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Original Article

Comparative evaluation of antioxidant levels in the serum of patients with periodontitis and diabetes mellitus after scaling and root planing

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Abstract

The present study was designed to estimate and compare the levels of Superoxide Dismutase (SOD), glutathione, catalase, and Total Antioxidant Capacity (TOC) in the serum of patients with periodontitis (CP) and diabetes mellitus type II (DM) before and after scaling and root planning (SRP). A single-blinded longitudinal study comprising 100 subjects, equally divided into four groups. Patients were categorized into group I (healthy patients), group II (systemically healthy patients with CP), group III (periodontally healthy patients with DM) and group IV (patients with CP and DM). Periodontal parameters such as gingival index, plaque index, clinical attachment level, and pocket probing depth were recorded. SRP was performed on all the patients. Serum samples were collected before and after SRP and sent for biochemical analysis. Results obtained were analyzed using ANOVA, Post hoc Tukey and paired t-test. The results showed significantly ($p<0.001$) lower levels of serum TAC, SOD, Glutathione, and catalase in DM, DMCP, and CP groups compared to healthy controls. Post-treatment values of TAC, Glutathione and Catalase increased in all groups but did not reach levels of healthy controls. Post-treatment values of serum SOD reached control levels only in the CP group, while values in the DM group did not vary significantly. The present study demonstrated the efficacy of scaling and root planing in improving serum antioxidant parameters by reducing the inflammatory load. The study further strengthens the inter-relationship between periodontitis and diabetes mellitus.

Keywords: antioxidants, inflammation, reactive oxygen species, diabetes, periodontitis.

Introduction

Periodontitis is a microbial-induced, host-mediated disease that destroys tooth-supporting structures. The tissue destruction in periodontitis is due to an imbalance between host response and microbial virulence [1]. This imbalance is due to a loss of equilibrium between the antioxidant cascade that protects and repairs vital tissue cells and reactive oxygen species (ROS) [2]. A respiratory burst occurs when powerful oxidizing agents are produced by an increase in oxygen uptake, resulting in reactive oxidants such as hydrogen peroxide, superoxide, and hydroxyl radicals. Prolonged and continuous exposure to ROS can initiate a wide range of pathologic responses resulting in tissue breakdown. These ROS are also found in increased concentrations in patients with diabetes mellitus. Consequently, patients with diabetes and periodontitis have increased periodontal tissue destruction and poor glycaemic control [3].

The immune system has developed certain protection and reparation processes to prevent the accumulation of harmful oxidatively damaged molecules. Antioxidants are molecules that significantly slow or prevent that substrate's oxidation when present in low concentrations compared to those of an oxidizable substrate [4]. When the oxidant-antioxidant balance is disrupted, favoring reactive oxygen species (ROS), tissue damage occurs. SOD is a superoxide dismutase enzyme that catalyzes the dismutation of superoxide to H_2O_2 and O_2. The most crucial antioxidant in mammalian tissue is SOD, which is measured intracellularly. Besides, several investigators have demonstrated the importance of extracellular SOD in plasma and other bodily fluids. Catalase eliminates hydrogen peroxide and primarily works intracellularly. Glutathione is involved in intracellular redox potential, consequent transcription activity, and the scavenging of reactive oxygen species (ROS). Total antioxidant capacity (TAC) outlines the biological interactions between distinct antioxidant types and quantifies the capacity of biological systems to tolerate oxidative attacks [5]. Because antioxidants work in harmony through chain-breaking mechanisms, measuring individual antioxidants may give a misleading impression. As a result, because total blood is constantly subjected to oxidative stress, TAC analysis is the most proper assessment (Figure 1).

According to earlier research, serum TAC levels were higher in periodontally healthy individuals, while systemically healthy individuals with chronic periodontitis (CP) had lower levels. The serum SOD levels in the DM-CP group were found to be the highest [6]. The current study aimed to assess Total Antioxidants (TAC), Superoxide Dismutase (SOD), catalase, and glutathione levels in individuals with chronic periodontitis and diabetes before and after scaling and root planing.

Material and methods

A total of 100 participants, aged between 30–60 years, who were willing to give consent to participate were selected for the study. The participants were equally grouped into Group A, systemically healthy individuals without periodontitis (control); group B, systemically healthy individuals with periodontitis (CP); Group C, patients with diabetes mellitus (Type 2) individuals without periodontitis (DM); Group D patients with diabetes mellitus (Type 2) individuals with periodontitis (CP-DM). A single physician evaluated the study participants' medical status, and diabetes history was documented as per physicians' records. Participants with a minimum of 20 natural teeth with at least 30% of sites with probing depth and CAL >3 mm with evident radiographic bone loss were included under the periodontitis category (retrospectively analyzed – generalized Stage III periodontitis). All the participants were diagnosed and under treatment for diabetes mellitus type 2 with ≤5-year duration and on oral hypoglycaemic medication. The participants were excluded for the following reasons: participants with systemic diseases or conditions apart from diabetes mellitus, smokers, participants who have undergone periodontal treatment in the last six months, and patients who have received antibiotics/anti-inflammatory drugs/steroids/vitamin or mineral supplements/antioxidants in the last three months.

The clinical periodontal measurements were recorded at six sites per tooth for probing depth and CAL. A single expert periodontist recorded all the periodontal parameters. The single-blinded operator performed scaling and root planning for all the study participants. Scaling and root planing were done for all participants, oral hygiene instructions were given, and on the 21st day, plaque and gingival index were recorded. Participants who did not follow oral hygiene instructions and in whom gingival and plaque scores were more than one were planned to be excluded from further participation.

Blood samples were collected at baseline and on the 21st day to estimate TAC, SOD, catalase, and glutathione. Five milliliters (5 ml) of venous blood sample was drawn from the individuals through disposable syringes and was transferred to the centrifuge tube. Blood samples were allowed to stay in a centrifuge tube undisturbed for 30 minutes. Later, they were centrifuged at 3000 rpm for 15 minutes and the supernatant serum was separated and sent immediately for biochemical analysis. TAC and SOD were estimated using the phosphomolybdenum method and the Nitro Blue Tetrazolium chloride reduction method (NBT). The glutathione and catalase levels were estimated as described in the previous study [7].

Statistical analysis

The data were entered into an excel sheet and verified for data entry mistakes. The mean and standard deviations were calculated. Quantitative evaluation of TAC, SOD, glutathione and catalase levels in serum was done using ANOVA, post hoc test and paired t-test for two timelines. Intra and Intergroup comparison was performed for different groups pre and post-treatment.

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Results

The mean age of the study population was 47.6 years. The study results show a statistically significant difference in terms of pre-treatment serum values of SOD, glutathione, catalase and TAC between the groups. The inter-group comparison between groups showed statistical significance in all parameters except SOD between CP and CP-DM groups (Tables 1–4). The post-treatment levels of TAC, SOD, glutathione and catalase among the four groups showed a statistical significance (Figure 1 A–D). Inter-group comparison between the groups showed statistical significance in all parameters except for SOD between health and CP groups. In the DM group, pre and post-treatment serum levels of antioxidants were statistically significant except for SOD. In CP and DM-CP groups, pre and post-treatment levels of all the measured antioxidants were found to be statistically significant.

Discussion

Periodontitis is the most prevalent disease of humans, affecting more than half of the global adult population and a complex cascade of destructive tissue pathways characterizes it [8]. The dysbiotic organisms colonizing the periodontal pockets trigger the release of pro-inflammatory cytokines, thereby promoting neutrophil accumulation, which produces reactive oxidants [9].

Periodontitis and diabetes together lead to a significant economic burden for the population [10, 11]. Many studies have revealed that a hyperactive innate immune response may be a precedent for both periodontitis and diabetes, which have a synergistic effect when they co-exist [12]. Besides, non-surgical therapy reduces the bacterial load, thereby reducing inflammation and having a positive effect in controlling periodontal destruction as well as diabetes status. Free radicals are formed excessively in diabetes by several pathways, such as non-enzymatic, enzymatic, and mitochondrial [4]. Free radical scavenging antioxidant enzymes like SOD and hydrogen peroxicrde degrading enzymes like catalase and glutathione peroxidase are essential to protect normal cells and matrix components from oxidation. Antioxidants can act in nexus through redox cycling reactions, renewing each other from their corresponding radical species and therefore can be measured as the total antioxidant capacity [13]. Hence, TAC, SOD, Glutathione, and Catalase level are expected to be

Table 1: Comparison of antioxidants among the groups pre and post Scaling & Root planing.

		Pre-treatment levels		Post-treatment levels	
		Mean±SD	P-value	Mean±SD	P-value
SOD	Healthy	4.14±0.63	<0.001	4.08±0.59	<0.001
	DM	2.89±0.41		2.94±0.49	
	CP	3.58±0.38		3.94±0.34	
	CP-DM	3.49±0.18		3.61±0.14	
Glut	Healthy	25.67±1.29	<0.001	25.34±1.17	<0.001
	DM	11.41±1.05		11.94±0.87	
	CP	13.60±0.99		19.22±0.44	
	CP-DM	20.34±1.05		23.86±0.89	
Cat	Healthy	2106.39±59.60	<0.001	2105.89±60.03	<0.001
	DM	1131.63±81.23		1146.74±96.96	
	CP	1652.89±63.74		1665.21±69.55	
	CP-DM	1577.20±74.17		1588.81±71.75	
TAC	Healthy	1.92±0.10	<0.001	1.88±0.12	<0.001
	DM	1.30±0.21		1.35±0.20	
	CP	0.56±0.04		1.58±0.05	
	CP-DM	0.67±0.04		0.91±0.07	

Table 2: Comparison of Pre-treatment levels of Serum antioxidants among the groups (post-hoc tukey).

Dependent variable	Groups		Mean Difference	Std. Error	P-value
SOD pre-treatment	Healthy	DM	1.25	0.12	<0.001
		CP	0.56	0.12	<0.001
		CP-DM	0.66	0.12	<0.001
	DM	CP	-0.69	0.12	<0.001
		CP-DM	-0.59	0.12	<0.001
	CP	CP-DM	0.10	0.12	0.843
Glutathione pre-treatment	Healthy	DM	14.26	0.31	<0.001
		CP	12.06	0.31	<0.001
		CP-DM	5.33	0.31	<0.001
	DM	CP	-2.20	0.31	<0.001
		CP-DM	-8.93	0.31	<0.001
	CP	CP-DM	-6.73	0.31	<0.001
Catalase pre-treatment	Healthy	DM	974.76	19.86	<0.001
		CP	453.50	19.86	<0.001
		CP-DM	529.19	19.86	<0.001
	DM	CP	-521.25	19.86	<0.001
		CP-DM	-445.56	19.86	<0.001
	CP	CP-DM	75.69	19.86	0.001
TAC pre-treatment	Healthy	DM	0.623	0.03	<0.001
		CP	1.364	0.03	<0.001
		CP-DM	1.253	0.03	<0.001
	DM	CP	0.74	0.03	<0.001
		CP-DM	0.629	0.03	<0.001
	CP	CP-DM	-0.11	0.03	0.006

Table 3: Comparison of Post-treatment levels of Serum antioxidants among the groups (post-hoc tukey).

Dependent variable	Group		Mean Difference	Std. Error	P-value
SOD post-treatment	Healthy	CP	0.20124	0.123422	0.367
		CP-DM	0.53644	0.123422	<0.001
	DM	CP	-1.0024	0.123422	<0.001
		CP-DM	-0.6672	0.123422	<0.001
	CP	CP-DM	0.3352	0.123422	0.039
Glutathione post-treatment	Healthy	DM	13.72761	0.26065	<0.001
		CP	6.446448	0.26065	<0.001
		CP-DM	1.808568	0.26065	<0.001
	DM	CP	-7.28116	0.26065	<0.001
		CP-DM	-11.919	0.26065	<0.001
	CP	CP-DM	-4.63788	0.26065	<0.001

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Table 3: Continued.

Dependent variable	Group		Mean Difference	Std. Error	P-value
Catalase post-treatment	Healthy	DM	959.6548000	21.4188621	<0.001
		CP	441.1840000	21.4188621	<0.001
		CP-DM	517.5795600	21.4188621	<0.001
	DM	CP	-518.4708000	21.4188621	<0.001
		CP-DM	-442.0752400	21.4188621	0.001
	CP	CP-DM	76.3955600	21.4188621	0.003
TAC post-treatment	Healthy	DM	0.5672	0.033882	<0.001
		CP	0.3416	0.033882	<0.001
		CP-DM	1.012	0.033882	<0.001
	DM	CP	-0.2256	0.033882	<0.001
		CP-DM	0.4448	0.033882	<0.001
	CP	CP-DM	0.6704	0.033882	<0.001

inversely related to periodontal disease severity. Due to the effects of smoking on various antioxidant species, this study sample was restricted to never-smokers. All serum parameters were assessed again at three weeks as it is associated with periodontal healing such as PD reduction and CAL gain [14]. The current study results demonstrate significantly lower levels of all the antioxidants mentioned above in serum of patients with CP with and without DM than healthy controls following scaling and root planing.

In the current study, pre-treatment serum SOD levels are significantly higher in healthy compared to CP group. The imbalance in the endogenous antioxidant cascade due to the excessive production of lipid peri oxidation products at inflammatory sites can be linked to a higher level of oxidative stress in patients with CP [15]. This study showed a significantly lower pre-treatment SOD activity (p<001) in the DM group compared to the healthy controls. Studies have shown that in conditions where chronic oxidative damage has

Table 4: Comparison of the pre and the post-treatment serum parameters within different groups.

	Paired Differences (Pre and post Treatment)			
Group	Antioxidants	Mean	Std. Deviation	P-value
DM	SOD	-0.05	0.28	0.415
	Glutathione	-0.54	0.57	<0.001
	Catalase	-15.11	36.91	0.052
	TAC	-0.06	0.03	<0.001
CP	SOD	-0.36	0.36	<0.001
	Glutathione	-5.62	0.83	<0.001
	Catalase	-12.32	15.91	0.001
	TAC	-1.02	0.05	<0.001
CP-DM	SOD	-0.12	0.089	<0.001
	Glutathione	-3.53	0.84	<0.001
	Catalase	-11.62	20.83	0.01
	TAC	-0.24	0.082	<0.001

Figure 1: Pre and post-treatment levels of antioxidants A – TAC; B – SOD; C – Glutathione; D – Catalase.

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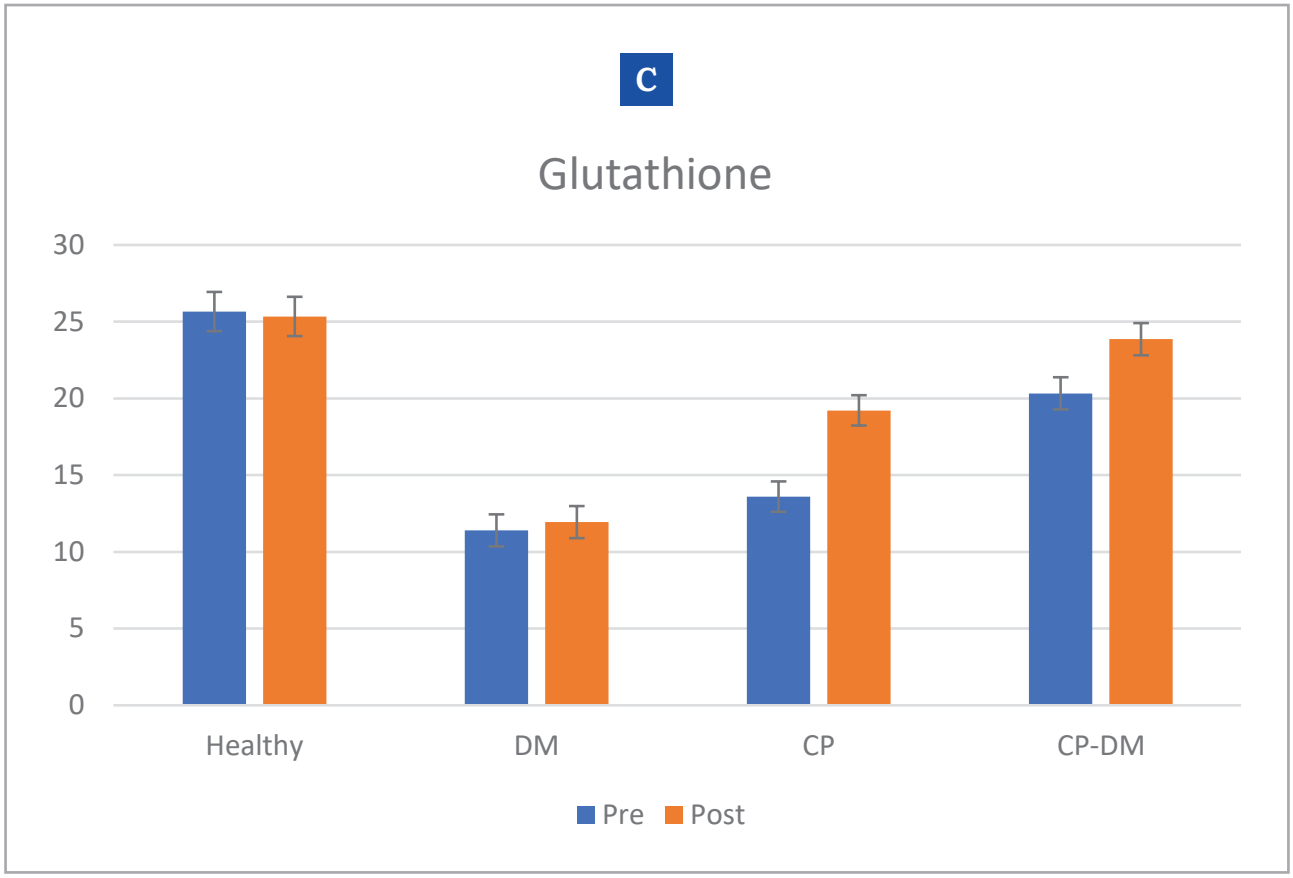

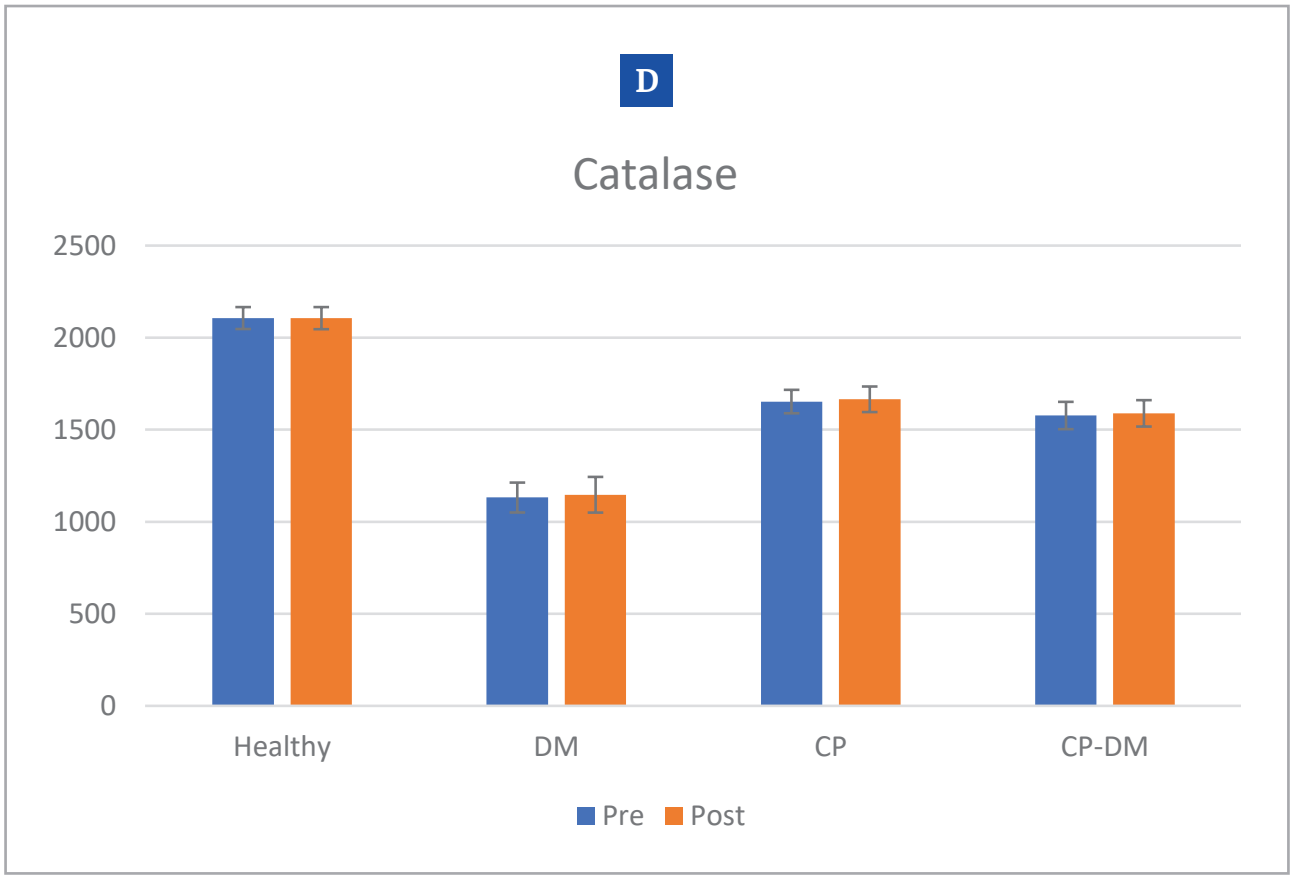

Figure 1: Continued.

occurred, the SOD enzyme adapts to the circumstances [16]. The difference in pre-treatment SOD activities in the CP and CP-DM groups was not statistically significant. This can be explained by reducing enzyme activities in DM and subsequent compensatory mechanisms in periodontitis, raising the levels similar to those in the CP group. Post-treatment, the serum SOD values increased significantly in all groups except the DM group. SOD values of systemically healthy with periodontitis were statistically not different from the values of the healthy controls.

The current study results showed a statistically significant ($p<0.001$) difference in pre-treatment levels of glutathione among the groups. Levels in healthy controls had higher levels than the CP group. This suggests that a large amount of glutathione was consumed during the ROS generation leading to a deficiency of antioxidants. Also, based on data of many microbiotas metabolizing glutathione, peri pathogens also reduce glutathione levels [17]. Post-treatment, there was a significant difference among all the groups. However, contrasting results were noted in some studies in terms of Glutathione levels measured in saliva and GCF.

Pre-treatment levels of catalase were statistically significant among the groups. However, the difference in catalase activities in CP and DM-CP groups was significant. This may be postulated that catalase levels in CP, though initially stimulated, are depleted secondary to excessive free radical production in microbial killing. However, in the case of diabetes mellitus, the body is not adapted for antioxidant formation, so the average level decreases because of enzymatic use [18]. Post-treatment, it was observed that there was a statistical difference in CP and CP-DM groups but not in DM alone group. However, none of the values reached the levels of healthy controls.

Generally, patients with CP and DM exhibit a lower serum concentration of total antioxidants due to oxidative stress [7]. In the current study, the pre-treatment levels of TAC were significantly lower in CP and DM than in the healthy controls. Besides, TAC in the DM-CP group was higher than in the CP group. Reduced TAC in DM-CP compared to DM alone suggests that periodontitis has a negative effect on the already compromised oxidative status of DM group. Oral hypoglycaemics have potent antioxidant effects by decreasing lipid peroxidation and its markers in both LDL and HDL [19]. This might be why a higher level of TAC is seen in periodontally healthy diabetic patients than CP and CP-DM groups. All the groups' post-treatment serum TAC values were significant compared to the healthy controls. The action of oral hypoglycaemic and changes in diet may explain why the DM group also showed a significant increase post-scaling and root planing.

The current study results align with a few studies, assessing one or two antioxidants considered in the current study and assessing the levels of antioxidants in saliva and GCF [20–22]. Besides, the current study results contrast the results of a few studies assessing antioxidant levels using different sampling methods and techniques [23–25]. The differences in results between the present study and others may be due to time-dependent changes in antioxidant activity and enzyme effects opposing oxidative stress by adaptation. Moreover, most of the studies assessing antioxidant parameters were case-control studies where the values were not assessed post-treatment. Furthermore, numerous studies employed various body fluids such as GCF and Saliva, tested for antioxidants in diabetes and periodontitis [26]. However, GCF and saliva are better to investigate alone or in cases of periodontitis, while serum concentration provides a broader picture of the disease and therapy involved.

Apart from the small sample size, a more detailed history into the diet of the patients could have been taken as these can affect the post-treatment values, especially serum glutathione and TAC levels. Most of the oral anti-hyperglycaemic drugs like metformin have potent antioxidant effects; hence, standardizing this factor could have strengthened evidence. Furthermore, multi-centre studies with a larger sample size are required to better understand the inter-relationship between oxidative disparity, diabetes, and periodontitis.

Conclusion

Periodontal tissue damage is caused by oxidative stress, which occurs either directly due to excessive ROS activity/antioxidant deficiency or indirectly due to the activation of redox-sensitive transcription factors and the creation of a pro-inflammatory state, which is exacerbated in the presence of diabetes mellitus. A clearer understanding of this relationship may assist healthcare providers in their effort to detect these diseases earlier and help reduce free radical-induced oxidative damage.

Conflict of interest

The authors declare no conflict of interest.

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Ethics approval

The approval for this study was obtained from the Ethics Committee of the AB Shetty Memorial Institute of Dental Science (approval ID: ABSM/EC/79/2012).

Consent to participate

Written informed consent was obtained from the participants.

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