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Research Article

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Assessment of nitrosative stress in Syrian patients with chronic periodontitis (Clinical laboratory study)

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ABSTRACT

Nitrosative stress is considered to be a strong feature in chronic periodontitis, It was found that the reactive nitrogen species (RNS) may play a role in periodntitis either by being catalysts or they are in most cases aggravating factors of periodontal disease that is already existing .The aim of this study is to examine the role of Nitrosative stress in periodontitis by assessing the levels of RNS in gingival crevicular fluid (GCF) samples taken from Syrian patients with chronic periodontitis and healthy controls. The study population comprised 35 subjects allocated within two groups: Chronic periodontitis (ChP) group (20 patients, aged 40-75) and Control group (RC) (15 subjects, aged 40-67). Nitrate/nitrite concentration in GCF was measured using Griess reagent. There was no significant difference in Total nitrite/nitrate (GCF) levels between (ChP) and (RC) groups (P> 0.05). Also, no significant correlations were found between the studied marker and the periodontal indices (P> 0.05) .The results of this study showed that the Nitrosative stress was not associated with chronic periodontitis in Syrian patients.

Key words: Nitrosative stress, chronic periodontitis, Nitric Oxide.

INTRODUCTION

Periodontitis is an inflammatory disease of the periodontium which affects the supporting tissues of the teeth; the disease is multifaceted and its etiopathogenecity is still not fully understood and therefore the treatment of different types of periodontal disease can be very difficult [1].

Strong body of evidence accumulated to support a role for RNS in a large number of physiological and pathological processes. Numerous recent studies showed that nitric oxide (NO) takes part in the etiopathogenesis of many diseases, including periodontal disease [2].

NO has been implicated either as trigger agents or more frequently, aggravators of the primary lesions in periodontitis [3] .

NO is synthesized from L-arginine by a family of enzymes called nitric oxide synthases. NO is a short living product of nitrogen metabolism, produced by many cells in the organism. [4]

Endothelial and neural cells constitutively produce NO. Furthermore, macrophages and other inflammatory cells can induce its synthesis and release. The most important inductors of NO synthesis are bacterial products [5].

NO is relatively unstable in the presence of oxygen and quickly auto-oxidize to produce nitrogen oxides[6].

Because of NO's reactivity and short-life, direct measurements of NO in cells and tissues are very difficult [7].

Although NO metabolites have a very short life, nitrate and nitrite are the relatively stable end products of NO oxidation. The total levels of nitrite and nitrate in biological fluids are generally used for adequate monitoring of the NO synthesis[8].

Nitrosative stress (NS) is defined as the ratio of nitrosants to antioxidants that has a value >1 [9].

Recent studies have examined NO metabolites levels in samples from saliva, in gingival crevicular fluid (GCF) and serum, (GCF) is seemed to be a more reliable source for the identification of periodontal disease. This presumption is based on that it is only affected by periodontal tissues surrounding the teeth comparing to the whole saliva that is secreted from the major salivary glands and therefore composed of GCF at a lower extent. Moreover, whole saliva may be more affected by systemic inflammatory and infectious conditions[10]

In a recent study, Abou Sulaiman & Shehadeh (2010) found that serum total antioxidant capacity (TAC) was lower in non-smokers Syrian patients with chronic periodontitis compared to healthy controls. Also, they reported that periodontal treatment restored TAS levels to normal levels similar to healthy controls.

The aim of this study is to assess the levels of RNS in gingival crevicular fluid (GCF) samples taken from chronic periodontitis patients and healthy controls. Subsequently, correlating these levels with the severity of periodontal disease in Syrian patients.

EXPERIMENTAL SECTION

A total of 35 subjects have been invited to participate in this study from the patients referred to the Department of Periodontology, Faculty of Dentistry, University of Damascus. The study has been approved by a specific Review Board. Subjects have been recruited according to specific inclusion criteria after completion of medical and dental history questionnaires. All subjects were of Syrian descent, systemically healthy, and had at least 20 teeth. Subjects were excluded from the study if 1) they had a course of non-steroidal anti-inflammatory drugs or antimicrobial drugs within a 3-month period before participation in the study; 2) were pregnant or lactating; 3) had used mouthwashes or vitamin supplements within the previous 3 months; 4) had a history of current or previous smoking or of recreational drug use; and 5) had special dietary requirements. All patients have signed a consent form after being advised about the nature of the study.

The selection of patients was made according of the criteria approved by the 1999 international world workshop for a classification system of periodontal diseases and conditions (Armitage 1999).

Subjects have been allocated into two groups:
- Chronic Periodontitis group (ChP): comprises 20 patients aged ≥ 40 years and have presence of ≥2 non-adjacent sites per quadrant that were not first molars or incisors, with probing depth (PD) ≥5 mm, which bleed on gentle probing. They demonstrated radiographic bone loss ≥30% of the root length. Patients also had poor oral hygiene and the amount of accumulated plaque commensurate with the amount of clinical attachment level (CAL).
- Resistant Control group (R): comprises 15 age-sex matched subjects who are ≥ 40 years, exhibit no signs of periodontal disease as determined by the absence of the evidence of interproximal (CAL ≤ 1mm), PD > 3 mm at any site, whole-mouth bleeding scores <10% and have no clinical signs of gingival inflammation . [11]

Clinical measurements:
A standard periodontal probe (UNC-15) has been used for recording periodontal indices at six sites per tooth. The examined clinical parameters include bleeding on probing (BOP), plaque index (PI), clinical attachment loss (CAL), periodontal pocket depth (PPD) and gingival index (GI).

Collection of samples
Subjects have been asked to refrain from brushing within 1 hour of sampling. Sites were isolated with cotton rolls and gently air-dried before sampling. GCF samples were obtained using standard paper strips (Periocol strips, Oraflow, NY, USA). 6 samples were collected from each individual (mesiofacial, distopalatal) sites from each examined tooth (incisor, premolar and molar) in the maxilla (Chapple et al. 2002). Paper strips were inserted into the collection sites until light resistance was felt and samples were harvested after 30 seconds. Subsequently, strips were put in PBS Buffer solution for 30 minutes then extracted and stored under minus 80.

Laboratory studies:
The stable end products of NO (nitrite and nitrate) were analyzed by Griess reagent using Nitric Oxide Colorimetric Assay Kit (BioVision, USA). It provides an accurate convenient measure of total nitrate/nitrite in a simple two-step

process. The first step converts nitrate to nitrite utilizing nitrate reductase. The second step uses Griess Reagents to convert nitrite to a deep purple azo compound. The amount of the azochromophore accurately reflects nitric oxide amount in samples.[12],

85 µl of GCF samples were mixed with 5 µl of the Nitrate Reductase and 5 µl of the enzyme cofactor using a micro plate, then incubated at room temperature for 1 hr to convert nitrate to nitrite. 5 µl of the enhancer was added and incubated for 10 min. Then, 5 µl of Griess reagent R1 and 5 µl of Griess reagent R2 were added. Later, After 10 minutes of incubation at room temperature, the absorption of each sample in microplate wells was determined at 540 nm [12]. Eventually, A standard curve was prepared using nitrate standard to calculate nitrite concentration in GCF.

Calculations: absorbance was plotted at 540 nm as a function of nitrate/nitrite concentration.

C = Sa/Sv = nmol/µl or mM nitrate (nitrite)

Where: Sa is sample amount from standard curve (in nmol).
Sv is sample volume added to the assay well (in µl) nmol/µl or mM nitrate (nitrite)

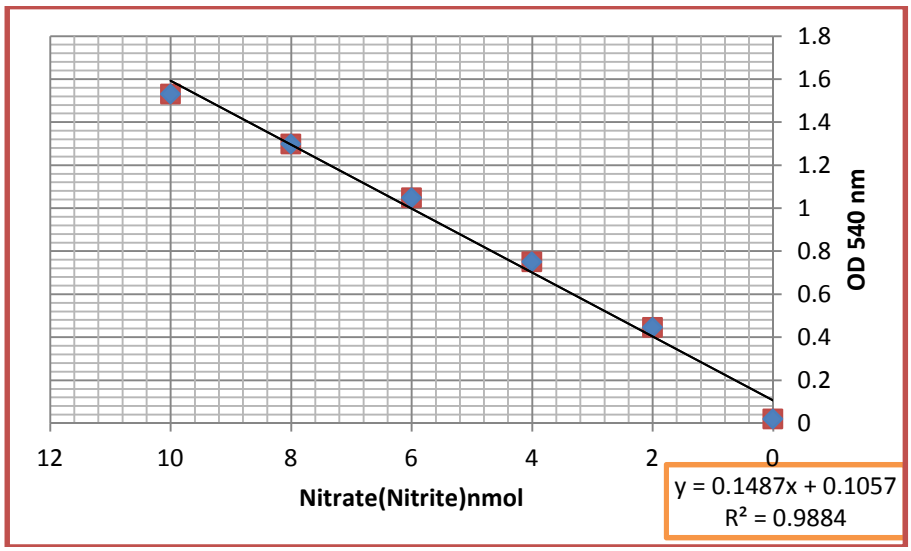

Chart (1) Standard Curve

Statistical Analysis: SPSS (version 17.0, Chicago, IL) was used to process the collected data and statistical testing. The concentration of NO levels was made by using Mean± STD.

Mann - Whitney U test was used to compare between mean concentrations and to know if the difference between the two groups was significant or it was a result of coincidence . Finally, the relationship between the levels of NO and the clinical indices was assessed by means of a Spearman rank correlation test. Values of P <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

A total of 35 subjects were enrolled .13 patients (65 %) were males and 7 patients (35 %) were females in(ChP) . There were 9 (60 %) male patients and 6 (40%) female in (RC). The mean age of (ChP) was 51±8 years ranging from 40 to 75 years. The mean age of (RC) was 50±8 years (40-67) as it showed in Table (1).

Table (1) Demographic Parameters of the Study Population

Patient Data	Control group (RC)	Chronic Periodontitis group (ChP)
N	15	20
Age (years) mean ± SD (range)	50±8 (40-67)	51±8 (40-75)
Sex (n, [%])		
Female	6 (40%)	7 (35%)
Male	9 (60 %)	13 (65 %)

The Total nitrite/nitrate (GCF) levels for all study groups are presented in Table 2. The mean nitrite/nitrate levels from patients with ChP was 0.489 ±0.449 compared to 0.477 ±0.824 nmol in RC. There was no significant difference in Total nitrite/nitrate (GCF) levels between study group and the control group (P =0.083).

Table (2) Mean Total nitrite/nitrate (GCF) Levels of Study Groups

	RC group	ChP group	*Z	*P value
Total nitrite/Nitrate (GCF) levels				
Mean ± SD (range)	0.410 ± 0.625 (0.01-1.97)	0.489 ± 0.449 (0.06 - 1.69)	0.083	- 1.734

** Mann-Whitney U test*

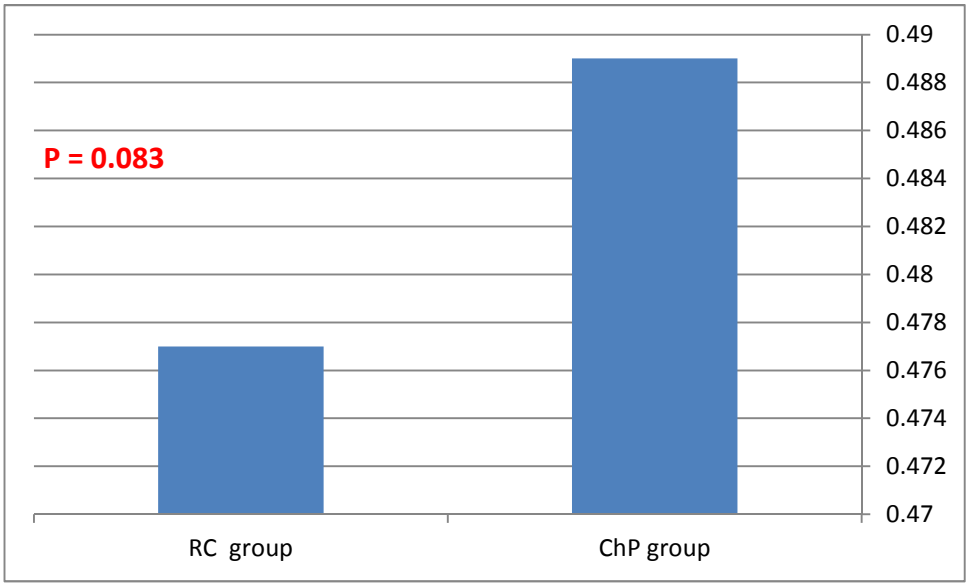

Chart (2) Total Nitrite/Nitrate (GCF) levels

No significant correlations were found between Total nitrite/nitrate (GCF) levels and age or sex (P = 0.785 and P = 0.097, respectively) among all study subjects. No significant correlations could be found between Total nitrite/nitrate (GCF) levels and any clinical measures among all study subjects (P > 0.05) table (3).

Table (3) Correlation Coefficient of Total Nitrite/Nitrate (GCF) levels in ChP

	GI	PI	BOP	CAL	PPD	Age	Gender
Correlation Coefficient	0.060	-0.316	0.025	-0.014	-0.140	- 0.065	- 0.382
P value	0.803	0.174	0.916	0.952	0.556	0.785	0.097

Spearman correlation coefficient

In the present study, we evaluated Total nitrite /nitrate levels in GCF from patients with chronic periodontitis and healthy control. The findings of this study demonstrated that GCF volumes exhibited clear increases at diseased sites compared to healthy sites. Also the present study nitrate levels did not differ significantly among study groups, and shows no increase in inflammatory condition GCF nitrite level in comparison with control subjects. probably the secreted substances in periodontitis suppress the production of NO[13]. Also, as a defense molecule, it may be consumed when disease progresses to combat the interfering oxidative and infectious process [13]. This could be due to the fact that consumption of NO as an antibacterial agent is for controlling the bacteria that reduce its level. In contrast to that of (Ali et al., 2014), (Bejeh-Mir et al., 2014) and (Hussain et al., 2015)[14] [15] [16]

(Ali et al., 2014) found that total GCF nitrite levels were higher in gingivitis and periodontitis groups (1.07 [SD 0.62] nmol and 1.08 [SD 0.59] nmol) than the control group (0.83 [SD 0.31] nmol) (P < 0.05) There were no relations between Total nitrite /nitrate levels in GCF and sex or age in the study groups and this result is compatible with (Ali et al., 2014)

CONCLUSION

Within the limits of the results that are obtained from the present study, it can be concluded that nitrosative stress is not associated in chronic periodontitis in Syrian patients.

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