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# Effect of Periodontal Therapy on Salivary Nitric Oxide Levels in Chronic Periodontitis Patients with and without Hypertension

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**ABSTRACT**

**Background:** Nitric Oxide (NO) is a multifunctional signaling molecule involved in the maintenance of metabolic, cardiovascular homeostasis and a potent endogenous vasodilator that suppresses the formation of vascular lesions in atherosclerosis. Through nitrate-nitrite reduction and some commensal oral bacteria can supply bioactive NO, which is essential for the endothelial cell function and regulation of arterial BP, it is thought that a decreased quantity of oral nitrate-reducing bacteria and an increased quantity of pathogenic bacteria are responsible for a correlation between oral hygiene and chronic periodontitis and at a later stage cardiovascular diseases(CVD).

**Aim:** To evaluate the effect of periodontal therapy on salivary NO levels in chronic periodontitis (CP) patients and chronic periodontitis patients with hypertension.

**Materials and methods:** A total of 60 subjects with age group of 20-55 years male and female subjects, divided into three groups, 20 each. Group I– control (Healthy), group II–CP (chronic periodontitis) and group III–CP with Hypertension. Subjects underwent clinical examination for Plaque Index, Gingival Bleeding Index, Probing Pocket Depth, Clinical Attachment Level using UNC15 probe and Blood pressure measurement using Sphygmomanometer. Salivary nitric oxide concentration detected using **human $^{N}$ Strips** developed from University of Texas health science. All the parameters were evaluated at baseline and 4weeks after initial periodontal therapy.

**Result:** Four weeks after initial periodontal therapy both groups II and III exhibited improvement in clinical periodontal parameters (OHI-S, GI, PPD, CAL) and at baseline salivary NO levels were depleted in group III and low in group II, after 4 weeks salivary NO was low and adequate respectively however, statistically significant differences were observed only with OHI-(S) and salivary NO values in both groups II and III ($p < 0.05$). However, when the post-treatment clinical periodontal parameters and salivary NO level of groups II and III were compared, statistically significant differences ($p < 0.05$) were observed except for PPD and CAL between the groups.

**Conclusions:** Study revealed depleted salivary nitric oxide levels in periodontitis with hypertensive group and low levels of nitric oxide levels with chronic periodontitis patients as compared to the control group at baseline and the NO levels were improved after 4 week in both the groups II and III improving the blood pressure levels.

Salivary Levels NO can be utilized as a indicator for assessment of the periodontitis as well as hypertensive patients. Periodontal therapy is effective in improving salivary levels of NO in both the groups.

**Clinical significance:** A co- relationship exists between periodontal disease and hypertension. NO is found to play a significant role in the pathobiology of both CP and hypertyension. Initial periodontal therapy seems to be beneficial in improving salivary NO levels along with periodontal parameters in CP patients with or without hypertension. However further studies are warranted to enhance our knowledge about the role of NO in periodontal diseases in the course of hypertension.

**KeyWords:** Hypertension, Periodontal Therapy, Periodontitis, Salivary Nitric oxide.

## I. INTRODUCTION

The role of oral microbiome in determining blood pressure levels has been linked to the active role of some bacterial species involved in the nitro-reducing process. [1,2] The oral bacterial species traditionally involved in the nitrate-nitrite converting process belong to the Veillonella, Actinomyces, Rothya, Stphylococcus and Propionibacterium species [3,4]; however, commensal Neisseria species are also well-known as powerful nitrate reducers [5]. Several studies state that nitric oxide (NO) produced in the oral cavity is a powerful resource for the human body, especially when NO-syntethase production is not adequate [6,7]; in fact, it is known that NO is a powerful systemic vasodilator [8-10], and its low concentration was considered a relevant cardiovascular risk factor and NO is essential for various physiological processes, such as homeostatic functions(including vasodilatation, inhibition of platelet adhesion and aggregation, regulation of vascular tone, and neurotransmission), host defense against infectious agents (such as bacteria, fungi, and parasites)and tumour cell killing [11,12].

As an uncharged molecule, NO passes freely across the cell membrane, inducing cell-to-cell communication [13].Unlike other intercellular messengers, NO shows no binding capability to receptors. Its half-life is in the order of seconds and its effects are transient and local [14]. Although NO is important in host defense and homeostasis, it also causes harm and has been associated with the pathogenesis of numerous inflammatory and autoimmune diseases [15-17]. In the literature, the association between periodontal disease and systemic inflammation and oxidative stress gains research attention [18]. As the NO production is associated with inflammatory diseases, it has been recognised as a marker of inflammation [19]. In different studies, the salivary levels of NO3, NO2 and NO of patients with periodontitis have been reported to be higher than those of patients with gingivitis and healthy individuals [20, 21,22]. On the other side, the effect of periodontal treatment on salivary NO levels remains controversial [20,23, 24 ]. Hence this study aimed to analyse the salivary NO levels in the chronic periodontitis (CP) patients with or without hypertension before and after initial periodontal therapy.

## II. MATERIALS AND METHODS

The study population consisted of 60 subjects aged 30-60 years, and were categorized into three groups (n = 20): Group-I (Healthy controls), Group-II (patients with CP), Group-III (Patients with CP and hypertension) patients for this study was selected from the Outpatient Department of Periodontics, Informed consent was taken from the selected subjects prior to the start of the study. Approval for the study was obtained from the Institutional Human Ethics Committee, College of Dental Sciences, Davangere, Karnataka India, in accordance with the Helsinki Declaration of 1975, as revised in 2000.

**2.1Inclusion criteria**: Who fulfill the criteria of chronic periodontitis given by 1999 classification, of age 30-60 years males and females, who fulfil the criteria of hypertension given by international society of Hypertension (2020), Pocket depth > 5 mm and Clinical Attachment Level > 4 -5 mm.
**2.2Exclusion criteria**: any periodontal therapy in past 6 months, any antibiotic and steroids 6 months prior to sampling, any systemic conditions other than hypertension, smokers. Pregnant and lactating women.

Sphygmomanometer device was used to check the patient Blood pressure,3readings was taken at 1 min intervals and average of 2 measurements were considered final, Thomas unger et all, 2020.Clinical periodontal parameters such as Plaque Index (Silness P and Loe H, 1964) Gingival Bleeding Index (Ainamo and Bay,1975), Probing Pocket Depth, Clinical Attachment Level using UNC 15 probe. All the parameters were checked before and after scaling and root planning, oral hygiene instructions were given to the patients.

**2.3SALIVARY NITRIC OXIDE CONCENTRATION LEVEL:** It is detected by using **human $^{N}$ Strips** developed from University of Texas health science centre. This method is non-invasive, the results are instant and easy to read. Easy to display results in just 10—15seconds.The strip is of 3.82 x 1.46 x 1.34 inches (9.7 x 3.7 x 3.4 cm); 0.95 Ounces (26.93 grams).

Patient is instructed to wash the hands then unstimulated saliva is collected in spit method, gather the saliva on the fingertip, place the strips on the fingertip, the results are compared from the colour change seen on the chart given.

## III.STATISTICAL ANALYSIS

The data obtained was analyzed by a software IBM SPSS (version 25). Paired t-test was used to determine the statistical significance within the group comparison (pre and post-treatment). ANOVA was used for multiple comparisons between groups. A post-hoc comparison test to compare as to which particular pairs of groups were statistically different and significance. A p value of < .05 was considered significant for all analysis.

## IV. RESULTS

The sample size of this study was 60, with a mean age of 45.00 ± 9.602 years, which were divided into three equal groups i.e. group I, being the control(n-20), group II – chronic periodontitis(n-20), group III- chronic periodontitis subjects with hypertension(n-20). Our study involved 30 males and 30 females. The average of the full mouth clinical parameters i.e. Plaque Index (PI), Gingival Bleeding 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 25 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. The salivary nitric oxide levels in various study groups is shown in Figure 1. 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 II and group III showed statistical significant difference (P value - 0.001). Similarly the comparison of gingival index in group I and group II showed statistical significance (P value - 0.001). Group III and group I comparison also showed statistically significant value (P value - <0.001. These results indicate a much higher GI score in diseased patients when compared with controls. Mean probing pocket depth values when compared between the group III did not show statistically significant values at baseline and after 4 weeks. In the case of mean clinical attachment level, group I and group II comparison as well as group I and group III comparison revealed no statistical significant difference (P value - <0.05). Analysis of salivary nitric oxide levels in various study groups showed the statistical significant difference (<0.001) between each Groups before and after initial treatment. The Nitric oxide was depleted in all the patients belonging to group III ‘chronic periodontitis with hypertension ‘low = chronic periodontitis, optimal =healthy group at baseline and after 4 weeks of therapy salivary nitric oxide levels were improved as shown in the fig 1.

## V. DISCUSSION

Nitrate-reducing oral bacteria have a direct role in the enterosalivary pathway of NO production and mediate the BP effect of dietary nitrate, boosting oral and plasmatic NO3 bioavailability. Nitrate/nitrite-reducing bacteria are cooperating synergistically creating an optimal oral bacterial community for NO generation, they are

protective on cardio metabolic health. Contrary to this periodontal pathogenic bacteria associated with an increased risk of adverse cardiovascular events[25]. Studies have investigated shared risk factors between periodontal disease and hypertension, such as age, gender, smoking, educational level, socioeconomic status, obesity and diabetes [27]. NO is also secreted by inflammatory cells in the oral cavity26], the NO levels may be used as markers of inflammation, disease severity and pathogenesis, requiring reliable and sensitive analytical techniques for quantitation of NO production and iNOS activity [28].

Analysis of NO can be performed by different direct and indirect methods, including gas and liquid chromatography, electron paramagnetic resonance and mass spectrometry. The reduced implementation of these methods for evaluation of biological samples [28, 29]in studies has been linked to short half-life, low in vivo concentrations of NO, unsuitableness for the clinical settings due to instrumentation requirements and inexpedience in processing large numbers of samples, To overcome these challenges, stable metabolites of NO are measured, such as NO2 and NO3, with NO2 as the only stable end product of the autoxidation [28]

Researchers in multiple targeted populations studies suggested that periodontal disease significantly increased the risk of hypertension by 1.50 times[odds ratio (OR) = 1.50, 95%CI = 1.27–1.78]. According to aurer at al, it is not clear whether decreased NO in periodontal disease is simply a marker of increased inflammatory activity and tissue destruction or whether it is causative in the pathogenesis of the disease. Aim of our interventional study was to see the levels of NO in chronic periodontitis patients with and without hypertension and results found significant association between periodontal disease and hypertension risk and association remained significant after seeing the salivary nitric oxide levels and periodontal parameters which was significantly reduced after initial periodontal therapy and study shows the association exists between periodontal disease and decreased salivary NO levels and hypertension. Even though both the groups in our study consisted of patients with chronic periodontitis, group III comprised of hypertensive patients, there by showing depleted levels of NO amongst these group. The depleted levels of NO amongst 2 subjects in group II showed undiagnosed case of hypertension they were later sent to the physician for the treatment.

Previous studies investigated the levels of NO metabolites in saliva of periodontitis patients and have found a varying result with both increased as well as decreased salivary NO metabolites in periodontitis patients [23,20,30,]. Cesar et al proved that Erythrocytes and whole blood nitrite levels diminished after periodontal treatment. Our study also finds a co-relation with aurer et al that showed local NO production is decreased in patients with periodontitis. [31] Studies also have suggested that polymorphonuclearleukocytes (PMNL) from patients with gingivitis and periodontitis suppress NO production and bioactivity (Akopov& Kankanian 1996). PMNL isolated from periodontal pockets were found to have a stronger suppressive effect as compared with PMNL from venous blood. [23,24].
The use of Strip method in our study to detect salivary NO levels may be useful for early intervention, diagnosis or chair side evaluation for individuals at risk for pre-hypertension/hypertension and however the present study used small sample size due to difficulties in the selection of patients and follow-up. Authors suggest different periodontal conditions are required to define the role NO levels in the management of periodontal diseases including larger sample size for further studies.

Table 1 **Intra group comparison of the index scores in each of the three groups using paired t-test.**

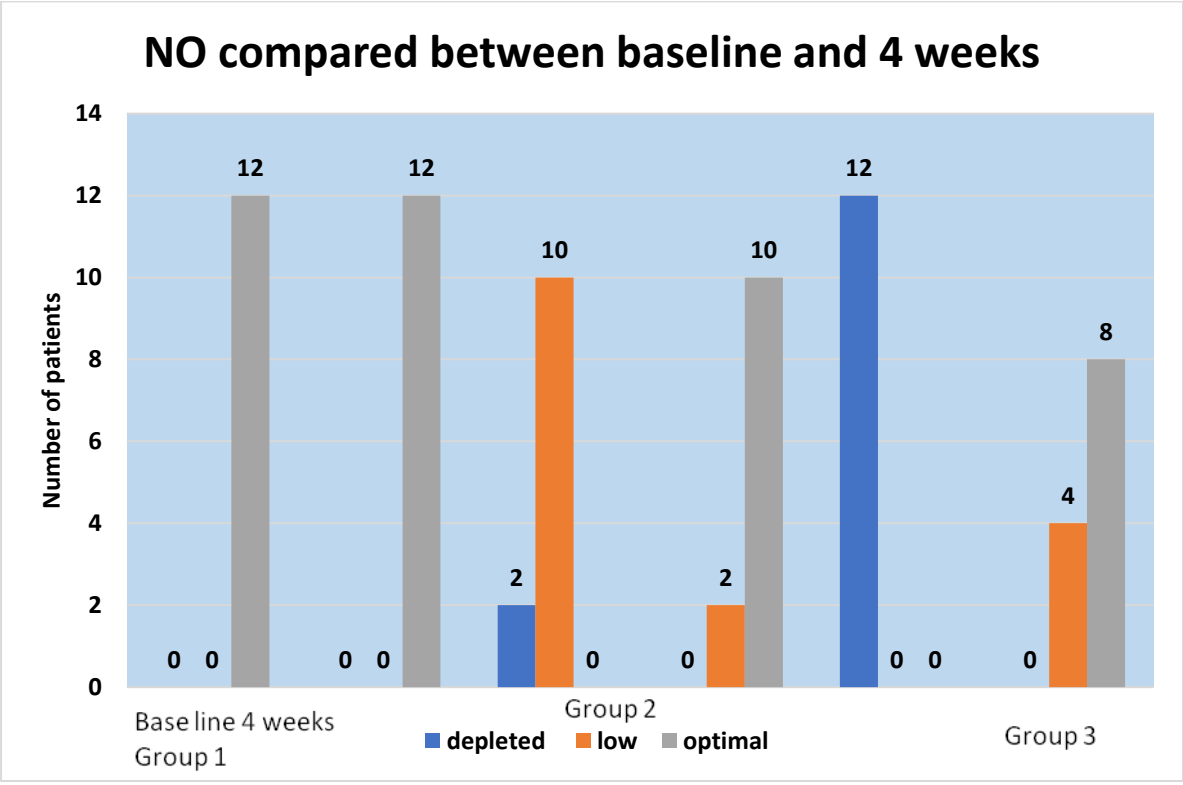
| **Index** | **Time** | **Group 1** | | **Group 2** | | **Group 3** | |
|---|---|---|---|---|---|---|---|
| | | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| Plaque index | Baseline | .32 | .29 | 2.47 | .26 | 2.50 | .41 |
| | 4 weeks | .128 | .07 | 1.12 | .22 | 1.16 | .32 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | P value | .033 | | <.001 | | <.001 | |
| Gingival bleeding index | Baseline | 20.83 | 17.94 | 85.42 | 16.71 | 87.50 | 13.05 |
| | 4 weeks | 4.17 | 9.73 | 29.17 | 20.87 | 31.25 | 11.30 |
| | P value | .001 | | <.001 | | <.001 | |
| Probing pocket depth | Baseline | 3.25 | .75 | 6.92 | 1.240 | 7.17 | 1.58 |
| | 4 weeks | 2.92 | .66 | 6.42 | .900 | 7.00 | 1.47 |
| | P value | .039 | | .007 | | .166 | |
| Clinical attachment loss | Baseline | .00 | .00 | 5.17 | 1.193 | 5.75 | 1.13 |
| | 4 weeks | .00 | .00 | 4.92 | .996 | 5.67 | .98 |
| | P value | - | | .082 | | .339 | |

The plaque index and gingival bleeding index scores reduced significantly from baseline in all the three groups, whereas the probing pocket depth reduced only in the control and chronic Periodontitis groups, but not in those patients with chronic periodontitis and hypertension. The clinical attachment loss scores did not vary from baseline in any of the three groups.

**Figure 1**

**Comparison of NO at baseline and at 4 weeks in each of the three groups**



## VI. Conclusions

To conclude, our interventional study had shown a possible link between hypertension and salivary NO levels and periodontitis, increase in salivary nitric oxide levels may be due to improvement in clinical periodontal parameters and there is decrease in blood pressure. The use of Strip method to detect salivary NO levels may be useful for early intervention, diagnosis or chair side evaluation for individuals at risk for pre-hypertension/hypertension. Evidences are required to find out whether changes in oral microbiome is a consequence or an important causal factor for the pathogenesis of hypertension. Future studies are needed to define mechanisms by which the oral microbiota and NO act on cellular targets to prevent or contribute to the pathogenesis/development of hypertension.

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