Original Article

Levels of nitric oxide in smokers and nonsmokers with chronic periodontitis before and after nonsurgical periodontal treatment

ABSTRACT

Introduction: The purpose of this study was to determine the level of nitric oxide (NO) in both serum and saliva of smokers and nonsmokers with chronic periodontitis before and after scaling and root planing, as well as to compare the same (NO levels) with the severity of periodontitis.

Materials and Methods: Sixty people took part in the study and were divided into three groups. The control group (Group I) involved 20 patients who were nonsmokers with healthy periodontium. The other two groups included 20 patients each, where Group II was nonsmokers with chronic periodontitis and Group III was smokers with chronic periodontitis. In addition, NO generation was quantified indirectly in this study using the Griess reaction to determine the nitrite level in serum and saliva.

Results: The mean value of salivary and serum NO was higher in Group III than in Group II, and NO decreased considerably ($P < 0.01$) in both Groups II and III after treatment compared to before treatment.

Conclusion: Serum and salivary NO levels can be used as a good predictor of the inflammatory condition of the periodontium in smokers.

Keywords: Chronic periodontitis, nitric oxide, smoking

INTRODUCTION

Nitric oxide (NO) is a free radical that plays a variety of organ-specific regulatory roles.[1] NO's role is complicated since it has benefits as an antimicrobial and antitumoricidal agent. However, on the other hand, it can show negative consequences when present in high amounts. NO is produced from L-arginine by NO synthases, which convert L-arginine, nicotinamide adenine dinucleotide phosphate, and O2 into NO and citrulline.[2] Endothelial NOS or NOS-1, inducible NOS (iNOS or NOS-2), and neuronal NOS are the three different types of NO synthase (NOS) (nNOS or NOS-3).[3] These isoforms are classified based on their primary sites of action in the body, inducibility, amounts of NO generation on activation, and calcium dependence.

The antitumor and antimicrobial role of NO is through various mechanisms such as its conversion to peroxynitrite (ONOO-), the formation of S-nitrosothiols, and the depletion of arginine. However, its cytotoxic and other detrimental effects on adjacent host tissues have been known because of different enzymatic activities.[4] Metalloproteinases and collagenases, released by activated macrophages, polymorphonuclear cells, and local fibroblasts, combine with NO to cause toxic effects. NO has also been exhibited to activate both the constitutive and inducible versions of

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the cyclooxygenase (COX) enzyme, increasing prostaglandins synthesis.[5]

NO produced by iNOS has been established to have immunomodulatory, cytotoxic, and antibacterial properties, suggesting that reactive oxygen and nitrogen species may play a role in periodontal tissue injury. Both anti-inflammatory and pro-inflammatory activities of NO are regulated through cytokines as it modulates many humoral and cellular responses during inflammation.[6]

The susceptibility of periodontal diseases in cigarette smokers has been documented due to the high free radicals concentration. Because smoking may increase the incidence of periodontitis, the current study examined the NO levels in smokers and nonsmokers with chronic periodontitis. In addition, the effect of smoking on nonsurgical periodontal therapy outcomes and NO levels was also assessed. The study of NO's role in periodontal disease will help us better comprehend the complexities of periodontal disease progression before and after therapy.

MATERIALS AND METHODS

The research was carried out after approval by the faculty of medicine's Institutional Ethics Committee. The study was an interventional biochemical investigation in smokers and nonsmokers with chronic periodontitis. Salivary and serum NO levels were re-estimated following therapy and after a healing interval in patients. The periodontal state and NO levels in smokers and nonsmokers with chronic periodontitis were examined. The study enlisted 60 participants, both male and female, who were 25 years old or older. The study included a control (Group I) ($n = 20$) with no periodontal disease and two experimental study groups with nonsmokers (Group II) ($n = 20$) and smokers (Group III) ($n = 20$) individuals with chronic periodontitis. Patients assigned to any of the following experimental groups were treated with nonsurgical scaling and root planning. The inclusion and exclusion criteria were fixed before involving any patient in sampling. The individuals had a minimum of 20 teeth, 30% of locations with a periodontal probing pocket depth of 4 mm and CAL of 4 mm for chronic periodontitis patients, and no smoking habit was included in Group II.[7] In Group III, people who had been smoking for at least 6 months and smoked at least 10 bidis or cigarettes per day were included in addition to the same periodontal findings as Group II. The pregnant/lactating women, subjects treated for periodontitis in the past 6 months, patients on antibiotics, anti-inflammatory therapy, vitamin/nutritional supplements, with systemic diseases such as diabetes mellitus, hypertension, renal disease, rheumatoid arthritis, and periapical infection with any tooth or any other form of systemic inflammatory involvement were excluded from the study.

Before obtaining a sample or commencing the treatment, all patients gave their informed consent. The periodontal status of the chosen participants was investigated. The clinical periodontal parameters plaque index (PI), gingival index (GI), probing pocket depth, and clinical attachment level were recorded for all teeth (after 6 weeks) at baseline and 4 weeks following scaling and root planing.

Treatment protocol

Oral hygiene instructions were given to all of the patients involved in the study. In addition, nonsurgical periodontal therapy, which included dental hygiene instructions and supra and subgingival debridement (scaling and root planing), was performed in all the individuals in experimental groups. The treatment plan consisted of four sessions spread out over 2 weeks. Following the completion of scaling and root planing, the periodontal state was re-evaluated 4 weeks later.[8]

For measuring NO, serum and saliva samples were taken at baseline and 6 weeks after nonsurgical periodontal treatment, that is, scaling and root planing. First, 2 ml of venous blood were drawn from patients in all the groups using a disposable syringe and needle in a Vacutainer under strict aseptic conditions. After that, blood samples were centrifuged at 3000 rpm for 5 min in a centrifugation machine for serum separation. Finally, the Griess colorimetric technique was used to determine the biochemical concentration of NO in the obtained serum. The spitting method collected approximately 4 ml of unstimulated saliva from all patients and controls in a sterile plastic vial. The saliva was then centrifuged for 5 min at 2500 rpm. Then, the Griess colorimetric reaction was used to estimate NO in saliva in the supernatant obtained after centrifugation.

The assessment of stable decomposition product nitrite (NO2), using the Griess reaction according to the method of Green *et al*., was used to assess the estimation of NO in serum/saliva (1982).[9] The serum and saliva samples were processed on the same day that the assay was performed. Microwells were filled with 100 ml of standard test samples. In each well, 50 ml of Griess reagent I and 50 ml of Griess reagent II were added and thoroughly mixed by tapping the sides. The absorbance was measured at 540 nm using a Bio-Rad iMark Microplate reader after 10 min of room temperature incubation. The nitrite content of samples was evaluated

using a linear regression equation to generate a standard curve from known standard concentrations and their corresponding absorbance values.

One-way analysis of variance was used to compare groups, and Tukey's HSD *post hoc* test was used to determine the significance of mean differences between groups. STATISTICA (Windows version 6.0) software was used for all analyses into BioEstat (version 4.0; Mamiraua Institute, Belem,Brazil) software was used for all analyses.

RESULTS

Table 1 summarizes the PI, GI, pocket depth, and clinical attachment levels of three groups before and after treatment. The pretreatment means of related parameters in Groups II and III were comparatively greater than in Group I, with Group III having the highest mean. However, after the therapy, mean values in all measured parameters dropped (improved) in Groups II and III. Furthermore, the reduction (improvement) was noticeably greater in Group II than in Group III.

When comparing the pre and posttreatment values of Groups II and III, it was found that they were significantly ($P < 0.001$) different and higher than Group I. When comparing the mean matching values for each group, the PI, GI, pocket depth, and clinical attachment levels in Groups II and III decrease considerably ($P < 0.001$) after therapy compared to before treatment.

Table 2 summarizes the pre and posttreatment NO in serum and saliva of three groups. The mean NO in serum decreased (improved) after the treatment in Group II and Group III. The reduction (improvement) was higher in Group II as compared to Group III. When comparing the mean NO in serum and saliva of Group II and Group III before and after therapy, both groups showed a substantial ($P < 0.001$) decrease ($P < 0.001$) [Tables 1 and 2].

DISCUSSION

The periodontal disease treatment has always been centered on mechanical or chemotherapeutic techniques to eliminate or change the subgingival microbial complexes. Despite their effectiveness in disease control, these modalities were ineffective in halting disease in a significant section of the population. In addition to traditional interventional therapy, host modulation therapy has been shown to interfere with periodontal disease etiopathogenesis.[9] Most host tissue destruction in periodontitis is caused by the excessive or prolonged production of neutrophil enzymes and reactive oxygen species.[10] Modification of host-pathogen interactions is, therefore, critical in the prevention and treatment of periodontal disease. As a result, the research was designed to modify the host's involvement in the illness process.

Several important mediators, such as NSAIDs, low doses of doxycycline, and bisphosphonates, have been studied, with several showing promises results in preclinical and clinical trials.[10-12] NO is currently garnering a lot of interest because of its link to a variety of chronic inflammatory diseases, including periodontal disease.[13] Our research was aimed to highlight or uncover the role of NO in the etiology and pathophysiology of periodontal disease in smokers. To ensure the validity of our study, we measured and compared NO levels in serum and saliva, which were markers of systemic and localized inflammatory alterations, respectively.[14]

Table 1: Various clinical parameters used for clinical examinations of three groups (mean±standard deviation)

Parameters	Group 1 (control)	Group 2 (nonsmokers)		Group 3 (smokers)	
		Pre (n=20)	Post (n=20)	Pre (n=20)	Post (n=20)
Plaque index	0.64±0.18 (0.40-1.00)	1.70±0.19 (1.30-2.10)	0.99±0.19 (0.60-1.40)	1.92±0.18 (1.60-2.20)	1.38±0.26 (1.00-1.90)
Gingival index	0.44±0.10 (0.30-0.60)	1.71±0.21 (1.30-2.20)	0.96±0.19 (0.60-1.50)	1.38±0.18 (1.10-1.70)	0.91±0.11 (0.70-1.20)
Probing pocket depth (mm)	0.84±0.21 (0.40-1.20)	4.35±0.56 (3.40-5.20)	2.45±0.42 (1.80-3.20)	4.59±0.48 (3.80-5.80)	3.43±0.36 (3.00-4.00)
Clinical attachment levels (mm)		6.51±0.40 (5.80-7.20)	3.61±0.44 (3.00-4.40)	6.53±0.41 (6.00-7.20)	4.23±0.48 (3.60-5.20)

n: number of patients in particular group; Numbers in parenthesis indicate the range (minimum–maximum)

Table 2: Pre and posttreatment nitric oxide levels in serum and saliva of two groups

	Group 1 (control)	Group 2 (nonsmokers)		Group 3 (smokers)	
		Pre (n=20)	Post (n=20)	Pre (n=20)	Post (n=20)
Nitric oxide levels in serum	5.33±1.42 (2.77-8.56)	9.38±3.11 (4.47-16.14)	6.48±2.35 (3.37-11.63)	19.03±5.20 (12.23-29.18)	15.31±4.53 (9.93-27.90)
Nitric oxide levels in saliva	27.70±8.04 (13.67-45.36)	79.52±24.88 (40.25-135.82)	57.42±18.73 (33.01-104.66)	153.84±44.04 (99.87-246.81)	132.94±45.79 (91.78-240.85)

n: number of patients in particular group; Numbers in parenthesis indicate the range (minimum–maximum)

The adverse consequences of tobacco use are caused by interfering with vascular and immunologic processes, undermining the periodontal tissues' supportive activities.[15] Due to the high concentration of free radicals in cigarette smoke, it has been suggested that smoking may increase sensitivity to periodontal infections. In periodontitis, cigarette smoke exposure is linked to increased lipid peroxidation and lower antioxidant levels. As a result, it is possible that smoking exacerbates periodontal tissue damage by increasing oxidative stress or burden. In addition, smokers have more bone and attachment loss, deeper periodontal pockets, and more tooth loss than nonsmokers.[16] Tobacco smoke contains nicotine and carbon monoxide, which have a harmful impact on wound healing. That could be one of the reasons for treatment failures and disease relapses, which are more common in smokers.[14]

The smokers' lower reduction in probing pocket depth and clinical attachment gain after treatment fits with prior studies, which found that smoking affects healing and regeneration and that nonsurgical treatment outcomes are less favorable than nonsmokers. The delayed clinical recovery in smokers may be attributed to vascular reactions and impaired immunoinflammatory and fibroblastic responses.

CONCLUSION

Within the limitations of the study, the following conclusion has been drawn:

1. The significantly higher pretreatment NO levels in smokers' blood and saliva samples with chronic periodontitis than nonsmokers indicate that NO can be considered a good indicator of the periodontium's inflammatory condition
2. In smokers and nonsmokers with chronic periodontitis, betterment of periodontal health and dopamine in nitric oxide levels was observed after nonsurgical periodontal therapy during a 4-week healing period.

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Conflicts of interest

There are no conflicts of interest.

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