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EFFECT OF NON SURGICAL PERIODONTAL THERAPY COMBINED WITH DOXYCYCLINE ON THE LEVELS OF GINGIVAL CREVICULAR FLUID OF NITRIC OXIDE

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ABSTRACT

Reactive oxygen species (ROS) and antioxidant activity have been involved in pathogenesis of periodontitis and investigated by many studies. Nitric oxide (No) one of ROS plays important role in increase pathogenesis of periodontal disease. Tetracycline derivatives like doxycycline is considered a ROS scavenger. It works as antioxidant, lessen oxidative stress and improve NO bioavailability.

INTRODUCTION

Periodontitis is multifactorial disease caused by the interaction of multiple microbial agents found in the bacterial plaque, host susceptibility, and environmental factors. It results in progressive destruction of the periodontal ligament, alveolar bone with pocket formation, attachment loss and recession[1-3]. The detection of oxygen dependent production of reactive oxygen species (ROS) and antioxidant activity involvement in pathogenesis of periodontitis have been investigated by many studies. ROS are oxygen-derived free radicals such as superoxide, hydroxyl, nitric oxide, hydrogen peroxide and hypochlorous acid[4-6]. NO is a short-lived, reactive free radical synthesized from the conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS). NO is relatively unstable in the presence of oxygen, so it quickly auto- oxidized to produce nitrogen oxides. Nitrate and nitrite are the stable end products of NO oxidation[7,8]. Gingival crevicular fluid (GCF) is an exudate creating from serum and can be collected from the gingival sulcus surrounding natural teeth . The flow of this biological fluid is an essential element for determination of the status of periodontal tissues, which reflects

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the cellular response in the periodontium by the constituents of serum and contributions from the gingival crevice[9-11]. Doxycycline is considered the most potent MMP inhibitor of tetracyclines. Doxycycline also work as a ROS scavenger and this may contribute to the improvement of some cardiovascular diseases associated with increased concentrations of ROS. It also lessen oxidative stress and improves nitric oxide (NO) bioavailability[12].

The aim of the present study was to compare the effect of non surgical periodontal therapy alone or combined with doxycycline on the level of nitric oxide in GCF of patients affected by chronic periodontitis.

SUBJECTS AND METHODS

40 subjects were selected from the out patients clinic of the Oral Medicine, Oral Diagnosis and Periodontology Department –Faculty of Dentistry Minia University and Nahda University.The 40 patients were diagnosed with moderate and advanced chronic periodontitis. 20 volunters healthy subjects were included in the study as a control group. The 60 subjects were grouped into three groups: Group I: 20 subjects received non surgical periodontal therapy consisted of scaling and root planning (SRP)+ doxycycline 100 mg once daily* (for 2 weeks). Group II: 20 subjects received only SRP.Group III: 20 healthy subjects. The inclusion criteria were Chronic periodontitis groups with PD≥ 5 mm and CAL≥ 4 mm in at least two sites, age from 25-55, systematically free patients, Non smoker and no prior use of antibiotics in the last 6 months. The exclusion criteria were allergy to tetracycline, Female patients who are pregnant or of child bearing potential and not utilizing birth control and Immunocompromised patients. The two groups of chronic periodontitis was subjected to non surgical periodontal therapy compromising of three sessions of scaling and root planning for 2 weeks including full mouth supra and sub gingival scaling and root planning using hand instruments and ultrasonic scalers. The pocket depth(PD), clinical attachment loss(CAL), plaque index(PI) and gingival index(GI) were recorded at baseline, day 15 and day 30. Gingival crevicular fluid (GCF) sample was collected from the two groups of chronic periodontitis to determine the inflammatory marker nitric oxide using the absorbent filter paper strips at baseline , day15 and day30 after treatment. Using scalpel blades,chromatography papers were cut to the dimensions of 2 x 8 mm [13]. Sampling was collected from the deepest probing site, taken from 3 sites.

After isolation and drying of the test site, pre-sterilized filter paper strips was held in place for 30 seconds[14]. Then stored in eppendorph tubes at -20C until be used for the laboratory analysis. All collected samples of GCF from 3 groups were assayed using (ELISA) technique to assess the level of nitric oxide.

RESULTS

The mean value of clinical parameters are listed in Table 1. All the clinical parameters were found significantly higher in chronic periodontitis (CP) groups compared to the control group where ($p<0.001$).

When comparing group 1 to group 2,there was improvement in clinical parameters by time from baseline to day 30.

All the mean values of nitrite, nitrate and nitric oxide levels were higher in CP groups compared to control group .When comparing group 1 to group 2, there was improvement in nitrite, nitrate and nitric oxide levels by time from baseline to day 30.

TABLE (1) Comparison between clinical parameters:

Clinical Parameters	Group1 (SRP+Vibramycin)	Group 2 (SRP)	Group 3 (Healthy Control)
PI Baseline PI Day 15 PI Day30	1.65±0.42 0.88±0.44 0.33±0.17	1.64±0.35 0.83±0.44 0.35±0.17	0.16± 0.04
GI Baseline GI Day 15 GI Day30	1.73±0.49 0.94±0.34 0.49±0.37	1.81±0.57 0.97±0.57 0.48±0.36	0.19± 0.06
PD Baseline PD Day 15 PD Day30	3.98±0.90 3.47±0.98 2.56±0.80	3.91±0.89 3.42±0.87 3.17±0.76	1.50±0.51
CAL Baseline CAL Day 15 CAL Day30	3.36±0.71 3.22±0.66 3.09±0.63	3.45±0.91 3.37±0.88 3.26±0.86	0.00±0.00

TABLE (2) Comparison between biological mediators:

Biological mediators	(SRP+Vibramycin) Group1	(SRP) 2 Group	3 Group (Healthy Control)
Nitrite Baseline Nitrite Day15 Nitrite Day30	0.80±5.73 0.26±2.70 0.19±1.65	0.54±5.96 0.82±4.64 0.85±2.66	0.22±1.53
Nitrate Baseline Nitrate Day15 Nitrate Day30	0.25±2.73 0.18±1.75 0.13±1.17	0.55±2.91 0.74±2.31 0.29±1.76	0.18±1.05
NO Baseline NO Day15 NO Day30	0.80±8.46 0.34±4.44 0.36±2.93	0.88±8.87 1.32±6.95 0.96±4.42	0.37±2.57

DISCUSSION

Several studies have investigated the role of NO in the progression of periodontal diseases. Chen et al. revealed an increase in NO expression in periodontitis where as Lohianai et al. showed that enhanced formation of NO played a significant role in the pathogenesis of periodontitis[15]**.** The source of anti-oxidant defence system against reactive oxygen species as nitric oxide may be endogenous as Vitamin A, B, C and E, carotene, and enzymatic oxidants such as superoxide dismutase, catalase, and myeloperoxidase or exogenous as drugs[16]**.** Vibramycin scavenges radicals and reduces oxidative stress. It is used as antioxidant drug. Whiteman and Halliwell demonstrated that doxycycline protected against peroxynitrite-induced degradation

of α1-antiproteinas[17]. Another investigations by Yagan et al. suggested that doxycycline decreased the oxidative stress index of gingival tissue in rats with periodontitis[18]. The flow of gingival crevicular fluid is an important determinant for the status of periodontal tissues. Reports have found that several crevicular biomarkers could be used to detect and/ or predict periodontal disease activity[19]. It has been previousely found by Ali et al. that nitrite and nitrate levels(metabolities of nitric oxide) in gingival crevicular fluid can be used as early detection marker in periodontal inflammation[20]. The present study employed non surgical periodontal therapy since scaling and root planning treatment approach in periodontal therapy is an efficient method to reduce the amount of calculus and biofilm bacteria attached to the subgingival root surface. It also induces beneficial changes to the periodontal tissues, as reduction of the gingival inflammation, reduction of probing pocket depth, and gain in clinical attachment[21]. Although serum has been used to evaluate the risk for an individual to develop periodontal disease and to monitor the host response to periodontal therapy, in this study measurement of nitric oxide level was performed in gingival crevicular fluid rather than serum as gingival crevicular fluid has the benefit of being closely approximated to the site of destruction and thus provide more information than markers in the serum[22]. Gingival crevicular fluid sampling offers several advantages as ease of access, atraumatic technique and enables repeated sampling at different times from the same site[23]. In the present study vibramycin has been used in adjunct to non surgical periodontal therapy in one group and the other group received only non surgical periodontal therapy in order to show the effect of doxcycyline as antioxidant to nitric oxide which is involved in pathogenesis of periodontal disease. Doxcycycline was used systemically as 100 mg oral tablet once daily for two weeks.It has many properties other than the antimicrobial. It combats oxidative stress, mop up free radicals and inhibit an excessive inflammatory response secondary to an antigenic stimulus.The present results demonstrated that group I and group II showed decrease in nitric oxide level that where statisticaly significant when compared to baseline.When comparing the two groups the decrease in nitric oxide level were more favorable in group I as compared to group II at each follow up period.

Furthermore, statistical analysis of data showed non significant differences in the clinical parameters between the two groups at Day_{30} after treatment as regard GI and PI. This may be attributed to the comprehensive treatment modality and oral hygiene maintenance that offered coverage of the major etiologic factors of both groups. The present study showed a reduction of GI after day_{15} and day_{30} in group 1. The exact explanation of these findings may be attributed to the anti-inflammatory effect of doxcycycline and its ablility to scavenge ROS[24]. A significant decrease in PD and gain in CAL were found within both groups (group 1 and group 2) at day_{15} and day_{30} compared to baseline. When comparing group 1 and group 2, the decrease in PD was more favorable in group 1 as compared to group 2 at follow up period day_{30} . This is attributed to anti-collagenase property of tetracycline[25]. In the present study, nitric oxide level was reduced in both groups (group1 and group 2) after day_{15} and day_{30} .

There was insignificant difference between group 1 and group 2 at baseline , on the other hand group 1 showed more reduction in the nitric oxide level than group 2 at follow up period day_{15} and day_{30}. Group 1 showed insignificant difference in nitric oxide level when compared to group 3 (healthy control) at day_{30} . The tenable explanation of these findings is attributed to the antioxidant effect of doxcycycline. The anti-proteolytic property, doxcycycline inhibition of neutrophil collagenase may also prevent other proteolytic events because neutrophil collagenase (MMP-8) as well as neutrophil-derived reactive oxygen species, i.e., hydrogen peroxide, and nitric oxide can degrade and inactivate α-1 proteinase inhibitor [26].

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