ESTIMATION AND COMPARISON OF SALIVARY NITRIC OXIDE LEVELS IN SMOKERS AND NONSMOKERS WITH CHRONIC PERIODONTITIS

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ABSTRACT:

Background: *Chronic periodontitis is the most frequently occurring form of periodontitis. Risk factors for periodontitis can be systemic or local. Nitric oxide (NO) is involved in pathogenesis of periodontitis. Smoking is a major risk factor in periodontal diseases. The aim of present study was to determine and compare the salivary levels of NO in healthy controls, smokers and nonsmokers with chronic periodontitis.*

Method: *A total of 120 subjects were involved in the present study and divided into 3 equal groups of 40 control patients, 40 nonsmokers with chronic periodontitis and 40 smokers with chronic periodontitis. Periodontal disease status was determined by recording the plaque index(PI) by Silness and Loe, gingival index(GI), Mean probing pocket depth(PPD) and Mean clinical attachment level(CAL). Saliva was collected from patients by spit method. Nitric oxide activity was estimated by Griess colorimetric reaction.*

Results: *The data was analyzed using statistical software package SPSS 16.0 for Windows version. The full mouth clinical indices values were analyzed by mean and standard deviation, one way ANOVA test. The comparison of the three groups and each parameter was carried out by post hoc (Tukey) test. Analysis of salivary nitric oxide levels in various study groups showed statistically significant difference (P value <0.001) between the control and non smokers with chronic periodontitis. Further, increased NO levels were noted in smokers with chronic periodontitis compared to nonsmokers.*

Conclusions: *This study indicated that salivary NO is increased in patients with chronic periodontitis, which may have a role in disease progression. Additionally increased salivary NO levels in smokers with chronic periodontitis may further contribute to periodontal breakdown. Thus, estimation of salivary NO levels in smokers as well as non smokers with chronic periodontitis may be useful for the progression of periodontal disease.*

Keywords: *Nitric oxide, Smoking, Chronic periodontitis*

INTRODUCTION

Periodontitis is a chronic inflammatory disease. Chronic periodontitis is a multifactorial disease initiated by overgrowth of gram negative bacteria in the dental pocket and leads to the periodontal tissue destruction and tooth loss.[1] The dental plaque consists of enzymes, toxins and metabolites of the bacteria present in it which play a key role in propagating the inflammatory process.[2] Risk factors for periodontitis can be systemic or local with one of the most important risk factors is smoking.[3]

Advances in diagnostic research have demonstrated that periodontal risk can be identified and quantified by objective measures such as biomarkers. Saliva has proved to be a useful diagnostic fluid for oral related diseases. Moreover saliva collection is simple, well accepted by the patient making it a preferred method for epidemiological surveys.[4]

NO is an ubiquitous intercellular messenger molecule which is highly reactive, gaseous, colourless and short lived in nature. It plays a key role in regulating a number of physiological and pathological mechanisms in the body. It is important in host defense and homeostasis, also it modulates the inflammatory response in periodontitis, leading to harmful effects. NO plays an important role in progression of periodontal disease. An increase in the expression of iNOS and NO production are seen in gingival tissue. [5]

NO synthases (NOS) is a collective term for a group of isoenzymes that produce NO in mammalian cells. In mammalian cells. Recent evidence indicates NO related cytotoxicity is attributed more to the formation of peryoxinitrite as compared to NO produced independently [6.] It is an established fact that excessive production of NO or peroxynitrite can lead to cytotoxicity toward the host tissues. [7]

A frequently used measure in the production of NO in the biological fluids constitutes of the stable end products of NO, nitrite and nitrate.[8] It has been postulated that the pharmacological inhibition of NO or its actions may be therapeutically valuable in the disease management. The severity and states of the underlying disease process can be judged by the NO production.[2]

Previous studies investigated the levels of NO metabolites in saliva of periodontitis patients and

have demonstrated a perplexing result with both increased as well as decreased salivary NO metabolites in periodontitis patients.[9,10,11,12,13]

The aim of the study is to evaluate and compare the salivary nitric oxide status in healthy individuals, non smokers with chronic periodontitis and smokers with chronic periodontitis

MATERIALS AND METHODS

The present study included 120 individuals in the age group of 18-60 years, reporting to the Department of Oral Medicine and Radiology. Ethical committee approval of the institution was obtained for the study. Informed consent was obtained from study subjects. Data regarding the personal history, medical, dental, habit history was recorded in a proforma. After screening, the patients were selected for the study. Based on the selection criteria mentioned below, they were divided in 3 groups. Criteria for test group and control group were as follows.

Group A (Control n=40): Nonsmokers and Systemically healthy individuals without periodontitis.

Group B (Test group n=40): Nonsmokers with chronic periodontitis. Patients having probing depth ≥ 5mm and clinical attachment loss.

Group C (Test Group n=40): Smokers with chronic periodontitis. Patients with probing depth ≥ 5mm, clinical attachment loss and smoking history of smoking more than ten cigarettes per day for more than ten years. Smokers were categorized as per the methodology of Queiroz DA et al. 14

Subjects with any chronic illness, on any medication from last 6 months, patients who have undergone or undergoing any periodontal therapy were excluded from the study.

Examination of periodontal disease status: Periodontal disease status was determined by recording the plaque index (PI) by Silness and Loe, gingival index(GI), Mean probing pocket depth(PPD) and Mean clinical attachment level(CAL). PD and CAL were recorded to the nearest millimeter using the Williams graduated periodontal probe at four sites around each tooth (messiobuccal, midbuccal, distobuccal and midlingual), excluding the third molars. One calibrated examiner obtained all the measurements so as to reduce intra-examiner variability.

Collection of samples: Saliva was collected from patients by spit method. Early morning samples were collected and centrifuged at 10,000X g for 10 minutes at 4 degrees Celsius and the supernatants were stored immediately at -20 degrees Celsius to be used later to determine the nitric oxide levels activity.

Nitric oxide level will be estimated by Griess reaction method: Nitric oxide concentration was measured as total nitrates and nitrites (NO_2+NO_3) by the Griess reaction method. Absorbance is read at 550nm. Concentration is determined using standard graph.

RESULTS

The sample size of this study was 120, with a mean age of 32.000 ± 9.602 years, which were divided into three equal groups i.e. group A, being the control(n-40) , group B –non smokers with chronic periodontitis(n-40), group C- smokers with chronic periodontitis (n-40). Our study involved 73 males and 47 females. Details are given in table 1. The average of the full mouth clinical parameters i.e. Plaque Index (PI), Gingival Index(GI), Mean probing depth (PPD) and Mean clinical attachment loss (CAL) were measured. The levels of salivary nitric oxide was calculated for all subjects. The data was analyzed using statistical software package SPSS 16.0 for Windows version. The measurements of full mouth clinical indices are depicted in figure 1. The clinical parameters were analyzed using one way ANOVA test. Post hoc (Tukey) was used to carry out the in between comparison of the three groups and each parameter which is shown in Table 2.The salivary nitric oxide levels in various study groups is shown in table 3 and figure 2. Clinical parameters such as PI, GI, CAL, PPD were compared between the groups. A statistical significance was observed between all the three parameters (p value <0.05). Posthoc tukey test analysis was done to determine the statistical significance between the subgroups.

The comparison of plaque index between only between group B and group C showed statistical significant difference (P value - 0.001). Similarly the comparison of gingival index in group A and group B showed statistical significance (P value - 0.000). Group C and group A comparison also showed statistically significant value(P value - <0.000). However group B and group C did not show statistically significant difference(P value - 0.991). These results indicate a much higher GI score in diseased patients when compared with controls. Mean probing pocket depth values when compared between the three groups (group A, group B, group C) showed statistically significant values. In the case of mean clinical attachment level , group A and group B comparison as well as group A and group C comparison revealed statistical significant difference (P value - <0.000). Values in group B and group C were also found to be statistical significant (P value - 0.006). Analysis of salivary nitric oxide levels in various study groups showed the statistical significant difference (<0.001) between each group (table 3 and figure 2)

Table 1: Number of males and females in the three study groups

	Males	Females	total
Group A	15	25	40
Group B	27	13	40
Group C	31	9	40
			120

Table 2: Post hoc test to determine which subgroup is statistically significant

Dependent Variable	Group I	Group J	Mean difference(I-J)	P value
Plaque index	Group A	Group B	-0.05675	0.535
		Group C	-0.31025	0.000
	Group B	Group C	-0.25350	0.000
Gingival index	Group A	Group B	-0.51625	0.000
		Group C	-0.52500	0.000
	Group B	Group C	-0.00875	0.991
Mean Probing pocket depth	Group A	Group B	-1.53875	0.000
		Group C	-2.20325	0.000
	Group B	Group C	-0.66450	.000
Mean clinical attachment level	Group A	Group B	-1.8995	0.000
		Group C	-2.39625	0.000
	Group B	Group C	-0.49675	0.006
Salivary nitric oxide levels	Group A	Group B	-6.14025	0.000
		Group C	-7.96075	0.000
	Group B	Group C	-1.82050	0.040

P value<0.05 is statistically significant, GROUP A-control , GROUP B- nonsmokers with chronic periodontitis , GROUP C- smokers with chronic periodontitis

Table 3: Salivary nitric oxide levels (IU/ml) in different study groups

	GROUP A	GROUP B	GROUP C	P VALUE
Salivary nitric oxide	17.8472±3.98703	23.9875±1.81809	25.8080±3.69512	<0.001

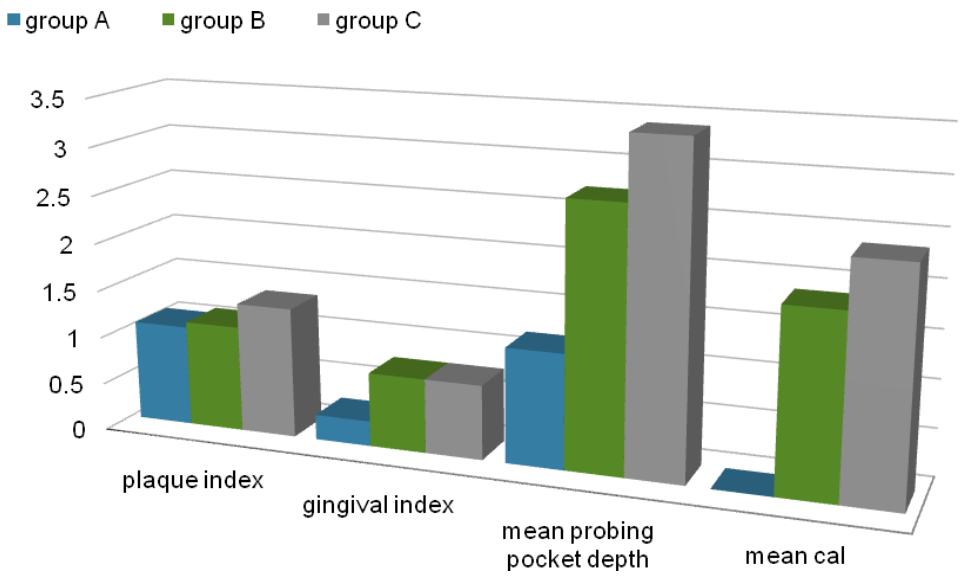

Fig. 1: Full mouth clinical indices in three study groups

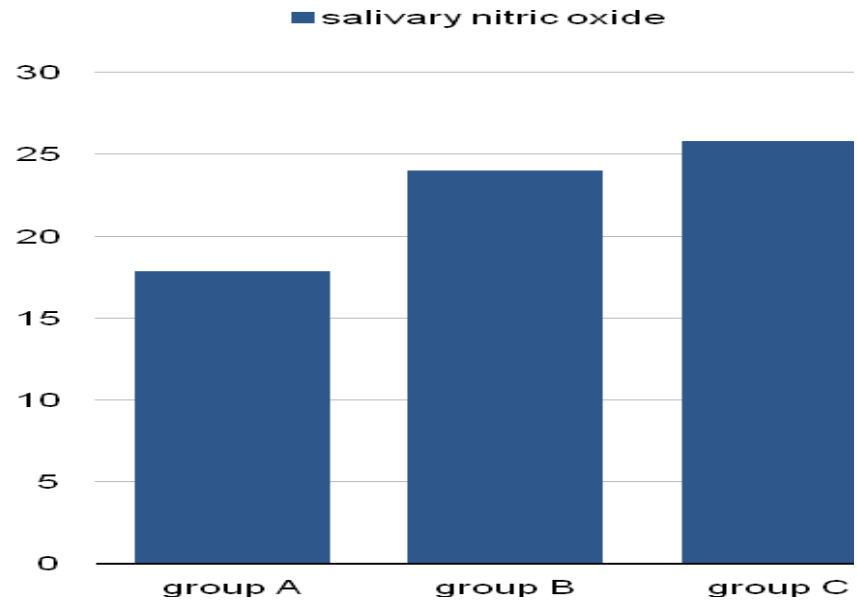

Fig. 2: Salivary Nitric Oxide Levels in different groups

DISCUSSION

Periodontal diseases have multifaceted modes of pathogenesis, characterized by inflammation and bone loss.[15] Destruction of periodontal tissues caused by periodontitis has been reported to be either by host or microbial derived enzymes including collagenase, arginase, nitric oxide synthase. [11]

Total of 120 subjects were involved in our study, of which 40 subjects were control patients, 40 non smokers with chronic periodontitis and 40 smokers with chronic periodontitis. The age range of our study subjects was 20 to 52 years with the mean age of 38 years. The total number of females in our study is 47 and73 males. This difference in the gender values may be attributed to the fact that smoking is largely seen in males as compared to females. Levels of NO were found to be higher in males than in females.[1] Our study does not analyze the variations of nitric oxide levels with regard to gender due to discrepancy in number.

A study carried out by Rathnayake N et al [4] indicates that certain salivary biomarkers can be used as markers for oral diseases within the saliva samples. Salivary biomarkers may also be used to determine the progression of periodontal diseases.[16] Saliva collection is noninvasive and is well accepted by patients.[17]A positive correlation between the

increase in NO levels in the saliva as well as in serum of smokers with chronic periodontitis was shown in a study. [3] Our study focuses on salivary nitric oxide estimation as it is economical and noninvasive in procedure.

Previous studies have stated that nitric oxide levels are affected by smoking. Therefore our study includes both smokers as well as nonsmokers. Nitric oxide plays an important role in the progression of periodontal diseases. Studies conducted by Batista et al [18] demonstrated a significant increase in the inducible Nitric Oxide synthases levels in periodontitis patients as compared to gingivitis samples. Another study conducted by Menaka et al[2] also revealed subjects with periodontitis had significantly higher levels of nitrite in serum than in healthy subjects.[2] Our study results were also in accordance to these studies.

Although many studies show an increase in salivary nitric oxide levels in periodontitis patients. A study by Aurer et al[10] found low levels of nitric oxide in periodontitis patients. This inconsistency in the nitric oxide levels in saliva may be attributed to the fact that the composition of saliva is very complex, and blood, GCF, food debris, bacteria are only a few of its components.[19] Thus, NO levels in saliva may be affected by various intrinsic or extrinsic factors due to which, in our study patients were instructed to rinse the mouth with 10ml water prior to saliva collection. They were also instructed not to consume any food or liquids 2 hours before sample collection.

Our study also demonstrated a significant difference in the NO levels amongst groups B and C. Although both these groups consisted of patients with chronic periodontitis, group B comprised of non smokers where as group C, of smokers, there by attributing the increase in levels of NO to smoking amongst these groups. Adverse effects of smoking undermine the supportive functions of the periodontal tissues and also interfere with the vascular and immunologic reactions. [19]A wide variety of ROS is depicted as the cause for smokers having high levels of oxidant stress. A study carried out by OjimaM[20] indicates that exposure to cigarette smoke in periodontitis leads to lipid peroxidation. A study states that the interaction between NO and hydrogen peroxide leads to the formation of an active molecule responsible for the lipid peroxidation. [21] Previous study mentioned that smoking acts as the perpetrator in enhancing the oxidative burden in periodontitis.[22]

The determination of the role and levels in NO in periodontitis can be used as an approach for the treatment of periodontitis. Inhibition of high levels of NO and increasing the levels of antioxidants through local drug delivery can be used as a treatment modality for periodontitis in smokers. [23] However, more studies need to be conducted for a better understanding of the nitric oxide mechanism in periodontal damage and for further exploration of new treatment approaches.

CONCLUSIONS

Present study revealed increased salivary nitric oxide levels in patients with chronic periodontitis as compared to the control group. Furthermore, the salivary nitric oxide levels were increased in smokers with chronic periodontitis. Study indicated that smoking does manifest undesirable effects on the periodontium. Thus, estimation of salivary NO levels in smokers and non-smokers with chronic periodontitis may be useful for the detection of progression of periodontal disease.

REFERENCES:

1. Andrukhov O, Haririan H, Bertl K, Rausch WD, Bantleon HP, Mortiz A et al. Nitric oxide production, systemic inflammation and lipid metabolism in periodontitis patients: possible gender aspect. J ClinPeriodontol 2013;40(10): 916-923.
2. Menaka KB, Ramesh A, Thomas B, Kumari NS. Estimation of nitric oxide as an inflammatory marker in periodontitis.J Indian Soc Periodontol.2009;13(2):75-78.
3. Wadhwa D, Bey A, Hasija M, Moin S, Kumar A et al. Determination of levels of nitric oxide in smoker and non-smoker patients with chronic periodontitis. J Periodontal Implant Sci.2013 43(5):215-220.
4. Rathnayake N, Akerman S, Klinge B, Lundegren N, Jansson H, Tryselius Y et al. Salivary biomarkers of oral health:a cross sectional study. J ClinPeriodontol.2013; 40(2):140-7.
5. Rausch-Fan X, Matejka M. From plaque formation to periodontal disease, is there a role for nitric oxide?. Eur J Clin Invest 2001:31(5):833-835.
6. Lohinai ZM, Szabo C. Role of nitric oxide in physiology and pathophysiology of periodontal disease. Med Sci Monit.1998;72:1089-95.
7. FostermannU ,Schimdt HH, Pollock JS, Sheng H, Mitchell JH, Warner TD. Isoforms of NOS, characterization and purification from different cell types. Biochem Pharmocol.1991;42(10):1839-57.
8. Sundar NM, Krishnan V, Krishnaraj S, Hemalatha VT, Alam NM. Comparison of the Salivary and the Serum Nitric Oxide Levels in Chronic and Aggressive Periodontitis: A Biochemical Study. J ClinDiagn Res.2013;7(6):1223-7.
9. Hegde S, Nunes C, Harini K, Babu S, Kumari S, Ajila V. Estimation of Salivary Arginase levels in smokers and non-smokers with chronic periodontitis: A biochemical study. Dent Oral Craniofac Res, 2015;1(1):15-18.
10. Aurer A, Aleksic J, Ivic-Kardum M, Aurer J, Culo F. Nitric oxide synthesis is decreased in periodontitis.JClin Periodontol.2001;28(6):565-568.
11. LeylaOzer, ElgunSerenay, OzdemirBurcu, PervaneBeste, OzmericNurdan. Arginine-Nitric oxide-Polyamine metabolism in periodontal disease. Journal of Periodontology.2011;82(2) 320-328.
12. Reher VG, Zenobio EG, Costa FO, Reher P, SoaresRV.Nitric oxide levels in saliva increase with severity of chronic periodontitis..J Oral Sci .2007;49(4):271-276.

13. Parwani SR, Chitnis PJ, Parwani RN. Salivary nitric oxide levels in inflammatory periodontal disease disease - a case control and interventional study.Int J Dent Hyg 2012 ;10(1):67-63.
14. Queiroz DA, Cortelli JR, Holzhausen M, Rodrigues E, Aquino DR, et al. Smoking increases salivary arginase activity in patients with dental implants. Clin Oral Investig 2009; 13: 263-267.
15. Di Beneditto A, GiganteI, ColucciS, Grano M.periodontaldisease: linking the primary inflammation to bone loss. Clin Dev Immunol,2013;2013:503754.
16. Nomura Y et al. Salivary biomarkers for predicting the progression of chronic periodontitis. Arch Oral Biol.2012 Apr;57(4):413-20.
17. Haririan H, Laky M, Matejka M, Andrukhov O,X Rausch. Fan. smoking increases salivary histamine levels in periodontital disease. Oral diseases 2012;18,410-416.
18. Batista AC, Silva TA, Chun JH, Lara VS. Nitric oxide synthesis and severity of human periodontal disease. Oral Dis. 2002;8(5):254-60.
19. Kaufman E, Lamster IB. The diagnostic applications of saliva-A review. Crit Rev Oral Biol Med 2002;13(2):197-212.
20. Ojima M, Hanioka T. Destructive effects of smoking on molecular and periodontal disease. TobInduc Dis.2010;8:4.
21. Epperlein MM, Nourooz-Zadeh J, Noronha Dutra AA, Woolf N. Nitric oxide in cigarette smoke as a mediator of oxidative damage. Int J Exp Pathol.1996;77(5):197-200.
22. Dhotre PS, Suryakar AN, Bhogade RB. Oxidative stress in periodontitis. Eur J Gen Med.2012;9:81-84.
23. Gadage JS, Chava VK, Reddy VR. Green tea extract as a local drug therapy on periodontitis patients with diabetes mellitus: A randomized case-control study. J Indian Soc Periodontol. 2013;17(2):198-203.