causes increased alveolar bone resorption.[24]

The use of NOS inhibitors such as isosorbid, mercaptoethylguanidine (MEG) and aminoguanidine may prove beneficial in treatment of periodontal diseases by preventing the participation of NO in a variety of pathways. These pathways include modification of prostaglandin and cytokine production, induction of oxidative and peroxidative damage, activation of PARP, and ultimately depletion of cellular energy and cell necrosis.[25] In the modulation of experimental gingivitis in beagle dogs, Paquette and co-workers found that twice daily topical application of gels containing selective iNOS inhibitors, MEG (6mg) or guanidinooethyldisulfide (GED) (6mg), significantly reduced the clinical signs of gingival inflammation, including gingival index scores and bleeding responses at 8 weeks.[26] In 1998, Lohinai demonstrated in a rat model of experimental periodontitis that treatment with the iNOS inhibitor MEG, 30mg/kg intraperitoneally four times daily, reduced inflammatory extravasation and osteoclastic bone resorption at study termination on day 8.[25] Most recently, Leitao and co-workers demonstrated that daily intraperitoneal administration of aminoguanidine and L-arginine methyl ester (L-NAME, a non-selective NOS inhibitor), using 5mg/kg and 20mg/kg respectively, significantly reduced the extent of the inflammatory cellular infiltrate and maxillary alveolar bone resorption in ligature-induced periodontitis in Wistar rats at 11 days.[27] But insufficient or excessive inhibition of iNOS may exacerbate the inflammatory process.

CONCLUSION

Patients with periodontitis show higher systemic oxidative stress and inflammation as compared to healthy controls. NO and MDA can be considered as a biological marker, the presence of which in serum can partially determine the extent of periodontitis. Finally the use of NOS inhibitors and antioxidants can prove beneficial in limiting the progress of disease.

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