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Research Article

RELATIONSHIP OF SALIVARY LEVEL OF NITRIC OXIDE IN OBESE PATIENTS WITH CHRONIC PERIODONTITIS:A BIOCHEMICAL STUDY

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ABSTRACT

Background: Nitric oxide (NO), a free radical gas, is a noxious chemical in the atmosphere acts as a physiological and pathophysiological mediator and it plays an important role in the biological systems. The levels of nitric oxide may provide intimation about the severity and the state of the underlying disease process. Hence, the aim of the present study was to assess the salivary levels of nitric oxide in obese patients with and without chronic periodontitis and non obese healthy patients. **Methods**: A total of 45 patients were divided into 3 groups. Group 1: Non‑obese healthy, Group 2: obese without chronic periodontitis and Group3: obese with chronic periodontitis. Whole saliva samples were collected, and NO levels were evaluated by using the griess method. The results were analyzed by SPSS and Mann–Whitney analysis. RESULTS: The highest nitric oxide levels from the Saliva were detected in group 3 while the lowest were detected in group 1. A significant difference in nitric oxide levels in the saliva was found when group 1 and group 2 was compared with group 3 (P<0.0001).**Conclusion**: The level of nitric oxide was higher in the saliva of chronic periodontitis patients. Nitric oxide may be used as an inflammatory marker for detection of periodontal disease.

Key words:- Nitric oxide, Obesity, Periodontitis, Saliva.

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INTRODUCTION

Periodontal disease is a chronic disease of the oral cavity comprising a group of inflammatory conditions affecting the supporting structures of the dentition.[1] The development of periodontitis cause a series of clinical manifestations including gingival bleeding, periodontal pocket formation, alveolar bone absorption, and eventually teeth loss.[2] The association between periodontal diseases and systemic conditions appears to be attributable to a low‑grade inflammatory

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burden that links them through a common pathophysiologic mechanism.[3] The progression of chronic periodontitis (CP) is mediated by both bacterial invasions and host immunoinflammatory responses which plays a critical role in the loss of connective tissue and the supporting alveolar bone.[4]

The World Health Organization has recognized obesity as a predisposing factor for major chronic diseases ranging from cardiovascular disease to cancer.[4] Obesity is characterized by the presence of chronic subclinical inflammation with increased concentration of proinflammatory mediators.[5] It is a condition similar to overall inflammation, occurring with metabolic and immune characteristics, rendering one susceptible to periodontal disease.[6]

Saliva is used as a diagnostic tool to evaluate various biomarkers associated with periodontal disease.[7] Assessment of the composition of saliva may provide valuable information about biochemical markers for assessment of periodontal diseases.[8]

Nitric oxide (NO) is an intercellular messenger molecule which shows its pervasiveness along with many applications in cardiovascular, neurological and immune functions.[9] Nitric oxide, a free radical gas, is a noxious chemical in the atmosphere, but in small controlled concentrations in the body, it acts as a physiological and pathophysiological mediator and it plays an important role in the biological systems.[10] NO is synthesized from L-arginine by a family of isoenzymes called NO-synthases(NOS).[11] NOS exists as three distinct isoforms, namely, endothelial NOS (eNOS), neural NOS (bNOS), and inducible NOS (iNOS).[12] eNOS and bNOS are collectively known as constitutive (cNOS) and release small amounts of NO for short periods followed by the stimulation of any of the receptor. When compared to cNOS, iNOS is expressed in response to proinflammatory stimuli and produces large amounts of NO for a sustained duration. NO, when produced in greater concentrations proves to be crucial in nonspecific host defense, along with its various cytotoxic activities against fungal, bacterial, and protozoal organisms as well as tumor cells.[13] Nitric oxide have been proved to be potential inflammatory markers. These biomarkers of host response can be found in gingival crevicular fluid (GCF), saliva and serum samples and can potentially be used as diagnostic markers.[14] NO has been linked to the etiopathogenesis of periodontal disease[15,16] and is expressed in salivary glands as well as in their product.[17,18]

In the light of the above facts, the current study was designed with an aim to assess the level of salivary nitric oxide in obese patients with and without chronic periodontitis and non obese healthy patients.

MATERIALS AND METHODS

This case–control, cross‑sectional study was performed from January 2017 to July 2017 in the Department of Periodontology after obtaining ethical approval from the institutional review board of the college. A total of 45 male and female subjects aged between 25 and 50 years participated in this study. Written informed consent was taken from all the participants before the start of the study.

ANTHROPOMETRIC MEASUREMENT

Measurements were taken uniformly according to a standard protocol. BMI was calculated as the ratio of body weight(kg) divided by the square of the height (m). Obesity is usually defined as body mass index. Overweight is defined as a BMI between 25.0-29.9 kg/m^2 and obesity is defined as a BMI of ≥ 30.0 kg/m^2.

The WHO STEPS protocol for measuring waist circumference instructs that the measurement be made at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Waist Circumference is used to measure the body fat distribution, the cut off point for abdominal obesity in men 102 cm and in women 88 cm. The waist hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm).

Participants were categorized into three groups based on the Gingival index(GI), pocket probing depths (PPD), body mass index (BMI) and waist circumference (WC).

• Group 1 (healthy) consisted of 15 individuals with healthy gingiva of probing depth ≤3mm and a GI =<1 mm.

• Group 2 (Obese with healthy gingiva) consisted of 15 individuals (BMI ≥ 30.0) with healthy gingiva of probing depth ≤3mm and a GI =<1 mm.

• Group 3 (Obese with chronic periodontitis) consisted of 15 (BMI ≥ 30.0) individuals who had signs of clinical inflammation, presence of at least 12 teeth, excluding third molars; and a diagnosis of CP with GI > 1and PPD ≥ 5 mm.

EXCLUSION CRITERIA

(1) cigarette smoking or tobacco use and alcoholism; (2) systemic diseases such as diabetes mellitus, hypertension, and rheumatoid arthritis; (3) pregnancy; (4) systemic bacterial, viral, or fungal infection; (5) history of antibiotic therapy or use of anti‑inflammatory medications during the past 6 months; (6) periodontal therapy during the past 2 years; and (7) patients with aggressive periodontitis.

SAMPLE COLLECTION

Subjects were requested to refrain from eating and drinking for at least 2 h before saliva collection. Using the spitting method, unstimulated saliva was collected between 11:00 am and 13:00 pm for 5 min (one spit per minute). The saliva was collected in sterile tubes and the samples were centrifuged at 3,000 rpm for 10 minutes and the supernatant was analyzed. The sample were stored at −70°C until the experiment. The NO level

was measured using Griess method [15] Griess reagent is a mixture of 1% sulfanilamide, 1% naphthylethylene diamine dihydrochloride, and 2.5% phosphoric acid. Griess reagent is very unstable as it reacts with the surface atmospheric nitrogen. Hence, it was freshly prepared before use. 0.5 ml of the prepared standard solutions of sodium nitrite were reacted with equal volumes of the Griess reagent in Eppendorf tubes and they were incubated at room temperature for 10 minutes to ensure that a complete reaction took place. The reaction mixture was then transferred into plastic cuvettes for measurement on a spectrophotometer which was connected to a computer, so that digital readings could be taken. By using these readings which were taken for the standard solutions, a graph of the absorbance versus the concentration was plotted, which constituted the standard curve. In a similar manner, the samples of the 45 subjects were added to the Griess reagent and they were then transferred to the spectrophotometer and their optical densities were recorded.

DATA ANALYSIS

Statistical analyses were performed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA). The salivary level of nitric oxide was compared between the two groups using the Mann–Whitney test and t‑test. $P < 0.05$ was considered statistically significant. Data were presented as mean ± standard deviation.

RESULTS

The mean values of the clinical and biochemical parameters were expressed as mean ± SD (Table 1). The highest nitric oxide levels from the Saliva were detected in group 3 (16.54 ± 2.47 µM), while the lowest were detected in group 1(4.78 ± 1.58 µM) (Graph 1).

Intergroup comparisons of the clinical and biochemical parameters are summarized in Table 2. A significant difference in nitric oxide in the saliva was found when group 1 was compared with group 3 (P<0.0001) and group 2 was compared with group 3(P<0.0001). Statistically significant differences were observed when comparison of gingival index and pocket probing depth scores were made with groups 1 and 2 versus the periodontally diseased groups 3. A significant difference in BMI, WC and WHR was found when group 1 was compared with group 2 and group 3.

Table 1. Values(mean ± SD) of Nitric oxide, PPD, GI, BMI, WC, WHR in the three Groups

Variable	Group 1	Group 2	Group 3
Nitric oxide (µM)	4.78±1.58	5.18±1.82	16.54 ± 2.47
PPD	1.47±0.57	2.0±0.73	6.80±1.21
GI	0.28±0.15	0.35±0.17	2.32±0.49
BMI	21.31±1.68	30.70±5.07	31.65±5.85
WC	73.20±5.84	99.47±8.43	97.24±7.98
WHR	0.76±0.09	0.94±0.12	0.91±0.15

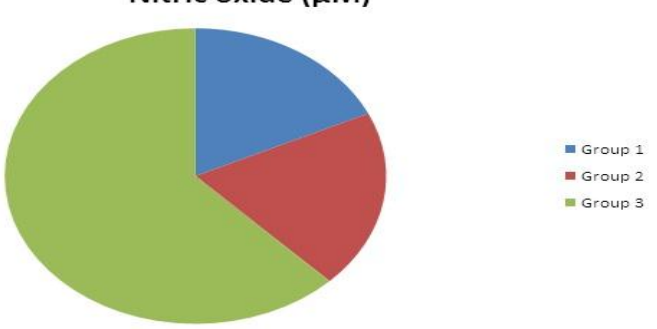
ǂ PPD – Pocket probing depth; GI – Gingival index; BMI – Body mass index; WC – Waist circumference; WHR – Waist‑hip ratio; SD - Standard deviation

Table 2. Consolidated Pairwise Comparison (p value) Among the Three Groups (p<0.05)

Variable	Group	Mean difference (95% CI)	t	df	p
Nitric oxide	Group1 vs Group2	-2.000 (−3694-−0.30)	-2.417	28	0.0224*
	Group1 vs Group3	2.370 (3.58-1.15)	3.991	28	0.0004*
	Group2 vs Group3	4.370 (5.83−2.90)	6.092	28	< 0.0001*
PPD	Group1 vs Group2	-0.530 (−1.01-−0.04)	-2.216	28	0.0350*
	Group1 vs Group3	-5.330 (−6.33-−4.31)	-10.878	28	< 0.0001*
	Group2 vs Group3	-4.80 (−5.83-−3.75)	-9.525	28	< 0.0001*
GI	Group1 vs Group2	-0.070 (−0.18-−0.04)	-1.196	28	0.2418
	Group1 vs Group3	-2.04 (−2.31-−1.75)	-15.418	28	< 0.0001*
	Group2 vs Group3	-1.970 (−2.24-−1.67)	-14.711	28	< 0.0001*
BMI	Group1 vs Group2	-9.390 (−12.21-−6.56)	-6.80	28	< 0.0001*
	Group1 vs Group3	-10.340 (−13.55-−7.12)	-6.580	28	< 0.0001*
	Group2 vs Group3	0.950 (−5.04-−3.13)	0.475	28	0.6383
WC	Group1 vs Group2	-26.270 (−31.69-−20.83)	-9.921	28	< 0.0001*
	Group1 vs Group3	-24.040 (−29.27-−18.79)	-9.415	28	< 0.0001*
	Group2 vs Group3	2.230 (8.36-−3.89)	0.744	28	0.4631
WHR	Group1 vs Group2	-0.180 (−0.26-−0.10)	-4.648	28	0.0001*
	Group1 vs Group3	-0.150 (−0.24-−0.05)	-3.321	28	0.0025*
	Group2 vs Group3	0.030 (−0.71-0.13)	0.605	28	0.5501

*Statistically significant at P<0.05; p – Probability; Independent sample t‑test; CI - Confidence interval, PPD – Pocket probing depth; GI – Gingival index; BMI – Body mass index; WC – Waist circumference; WHR – Waist‑hip ratio

Graph 1: Bar diagram shows the comparison of the nitric oxide values in three groups.



DISCUSSION

The potential role of saliva in the diagnosis of oral and systemic health is evident in researches. Salivary biomarkers could be used to screen periodontal health status and disease progression.[19,20]

Nitric oxide (NO) is a gaseous free radical with a short biological half-life, which is generated enzymatically from L-arginine by a family of the NO synthase (NOS) isoforms. Nitric oxide is a potent modulator of the inflammatory disease processes and under pathological conditions, has damaging effects.[21] The result of the present study revealed that the mean salivary nitric oxide level of group 3 was higher than that of group1 and group 2. The increased levels of nitric oxide in the periodontitis patients were attributed to the fact that there were increased levels of iNOS expressing cells during the inflammation of the periodontal tissue. The finding in this study are in accordance with the studies conducted by Ozar et al,[22] parwani et al,[23] meneka et al,[24] Matejka et al,[25] Kendall et al[26] and Carossa et al.[27] In contrast, a study report of Aurer A et al.,[28] showed reduction of salivary NO levels in the saliva of the periodontitis patients than in the healthy subjects. This may be due to the fact that NO is relatively unstable in the presence of oxygen and that it quickly auto oxidizes to produce nitrogen oxides. Moreover, because of NO's reactivity and short-life, directly measuring NO in the cells and tissues is very difficult.

Patients in group 1 and group 2 showed no statistically significant difference when the GI and PPD were compared. Statistically significant difference was observed when group 1 and group 2 were compared with group 3($p< 0.0001$). A mean probing depth of 6.80±1.21mm and a GI score of 2.32±0.49mm in group 3 established the presence of an active inflammatory component along with a destructive component to the prevalent periodontal disease.

The salivary nitric oxide levels were correlated with obesity measures such as BMI, WC and WHR but this correlation was not significant in Group 1 and Group 2. Further, a significant difference was noted in salivary nitric oxide levels when Group 1 and Group 2 were compared with group 3. Thus salivary nitric oxide levels increased with periodontal inflammation, but obesity did not affect the levels significantly when compared amongst the groups. In this study, the increase in the salivary nitric oxide level in obese patients with periodontitis could be enhanced by the preexisting active inflammation (chronic periodontitis). The possible causal relationship between obesity and periodontitis may be that adipose tissue secretes a variety of cytokines and hormones that are involved in inflammatory processes.[29]

At present there are several biomarkers, studied in relation to periodontitis and obesity. However, there are very few studies addressing the relation between chronic periodontitis and nitric oxide. In the current study, level of nitric oxide was higher in the saliva of chronic periodontitis patients. Nitric oxide may be used as an inflammatory marker for detection of periodontal disease. The limitation of this study was that we did not have information about the level of nitric oxide after treatment of periodontitis. Such information would be useful to understand the role of nitric oxide in periodontal regeneration. Further long-term and interventional studies with larger sample sizes are required to assess the efficacy of this biomarker for early detection of periodontal disease and prevention of its progression. Finally, for considering nitric oxide as biomarkers of inflammation in periodontitis, further studies are needed with more sample size in different populations.

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