

Salivary markers quantification of oxidative stress in patients with periodontitis, before and after intensive periodontal treatment: a one-blinded, randomized, clinical trial

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AIM: Periodontitis is one of the most common inflammatory and infectious diseases, significantly associated with circulating levels of oxidative stress biomarkers. A network of interacting molecular pathways comprising proinflammatory mediators and reactive oxygen species (ROS) are involved in the progression of periodontitis. The aim of the trial was to determine the effects of intensive periodontal treatment on the salivary levels of oxidative stress in patients with moderate periodontitis.

MATERIALS AND METHODS: A randomized, one-blinded, controlled clinical trial was conducted with 3 months of follow-up. A total of 58 patients were included in the study and randomized to receive intensive periodontal treatment (Group Test) or conventional adult prophylaxis (Group Control). Salivary samples for determination of total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NO), myeloperoxidase (MPO), glutathione (GSH), malondialdehyde (MDA) levels, and 8-hydroxy-2'-deoxyguanosine (X8-OHdG) concentrations were collected from each patient prior to the periodontal treatment and at 3 months after the therapy.

RESULTS: A significantly decreased NO, and MDA levels were registered in the Test Group compared to the Control Group ($p < .0001$), and the concentration of GSH was found to be significantly increased in the test group compared to the control group.

CONCLUSION: Intensive periodontal treatment mitigates salivary oxidative stress in patients with moderate periodontitis.

Key words: periodontitis; oxidative stress; salivary markers; prophylaxis; periodontal treatment

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Periodontitis is a common inflammatory chronic disease affecting the hard and soft tissues around the teeth and is the primary cause of tooth loss in adults (1-6). Periodontitis is due to a complex interaction between the host immune system and pathogenic bacteria that leads to the loss of attachment of the connective tissue from the root cementum and alveolar bone (7-12). Oxidative stress plays an essential role in the pathophysiology of the destruction of periodontium during inflammation (13-15). Oxidative stress is an imbalance of pro-oxidants and oxidants (16-18). An oxidative stress condition is established if homeostasis is disrupted in favor of ROS (19-23). The effect of oxidative stress in periodontitis can be explained by the increase in levels of systemic and local release of Tumor Necrosis Factor- α (TNF- α) and Interleukin 6 (IL-6) (24-29). ROS production is mandatory in all aerobic organisms with a complex antioxidant defence system (30-33). The critical biomarkers of oxidative stress are the total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NO), myeloperoxidase (MPO), glutathione (GSH), malondialdehyde (MDA) levels, and 8-hydroxy-2'-deoxyguanosine (X8-OHdG) (34-40). MDA biomarker is the main product of lipid peroxidation. It has been demonstrated that ROS directly impacts fibroblast and osteoblast cells and might be responsible for decreased collagen production in extracellular matrix producing cells (41-45). Several clinical trials have demonstrated that total salivary antioxidant status is decreased in patients with chronic periodontitis and that periodontal treatment improves this condition (46-49). The aim of this trial was to evaluate the impact of intensive periodontal treatment on oxidative stress of patients diagnosed with moderate periodontitis.

MATERIALS AND METHODS

This prospective, one-blind controlled clinical trial randomized 58 patients with moderate periodontitis to receive standard periodontal treatment plus gaseous ozone therapy (Test Group) or periodontal treatment alone. The study protocol was approved by the Institutional Review Board at the University of Tetovo in Macedonia (PR. NR. 09-154/3) and conformed with the ethical standards established in the Declaration of Helsinki. Informed

consent was obtained prior to randomization from study subjects. Patients who had at least 4 teeth with one or more sites exhibiting probing pocket depth (PPD) ≥ 4 mm, clinical attachment level (CAL) ≥ 4 mm, full-mouth plaque score and full mouth bleeding score $>35\%$ were enrolled in this study. In addition, patients were included in the trial if they met the following criteria: age >18 years; diagnosis of moderate periodontitis; ability to provide written informed consent. Patients who received periodontal treatment within 12 months prior to the start of the study were excluded, as were patients who received systemic antibiotics within the last 6 months, had uncontrolled hypertension, were pregnant, had a history of heart disease or stroke, or breast-feeding women, and patients unable to make informed consent.

Sample size calculation

A power analysis was conducted prior to the study based on preliminary data (1-4). Using an approximate two-sample proportion test, two-sided and $\alpha = 0.05$, we estimated $>80\%$ power with 25 patients per group to detect a significant difference (<0.001) in oxidative status.

Salivary markers assessment

Participants' clinical periodontal examination and oxidative stress assessment were performed at baseline and during a 3-month follow-up. Salivary samples were taken at baseline in non-fasting conditions and stored at -80°C for all patients. Then, they were transferred to the University of Tetovo for analysis. Salivary concentrations of glutathione (GSH), malondialdehyde (MDA), (X8-OHdG), total antioxidant status (TAS), and total oxidant status (TOS), nitric oxide and (NO) were measured by employing a Sciex API 3000 triple quadrupole mass spectrometer as described previously.

Statistical analysis

Data were expressed as means \pm SEM or median or median and range. Shapiro-Wilk tests were conducted to determine whether a normal distribution could have produced dependent variables for each group (Razali & Wah, 2011). The result of the Shapiro-Wilk test was significantly based on an alpha value of .05, $W = 0.81$, $p < .001$. This result suggests that the variables are unlikely to have been produced by a normal distribution. Levene's test was conducted to assess the homogeneity of variance. The result of Levene's test

was significant based on an alpha value of .05, $F(1, 85) = 13.73$, $p < .001$. This result indicates that the assumption of homogeneity of variance was violated. The Mann-Whitney U test assessed differences in GSH levels. All entered data were analyzed using SPSS software (Statistical Package for the Social Sciences version 15 USA).

RESULTS

Fifty-eight patients were enrolled. All participants completed the study. Group Test had an average of age of 58.25 ($SD = 8.46$, $SE_M = 1.28$, Min = 39.00, Max = 70.00). The observations for age for Group Control had an average of 47.26 ($SD = 8.50$, $SE_M = 0.91$, Min = 32.00, Max = 54.00). At baseline, about the variable X8-OHdG, the two-tailed Mann-Whitney U test was not significant based on an alpha value of .05, $U = 726$, $z = -1.87$, $p = .062$. The mean rank for group A was 39.00, and the mean rank for group B was 49.12; this suggests that the distribution of X8-OHdG for group A ($Mdn = 4,789.00$) was not significantly different from the distribution of X8-OHdG for the group B ($Mdn = 4,890.00$) category. Fig. 1 presents a boxplot of the ranks of X8-OHdG by the group. About the variable TOS, the result of the two-tailed Mann-Whitney U test was not significant based on an alpha value of .05, $U = 996.5$, $z = -0.43$, $p = .667$. The mean rank for group A was 45.15, and the mean rank for group B was 42.83, suggesting that the distribution of TOS at baseline for group A ($Mdn = 15.75$) was not significantly different from the distribution of TOS at baseline for group B ($Mdn = 15.50$) category. Figure 1 presents a boxplot of the ranks of each variable at baseline by the group. Regarding the variable NO, the result of the two-tailed Mann-Whitney U test was not significant based on an alpha value of .05, $U = 1109.5$, $z = -1.39$, $p = .165$. The mean rank for group A was 47.72, and the mean rank for group B was 40.20, suggesting that the distribution of NO at baseline for group A ($Mdn = 25.35$) was not significantly different from the distribution of NO for group B ($Mdn = 24.50$) category. The result of the two-tailed Mann-Whitney U test was significant based on an alpha value of .05, $U = 1331$, $z = -3.27$, $p = .001$. About the variable MDA, the mean rank for group A was 52.75, and the mean rank for group B was 35.05, suggesting that the distribution of MDA for group A was

significantly different from the distribution of MDA at baseline for the group B. The median for A ($Mdn = 233.84$) was significantly larger than the median for B ($Mdn = 190.34$). The two-tailed Mann-Whitney U test about the variable TAS was not significant based on an alpha value of .05, $U = 954$, $z = -0.07$, $p = .946$. The mean rank for group A was 44.18, and the mean rank for group B was 43.81; this suggests that the distribution of TAS at baseline for group A ($Mdn = 4.32$) was not significantly different from the distribution of TAS for the group B ($Mdn = 4.32$) category. Table I presents the result of the two-tailed Mann-Whitney U test. Fig. 1 presents the boxplot of the ranks of each variable at 3 months by the group.

At 3 months, the two-tailed Mann-Whitney U test for X8-OHdG was not significant based on an alpha value of .05, $U = 726$, $z = -1.87$, $p = .062$. The mean rank for group A was 39.00, and the mean rank for group B was 49.12, suggesting the distribution of X8-OHdG for group A ($Mdn = 4,789.00$) was not significantly different from the distribution of X8-OHdG for the B ($Mdn = 4,890.00$) category. TOS of the two-tailed Mann-Whitney U test was not significant based on an alpha value of .05, $U = 996.5$, $z = -0.43$, $p = .667$. The mean rank for group A was 45.15, and the mean rank for group B was 42.83; this distribution of TOS for group A ($Mdn = 15.50$) was not significantly different from the distribution of TOS for the group B ($Mdn = 15.50$) category. The two-tailed Mann-Whitney U test regarding the variable NO was not significant based on an alpha value of .05, $U = 1109.5$, $z = -1.39$, $p = .165$. The mean rank for group A was 47.72, and the mean rank for group B was 40.20, suggesting that the distribution of NO for group A ($Mdn = 25.35$) was not significantly different from the distribution of NO for the group B ($Mdn = 24.50$) category. The two-tailed Mann-Whitney U test about the MDA variable was significant based on an alpha value of .05, $U = 1331$, $z = -3.27$, $p = .001$. The mean rank for group A was 52.75, and the mean rank for group B was 35.05, suggesting that the distribution of MDA for group A was significantly different from the distribution of MDA for group B. The median for A ($Mdn = 233.84$) was significantly larger than the median for B ($Mdn = 190.34$). The two-tailed Mann-Whitney U test for TAS was not significant based on an alpha value of .05, U

= 954, $z = -0.07$, $p = .946$. The mean rank for group A was 44.18, and the mean rank for group B was 43.81, suggesting that the TAS distribution for group A ($Mdn = 4.19$) was significantly different from the distribution of TAS for the group B ($Mdn = 4.32$) category. Table II presents the two-tailed Mann-Whitney U test results at 3 months. Fig. 2 presents the boxplot of the ranks of each variable at 3 months by the group.

DISCUSSION

Periodontal tissue damage results from several pathologic mechanisms involving the immune response, direct stimulation of the pathogenic

microorganism, and the host system in response to this trauma (50-56). The primary etiologic agent is specific, predominantly gram-negative or facultative anaerobic bacteria within the subgingival biofilm; most periodontal tissue destruction is caused by an inappropriate host response to microorganisms and their products (41-44, 57-59). More specifically, a lack of balance between the proteolytic enzyme, inhibitors, reactive oxygen species (ROS), and antioxidant defence systems causes oxidative stress (60-68). Oxidative stress is believed to cause cellular and molecular damage, which further leads to tissue destruction. (69-74) ROS cause tissue damage through several mechanisms, which include

Table I. Two-Tailed Mann-Whitney Test for all variables at baseline

Variable	Mean Rank				
	A	B	<i>U</i>	<i>z</i>	<i>p</i>
X8-OHdG	39.00	49.12	726.00	-1.87	.062
TOS	45.15	42.83	996.50	-0.43	.667
NO	47.72	40.20	1,109.50	-1.39	.165
MDA	52.75	35.05	1,331.00	-3.27	.001
TAS	44.18	43.81	954.00	-0.07	.946

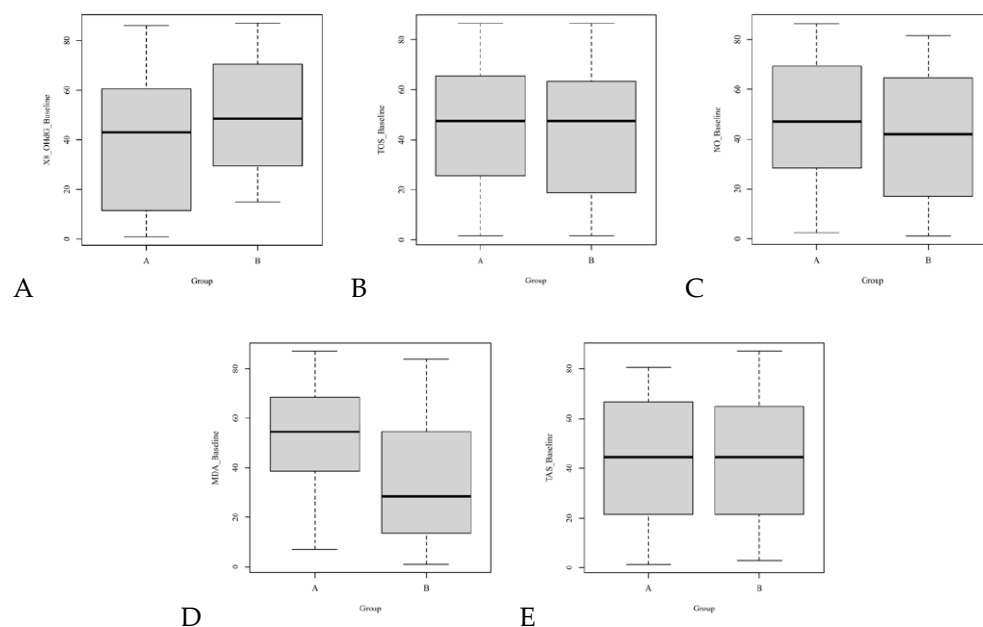


Fig. 1. Ranks of X8-OHdG (A), TOS (B), NO (C), MDA (D), TAS (E) at baseline by Group

DNA damage (75-80), lipid peroxidation (through activation of cyclooxygenases and lipoxygenases) (76), damage to proteins including gingival hyaluronic acid and proteoglycans, and oxidation of important enzymes (e.g., antiproteases, α -antitrypsin, stimulation of proinflammatory cytokine release by monocytes and macrophages by depleting intracellular thiol compounds and activating nuclear factor) (81-86). Oxidative stress generally appears after exposure to a relatively high concentration of ROS and/or a decrease in the antioxidant defence system against ROS (87). It has been implicated as a major contributor to more than 100 disorders and,

more recently, to periodontitis. Our study observed that oxidative stress levels in patients with moderate periodontitis were significantly decreased after intensive periodontal treatment compared with the control group. These results are consistent with other studies that have shown significant improvement in periodontal condition in conjunction with the reduction in oxidative stress (88-129).

Within the limitations of this study, it can be concluded that patients with periodontitis demonstrate a high level of oxidative stress, and intensive periodontal treatment reduces the salivary concentration of the key biomarkers.

Table II. Two-Tailed Mann-Whitney Test for all variables at 3 months

Variable	Mean Rank		U	z	p
	A	B			
X8-OHdG	39.00	49.12	726.00	-1.87	.062
TOS	45.15	42.83	996.50	-0.43	.667
NO	47.72	40.20	1,109.50	-1.39	.165
MDA	52.75	35.05	1,331.00	-3.27	.001
TAS	44.18	43.81	954.00	-0.07	.946

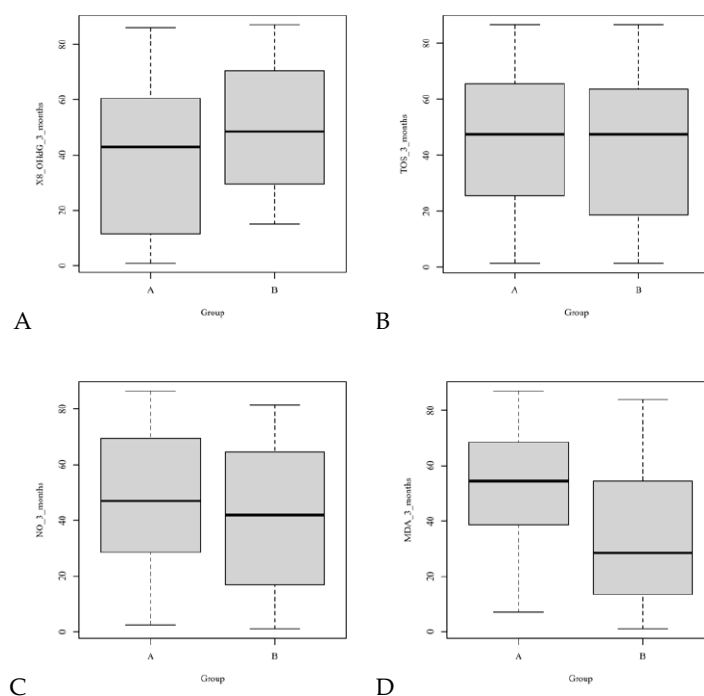


Fig. 2. Ranks of X8-OHdG (A), TOS (B), NO (C), MDA (D), TAS at 3 months by Group

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