Evaluation of co-relation between Hypertension and Salivary Nitric Oxide Levels in Chronic Periodontitis and Chronic Periodontitis Patients with Hypertension

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Abstract

Background : Nitric oxide   an ubiquitous, highly reactive, gaseous, colourless, intercellular messenger molecule which might play role in pathogenesis of both periodontitis and hypertension. Hypertension is a common condition in India which exerts substantial public health burden on cardiovascular health status and healthcare systems in India,hence it is important to study weather periodontal disease is having role in the development of hypertension and vice-versa.

Aim: Aim of our study was to find out   salivary nitric oxide levels in periodontitis and hypertension and their correlation with each other.

Materials and methods:
A total of 60 subjects divided into three groups, 20 each.Group I– Healthy -control, group II–CP and group III–CP with Hypertension.Subjects underwent for Clinical periodontal parameters Plaque Index, Gingival Bleeding Index, Probing Pocket Depth,Clinical Attachment Level using UNC15 probe. Blood pressure measurement using Sphygmomanometer. **human ^{N} Strips** developed from University of Texas health science centre used to detect Salivary nitric oxide concentration.

Results: The data was analyzed using statistical software package IBM SPSS 25. The full mouth clinical indices values were analyzed by mean and standard deviation, one way ANOVA test. The comparison of the 3 groups and each parameter was carried out by post hoc (Tukey)test. Analysis of salivary nitric oxide levels in 3groups showed statistically significant difference (P value <0.001) between the control and chronic periodontitis patients with and without hypertension.

Conclusions
Present study revealed depleted salivary nitric oxide levels in patients with chronic periodontitis with hypertension and low levels of nitric oxide levels with chronic periodontitis patients as compared to the control group. Study indicated that salivary nitric oxide and blood pressure levels has undesirable effects on the periodontium. Thus estimation of salivary NO levels in chronic periodontitis with and without hypertension may be useful for the detection of progression of periodontal disease. Salivary nitric oxide strips can be used as chair side evaluation method.

Keywords: Hypertension And Periodontitis, Salivary Nitric Oxide Levels In Chronic Periodontitis

Introduction

Nitric oxide (NO) is a gaseous free radical with a short biological half-life and it is physiological messenger molecule involved in various physiological processes, such as the regulation of vascular tone, inhibition of platelet aggregation, neurotransmission and immune response[1]. It is

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produced by the specific enzyme nitric oxide synthase (NOS) through the oxidation of L-arginine[2].In inflammatory reactions,pro inflammatory cytokines lead to expression of inducible NO synthesis in monocyte,macrophages,neutrophil granulocytes and other cells, in case of bacterial infection endotoxine is another strong inducer of expression of NO [3];The overproduction of NO acts as inflammatory mediator which can lead to tissue destruction. Depending on the concentration of NO levels it acts as pro-or anti inflammatory effects hence it is called double –edged sword or Jekll and Hide [4].

Chronic periodontitis is "an infectious disease resulting in inflammation within the supporting tissues of teeth, progressive attachment loss and bone loss"[5].Hypertension is defined when patient has elevated systolic blood pressure value greater than 140 mm Hg or diastolic blood pressure greater than 90 mm Hg.[6]It is a major global health disorder expected to be increased to 1.56 billion by the year 2025[6]. Hypertension has a complex etiology, with more evidence suggesting an increased role of inflammation in the development of this condition. As both hypertension and periodontitis have an inflammatory component these diseases may be associated and they share common risk factors, such as increasing age, smoking, stress and socio-economic status acting as confounding factors for association of the two diseases[7].NO might play role in pathogenesis of both periodontitis and hypertension.

Studies evaluated the association between periodontitis and hypertension, with different results, Holmlund et al[8] showed association and Buhlin et al[9] found no association. There are controversial results observed in some studies where they have investigated the levels of NO metabolites in saliva of periodontitis patients, including decreased and increased salivary levels of NO metabolites. Aurer et al [10],Reher et al,Ozer et al[11].,Parwani et al[12],Pamelo barbadoro et al[13] proved to have an association between oral microbiome and salivary nitric oxide levels and its relation to hypertension.

NO levels studies has been assessed using Griess reaction method, fluorescent probes, Strips method etc. but using Strips studies are not available in literarture.With our interest keeping that in mind, no study has been done to assess the level of NO using Strip method in literature, hence this study was to find out salivary nitric oxide levels in periodontitis and hypertension and their correlation with each other.

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**.**

Inclusion criteria: Who fulfill the criteria of chronic periodontitis given by AAP classification 2017,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. Exclusion 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 pt 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.

SALIVARY 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 finger tip,the results are compared from the colour change seen on the chart given (Fig 2).

Statistical Analysis

The data which was obtained was subjected to a statistical analysis by using IBM SPSS version 25.

1. Mean and standard deviation for each measured parameter.

2. ANOVA analysis of variance to determine the difference in the nitric oxide levels in different groups.

3. 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.

Results

The salivary nitric oxide levels in various study groups is shown in table 3 and figure 2.The nitric oxide levels were depleted with group III and low in group II and optimal in group I . The comparison of plaque index between group I and group II 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). However group II and group III did not show statistically significant difference(P value - 0.981). These results indicate a much higher GI and PI score in diseased patients when compared with controls. Mean probing pocket depth values when compared between the three groups (group I, group II, group III) did not show statistically significant values with group II group III. In the case of mean clinical attachment level , group I and group II comparison as well as group I and group III comparison revealed statistical significant difference (P value - <0.001). Values in group II and group III were also found to be statistical not significant (P value - 0.304).The blood pressure was increased with group III and group II, 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)

Discussion

Saliva is useful diagnostic fluid and well accepted by the patient making it a preferred method for studies, so we used saliva as medium to check the levels of nitric oxide.The relationship between oral health and the body's ability to produce nitric oxide is mainly part of a system known as an enterosalivary sequence. Nitric oxide (NO) levels in the oral cavity and saliva have been associated with various oral diseases. There are two main sources of salivary NO in the oral cavity; chemically from physiological reduction by the enzyme nitrate reductase, released by certain bacteria, that converts nitrate (NO_3-) to nitrite (NO_2-). The other is through a reduction process facilitated by certain bacterial products resulting from the dental plaque microflora, forming nitrogen oxide and nitric acid.[14]

Advances in diagnostic research have been demonstrated that periodontal risk can be identified and quantified by objective measures such as biomarkers like NO. Periodontal disease are chronic inflammatory infections associated with gram –ve bacteria ,including Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans which stimulate NO production.(15).NO may activate proinflammatory enzymes such as cyclooxygenase and metalloproteinases, which, in turn, may also contribute to periodontal tissue damage.

In periodontitis, iNOS expression plays a beneficial role may include antimicrobial activity, immune modulation, inhibition of microvascular thrombosis, as well as increased tissue perfusion.[16] On the other hand, iNOS expression plays a detrimental effects may include a cytotoxic action towards the host tissues, including the alveolar bone.

Pro- or anti-inflammatory properties may vary according to NO concentration, the potential for the formation of toxic derivatives, the site of the pathological process, and the adaptive response of the target cell.[17]

In this study comparison of plaque index between group I and group II 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). However group II and group III did not show statistically significant difference(P value - 0.981). These results indicate a much higher GI and PI score in diseased patients when compared with controls. Mean probing pocket depth values when compared between the three groups (group I, group II, group III) did not show statistically significant values with group II group III. In the case of mean clinical attachment level , group I and group II comparison as well as group I and group III comparison revealed statistical significant difference (P value - <0.001). Values in group II and group III were also found to be statistical not significant (P value - 0.304) and salivary nitric oxide levels were depleted with group III and found low levels in Group II and optimal with group I, our study was in accordance with a study by Aurer et al[10] found low levels of nitric oxide in periodontitis patients. Although many studies show an increase in salivary nitric oxide levels in periodontitis patients, study conducted by Menaka et al[17] revealed subjects with periodontitis had significantly higher levels of nitrite in serum than in healthy subjects.

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[9,10,11].Cesar et al[18] proved that Erythrocytes and whole blood nitrite levels diminished after periodontal treatment.

Studies in humans and animals have shown that the mechanisms by which the oral microbiota and NO act on cellular targets play a role to prevent or contribute to the pathogenesis/development of hypertension, including changes in the immunologic, hormonal and metabolic homeostasis [13].Different authors have reported the significant role of dental disease in influencing blood pressure levels [19], for instance, poor oral hygiene could lead easily to chronic periodontitis,that is well-known associated with hypertension [20-21]. Our study results also illustrated the influence of nitric oxide (NO) in hypertensive disease significantly higher level of NO was found in normotensive patients and depleted levels in Hypertensive with chronic periodontitis cases.

The results obtained from our study is important for early intervention, diagnostics and therapies for subjects with hypertension or periodontitis. Our study was the first to use strip method to detect salivary NO levels in periodontitis as well as periodontitis patients with hypertension. Study method was easy, noninvasive, most economical and can be used for chair side evaluation.Evidences is must to see whether change in oral microbiome is a consequence or an important causal factor for the pathogenesis of hypertension. So it is necessary to conduct further studies.

Conclusions

Present study revealed low levels of nitric oxide levels with chronic periodontitis patients as compared to the control group,depleted salivary nitric oxide levels in patients with chronic periodontitis with hypertension . Study indicated that salivary nitric oxide and blood pressure levels has undesirable effects on the periodontium.Thus estimation of salivary NO levels in chronic periodontitis with and without hypertension may be useful for the detection of progression of periodontal disease.salivary nitric oxide strips can be used as chair side evaluation method. In future, research can be done to study progression of periodontitis in isolated as well as associated cases with hypertension .

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

	MALES	FEMALES	TOTAL

GROUP I	10	10	20
GROUP II	10	10	20
GROUP III	10	10	20
			60

Table 2: Post-hoc comparison of the Indices.

Index			
	Group 1	Group 2	P value
Plaque index	1	2	<.001
	1	3	<.001
	2	3	.981
Gingival bleeding index	1	2	<.001
	1	3	<.001
	2	3	.946
Probing pocket depth	1	2	<.001
	1	3	<.001
	2	3	.875
Clinical attachment loss	1	2	<.001
	1	3	<.001
	2	3	.304

post-hoc analysis revealed that all the four index scores were significantly greater for the chronic periodontitis and chronic periodontitis with hypertension patients when compared to those of controls. P value<0.05 is statistically significant, GROUP A-control , GROUP B- chronic periodontitis , GROUP C- chronic periodontitis with hypertension.

Table3: Inter group comparison of salivary nitric oxide levels and blood pressure .

Group	N	Nitric oxide levels of 3 groups			Chi square	Mean blood pressure (mmhg)
	%	Depleted	Low	Optimal	(P value)	
1	N	0	0	12	61.71 (<.001)	120/80
	%	0.0%	0.0%	100.0%		
2	N	2	10	0		148/82
	%	16.7%	83.3%	0.0%		
3	N	12	0	0		162/92

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	%	100.0%	0.0%	0.0%		

The nitric oxide was depleted in all the patients belonging to group 3= 'chronic periodontitis with hypertensionpatients,significantly lower levels in groupII- Chronic periodontitis and optimal in Group I- healthy subjects.

Fig 1:Mean scores values of PI,GI,PPD,CAL of all 3 groups.
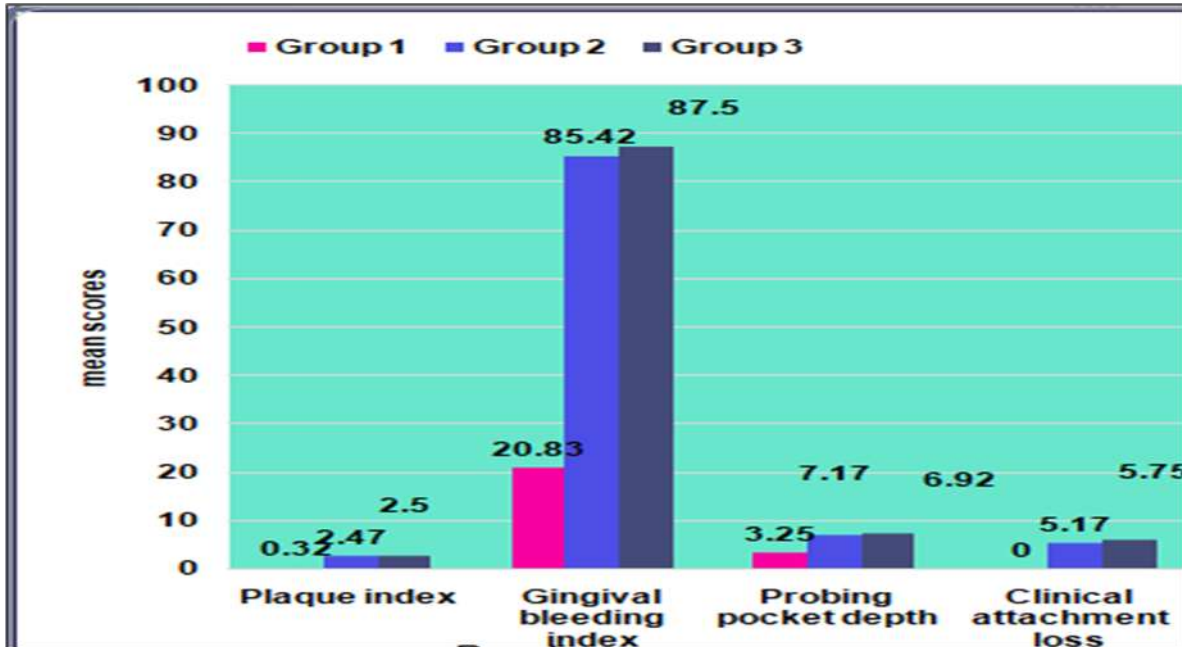
Fig 2: salivary nitric oxide levels chart.

Fig 3: Collection of samples.