

# Cellular and Biochemical Changes in Different Categories of Periodontitis: A Patient-based Study

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Received : 27-01-20.  
Revised : 17-02-20.  
Accepted : 27-02-20.  
Published : 07-05-20.

## ABSTRACT

**Objectives:** The aim of this study was to study the effects of periodontitis, diabetes mellitus (DM), and tobacco smoking and chewing habits (TBSCH) on the oxidative stress biomarker levels, namely malondialdehyde (MDA), and the mucosal genotoxic nuclear damage in the marginal gingival cells of subjects. Furthermore, the correlation of the biomarkers, MDA, and nuclear changes in the form of micronucleation (Mn) and binucleation (Bn) was investigated. **Materials and Methods:** Forty study participants were divided into five subject categories, which were established based on the presence of periodontitis, DM, and TBSCH. Whole saliva and marginal gingival smears collected from subjects were used to determine MDA levels and nuclear changes, respectively. A full-mouth assessment of periodontal pocket depth, clinical attachment loss, and bleeding on probing was performed for each subject to determine periodontal status. **Results:** MDA and Mn levels between control group and subjects with only periodontitis (MDA:  $P < 0.9990$ ; Mn:  $P < 0.8200$ ) showed no significant difference, whereas levels among subjects with DM, TBSCH, and periodontitis, and all other categories were statistically significant (MDA:  $P < 0.001$ ). DM and/or TBSCH superimposed on periodontitis cause an exponential increase in biomarker levels. Furthermore, MDA and Mn showed poor correlation ( $r = 0.162$ ;  $P = 0.318$ ). Periodontitis alone did not significantly increase oxidative stress levels compared to healthy controls, whereas DM and TBSCH resulted in augmented oxidative stress levels, implying that increased stress produced by DM and TBSCH aggravates or exaggerates periodontal inflammation. **Conclusion:** Poor correlation between MDA and Mn indicated that the mechanisms involved in their production are independent of each other.

**KEYWORDS:** Binucleation, diabetes mellitus, malondialdehyde, micronuclei, periodontitis, tobacco

## INTRODUCTION

Periodontal disease is a chronic and progressive oral inflammatory condition that affects majority of the world's population.<sup>[1]</sup> It comprises both reversible gingivitis, affecting the gingiva, and irreversible periodontitis, affecting the periodontal ligament, cementum of teeth, and the alveolar bone. Patients with periodontitis usually present with the following features: tenderness and bleeding from

the gums, redness and swelling, periodontal pocket formation, clinical attachment loss (CAL) and alveolar bone loss (horizontal or vertical), and ultimately loss of teeth. It has been identified as the world's leading cause

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**How to cite this article:** Shee F, Pralhad S, Natarajan S, Manaktala N, Arun S, Marathe A. Cellular and biochemical changes in different categories of periodontitis: A patient-based study. J Int Soc Prevent Communit Dent 2020;10:341-9.

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DOI: 10.4103/jispcd.JISPCD\_42\_20

of tooth loss.<sup>[2]</sup> Pathogenesis of periodontal disease involves complex interactions between pathogenic microorganisms present in adherent plaque and the host immune system,<sup>[1]</sup> along with environmental, dietary, and genetic factors. Although pathogenic microorganisms present in the adherent plaque are known to cause periodontal tissue destruction due to the activity of microorganisms and their toxic products, the host immune system has also been found to play a role in the process of periodontal breakdown due to the establishment of oxidative stress.<sup>[3-6]</sup>

Polymorphonuclear leukocytes (PMNLs) form the first line of defense against bacteria and bacterial products by oxygen-dependent and independent mechanisms.<sup>[3]</sup> As a part of the oxygen-dependent mechanism, PMNLs and monocytes have been reported to produce increased amounts of reactive oxygen species (ROS) during the phagocytosis of periodontopathic pathogens.<sup>[3,5,6]</sup> ROS are highly toxic substances that are formed under normal physiological conditions and subsequently removed by antioxidant systems, to prevent damage due to their deleterious effects. When ROS are produced in excess, or antioxidant system activity is decreased, oxidative stress results.<sup>[4-7]</sup> The increased levels of ROS initiate destruction of periodontal tissues, implicating a role of oxidative stress in the pathogenesis of periodontal disease.<sup>[5]</sup> Several studies have shown that there is an increase in ROS and impairment of antioxidant systems in patients with periodontal disease.<sup>[3-6]</sup> Habits such as tobacco smoking and chewing habits (TBSCH) and chronic systemic disorders such as diabetes mellitus (DM) are associated with systemic oxidative stress. TBSCH introduce numerous free radical species (FRS) directly into the body and induce ROS production within the tissues via immune-mediated inflammatory responses.<sup>[8,9]</sup> Systemic disorders such as DM, because of chronic hyperglycemia and associated dyslipidemia, also results in systemic overproduction of ROS.<sup>[10,11]</sup> Both TBSCH and DM further exacerbate the oxidative stress condition by impairing and suppressing the antioxidant systems, affecting various proteins and enzymes within the system (superoxide dismutase, glutathione peroxidase, and glutathione reductase).<sup>[8-11]</sup> ROS cause tissue destruction by a variety of mechanisms, including lipid peroxidation, deoxyribonucleic acid (DNA) damage, oxidation of important enzymes, protein damage, and liberation of pro-inflammatory cytokines by monocytes and macrophages.<sup>[3,5]</sup> One of the major end products of lipid peroxidation is malondialdehyde (MDA) and that of DNA damage is micronuclei (Mn), which

can be used as biomarkers to indicate the severity of genotoxic damage in patients with periodontitis.<sup>[2,12-14]</sup>

Micronucleus (Mn) is an extra small nucleus separated from the principal nucleus, generated during cellular division by late chromosomes or by chromosome fragments. It is generally formed due to DNA damage mostly by external agents or internal processes such as inflammation. Mn assay is a minimally invasive, inexpensive, and a highly sensitive test, which can be detected in simple buccal/gingival smears.<sup>[15]</sup> With this background, the aim of this study was to correlate the effects of periodontitis, DM, and TBSCH with the genotoxic damage cells (assessed using Mn and binucleation [Bn]) observed in marginal gingival mucosal as well as the salivary levels of MDA. In addition, this study assessed the correlation between the genotoxic biomarkers evaluated, namely MDA levels and nuclear changes observed.

## MATERIALS AND METHODS

### SUBJECT POPULATION

This cross-sectional case-control study was conducted among 40 subjects, in the age range of 23–75 years ( $36.52 \pm 13.6$  years [mean and standard deviation]), who were recruited to participate in the study. Subjects were selected from the Department of Periodontology and the Department of General Medicine. Assessment of nuclear changes was conducted in the Department of Oral Pathology, and the MDA assessment was performed in the Department of Biochemistry. The study was initiated after approval from the Institutional Ethics Committee. On the basis of the presence or absence of periodontitis, DM, and TBSCH, subjects were divided into the following five categories (eight subjects in each group):

1. Category 1: Controls (subjects with healthy periodontium, no DM, no TBSCH)
2. Category 2: Patients with periodontitis only
3. Category 3: Patients with periodontitis and TBSCH
4. Category 4: Patients with periodontitis and DM
5. Category 5: Patients with periodontitis, DM, and TBSCH

Information regarding the study was provided both orally and on written documents, and written consent was obtained before the subjects' participation in the study. Following an initial screening process, a complete periodontal examination was carried out along with saliva and smear collection.

### INCLUSION CRITERIA

Control category comprised subjects with healthy periodontium, just after scaling with a negative history of DM and tobacco-related habits. Subjects

who presented with pocket depth (PD)  $\geq$  5 mm and/or clinical attachment loss (CAL) were included as periodontitis cases, whereas subjects were said to have TBSCH if they presented with the habit, and DM if they had fasting blood sugar (FBS)  $<$ 100 mg/dL or glycated hemoglobin (HbA1c)  $<$ 6.5.

#### EXCLUSION CRITERIA

Patients undergoing chemotherapy or radiotherapy and patients with known premalignant/malignant lesion were excluded.

#### SALIVA COLLECTION AND MALONDIALDEHYDE ASSESSMENT

Saliva was collected in containers by “spitting method,” after allowing the saliva to pool in the mouth for 4 min. Approximately 2–3 mL of saliva was collected from each patient. *Precipitation reaction:* 0.5 mL of saliva was mixed with 2.5 mL of 10% phosphotungstic acid solution in a test tube and centrifuged at 2000 rpm for 10 min. This resulted in the formation of a precipitate and supernatant fluid. The test tube containing the precipitated solution was then refrigerated at  $-4^{\circ}\text{C}$  until analysis could be performed. MDA assessment was performed using thiobarbituric acid (TBA) assay put forth by Buege and Aust<sup>[16]</sup> in 1978, using spectrophotometry. The test involved the reaction of MDA in the collected supernatant samples with 0.5 mL of TBA. This was then boiled in a water bath for 45 min. After cooling, 0.05 mL of 5 M HCl was added to 0.4 mL of the supernatant—TBA solution and mixed well. It was then cooled and 1.0 mL of freshly prepared NaOH solution was added to eliminate the need for centrifugation. The absorbance of pink color was then measured at 535 nm against a blank containing distilled water.

MDA levels were calculated using the following equation:

Here  $\chi$  is the optical density obtained,

$$\text{MDA concentration} = \chi / 1.56 \times 10^8 \times 0.4 \text{ mol/mL}$$

#### MARGINAL GINGIVAL SMEAR COLLECTION

Marginal gingival smear was then collected from each patient using a sterile wooden tongue spatula. The tongue spatula was used to scrape the marginal gingival area around all teeth to collect cells, following which a smear was created by rubbing the spatula against the surface of a frosted slide (labeled with subject's name and details) in a circular motion till a flame-shaped smear of cells was created. Slides were stored in Coplin jars containing cytological fixative (50% ethanol and 50% ether) until analysis could be done. Saliva and marginal gingival smears were collected before measurement of periodontal parameters to ensure that bleeding from gingiva during probing or

examination did not contaminate the saliva or smear samples.<sup>[17]</sup>

#### ASSESSMENT OF NUCLEAR CHANGES

Nuclear changes (micronucleation [Mn] and Bn) were assessed from the marginal gingival smears using acridine orange staining and fluorescent microscopy. The stained cytoplasm appeared green or orange; nuclear material showed bright green fluorescence. Cells were counted in vertical loops (Battlement technique) to avoid double counting. Although cells were examined for all possible nuclear changes, only Mn and Bn were seen in the patient samples. Countryman and Heddle criteria were used to identify Mn in the cells (intracytoplasmic DNA bodies distinctly separate from the nucleus and are found in the same plane as nucleus: same or slightly less staining intensity, one-third to one-fifth of the size of the main nucleus, and placed within two nuclear diameters from the nucleus), whereas a single cell with two attached or unattached nuclei were categorized under Bn.<sup>[18]</sup>

#### PERIODONTAL PARAMETER MEASUREMENT

This was performed using mouth mirror and William's periodontal probe. Parameters measured were bleeding on probing (BOP), PD, and CAL, and the findings were recorded. All parameters were evaluated on four surfaces (mesiobuccal, mid-buccal, distobuccal, and palatal/lingual) of each tooth, for all teeth present in the oral cavity. All the examinations and measurements were performed by the same investigator. William's probe was walked through the sulcular region of all the teeth to detect BOP, PD, and CAL. PD was measured as the distance (in mm) from the gingival margin to the base of the probable crevice. CAL was measured as the distance (in millimeters) from the cemento-enamel junction to the base of the probable crevice. It was used to diagnose subjects as being healthy (control) or periodontitis cases, and also to classify the severity of periodontitis as being early, moderate, or severe, based on the criteria established by Armitage.<sup>[19]</sup> Severity of periodontitis was designated as mild (CAL: 1–2 mm), moderate (CAL: 3–4 mm), and severe ( $>$ 5 mm). Subjects were said to be healthy (control group) if there was no BOP, PD  $\leq$  2 mm, and CAL  $\leq$  1 mm.

#### STATISTICAL ANALYSIS

All the data obtained were compiled, and the mean values of MDA, Mn, Bn, and total nuclear abnormalities (TNA) (Mn + Bn) for each of the categories of subjects were calculated. The mean values of the categories were compared using the *post hoc* Tukey test and one-way analysis of variance (ANOVA) test. Pearson's correlation coefficient was calculated to identify any correlation present between the biomarkers evaluated,

Mn and MDA. A multinomial logistic regression analysis was also performed to check the association of nuclear abnormalities, periodontal status, and MDA levels using the control group as reference group.

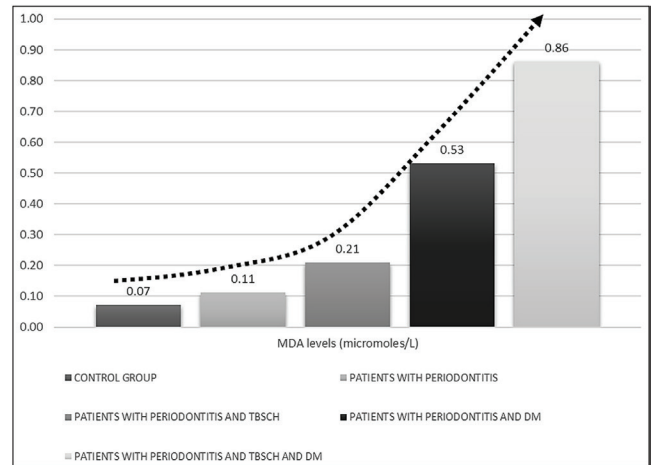
**RESULTS**

The mean values of salivary MDA levels between subject categories are shown in Table 1.

MDA levels showed an exponential increase with the inclusion of TBSCH and DM, but not with periodontitis [Figure 1]. Test of association of the mean values of MDA showed similar values between Category 1 and Category 2 [Table 1], Category 2 (0.11 µmol/L) being only slightly higher than the control group (0.07 µmol/L). Category 5 recorded the highest levels of MDA, 10 times that of the control group, which was statistically significant ( $P = 0.003$ ) compared to the other categories. The differences in MDA levels between the other categories were not significant.

The mean values of the Mn levels between subject categories are shown in Table 1, and are graphically represented (bar chart) in Figure 2. Corresponding with the observed MDA levels, Mn levels between the five categories show an overall trend of exponential increase. In addition, Category 1 and Category 2 have the same Mn levels. Although there was a statistically significant difference in Mn levels between the five subject categories using the one-way ANOVA test, *post hoc* Tukey test did not yield a significant variation between the subgroups.

The mean values of Bn and (TNA = Mn + Bn) are given in Table 1. The levels of Bn and TNA did not demonstrate the same exponential trend shown by Mn and MDA levels, and neither Bn nor TNA showed similarity in levels between Category 1 and Category 2. The mean values of Bn between the subject categories did not show significant correlation ( $P = 0.46$ ). TNA showed an overall increasing trend [Figure 3] but the levels between the subject categories were also not significant ( $P = 0.07$ ). Correlation of MDA and Mn counts using Pearson's correlation ( $r$ ) value produced



**Figure 1:** Levels of salivary malondialdehyde between subject categories and control group. Periodontitis alone did not significantly increase MDA levels compared to the control group, but an exponential increase in MDA levels occurred when periodontitis was compounded with DM and TBSCH

**Table 1: Comparison of levels of salivary malondialdehyde and nuclear abnormalities, age, and clinical attachment loss between subject categories (mean value ± SD, F, and P values)**

Subject categories	MDA levels (µmol/L)	Micronuclei levels (Mn)	Binucleation levels (Bn)	Total nuclear abnormalities (= Mn + Bn)
Category 1	0.07 ± 0.05 <sup>+</sup>	0.25 ± 0.463	0.63 ± 0.916	0.88 ± 0.991 <sup>×</sup>
Category 2	0.11 ± 0.072 <sup>±</sup>	0.25 ± 0.463	1.50 ± 1.773	1.75 ± 1.753
Category 3	0.21 ± 0.213 <sup>φ</sup>	0.75 ± 1.035	1.75 ± 1.389	2.50 ± 1.927
Category 4	0.53 ± 0.447 <sup>*</sup>	1.00 ± 0.535	1.50 ± 1.512	2.50 ± 1.852
Category 5	0.86 ± 0.533 <sup>+ φ *</sup>	1.50 ± 1.604	2.00 ± 1.852	3.50 ± 2.204 <sup>×</sup>
F value	6.356	3.411	0.925	2.383
P value	0.003	0.032	0.461	0.07
Subject categories	Age (years)	Clinical attachment loss (CAL) (mm)		
Category 1	23.25 ± 0.707 <sup>= * #</sup>	1.17 ± 0.150 <sup>Δ = ¥ °</sup>		
Category 2	42.0 ± 9.411 <sup>= φ □</sup>	1.97 ± 0.490 <sup>Δ</sup>		
Category 3	33.0 ± 9.289 <sup>φ ω</sup>	1.93 ± 0.541 <sup>= +</sup>		
Category 4	58.38 ± 12.082 <sup># φ ⊕</sup>	2.26 ± 0.299 <sup>¥</sup>		
Category 5	59.63 ± 8.782 <sup>* □ ω</sup>	2.71 ± 0.841 <sup>° +</sup>		
F value	52.834	9.319		
P value	<0.001	<0.001		

<sup>+ ± φ \*</sup> indicate significant difference between groups according to *post hoc* Tukey test

<sup>= # □ φ ⊕ Δ = ¥ + \* °</sup> indicate significant difference between groups according to *post hoc* Tukey test

the result,  $r = 0.162$  indicating poor correlation between them ( $P = 0.318$ ).

Stepwise multinomial logistic regression analysis was performed using the control group as the reference group. It showed a progressively increasing odds ratio for MDA and Mn levels with the inclusion of the variables of periodontitis, TBSCH, and DM [Table 2].

## DISCUSSION

To the best of our knowledge, this is the first study investigating the MDA levels and Mn frequency in subjects with periodontitis, with or without the presence of TBSCH and type 2 DM; attempting to correlate the biomarkers MDA and Mn and Bn. MDA is formed by the process of lipid peroxidation,<sup>[20]</sup> in which FRS attack lipids containing carbon-carbon

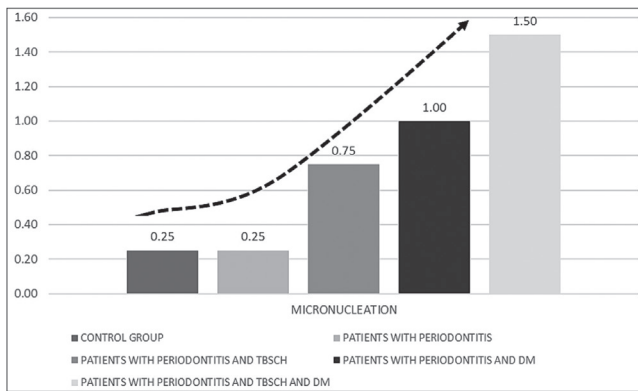
double bonds, especially polyunsaturated fatty acids (PUFAs), via nonenzymatic peroxidation processes. Mn occurs due to FRS-induced DNA damage resulting in fragmentation of chromosomes, the fragments of which are not incorporated into the nucleus and exist as acentric structures in the cytoplasm of the affected cells [Figure 4A]. In addition to Mn, other nuclear changes are observed in cells (due to FRS-induced chromosome fragmentation and pericentriolar matrix injury) such as nuclear budding, Bn, and multinucleation [Figure 4B].

This pilot study investigated the effect of periodontitis, TBSCH, and type 2 DM on the salivary levels of MDA and the cellular cytotoxic changes (Mn and Bn) observed in marginal gingival smears, and suggested that the biomarker levels represented the oxidative stress status of the individual, and that they would increase in the presence of periodontal disease, and further increase if periodontitis was compounded with TBSCH, DM, or both, when compared to healthy subjects. The observed results show a statistically significant difference in salivary MDA levels between Category 5 and all the other subject categories. One-way ANOVA test showed a statistically significant difference in the Mn levels between the subject categories, but *post hoc* Tukey test did not reveal any such difference.

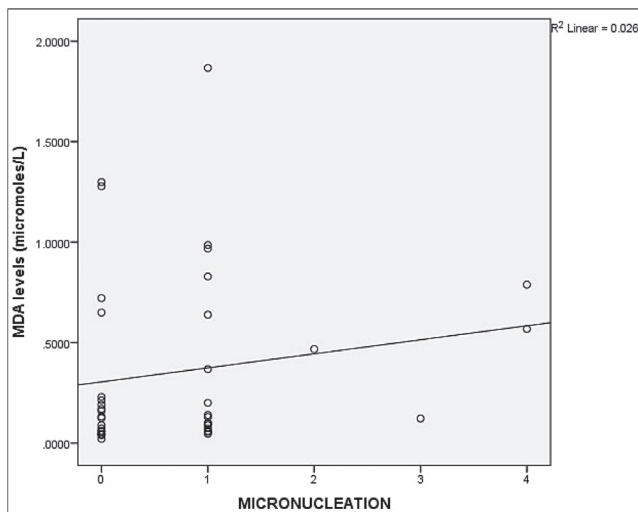
Several studies have reported findings of increased levels of lipid peroxidation and DNA damage in patients with periodontitis. Khalili and Biloklytska<sup>[12]</sup> reported statistically significant difference in salivary MDA levels between a group with generalized chronic periodontitis (GCP) and a healthy control group. Dalai *et al.*<sup>[6]</sup> also reported increased MDA levels between two groups of subjects with stage I and stage II periodontitis and a healthy control group; the comparison between the groups was statistically significant.<sup>[6]</sup> Significantly higher levels of salivary MDA were also reported in subjects with chronic periodontitis (CP) compared to healthy controls by Canakci *et al.*<sup>[5]</sup> Zamora-Perez *et al.*<sup>[13]</sup> reported that subjects with CP presented significantly increased levels of Mn and nuclear abnormalities in buccal mucosal cells compared with control group. The results of this study revealed the following:

- There was no significant increase in salivary MDA levels between Category 2 (subjects with periodontitis only) (0.11  $\mu\text{mol/L}$ ) and Category 1 (control group) (0.07  $\mu\text{mol/L}$ )
- The Mn levels between Category 1 and 2 were the same (0.25)

This suggests that the presence of periodontitis alone does not cause significant increase in oxidative stress



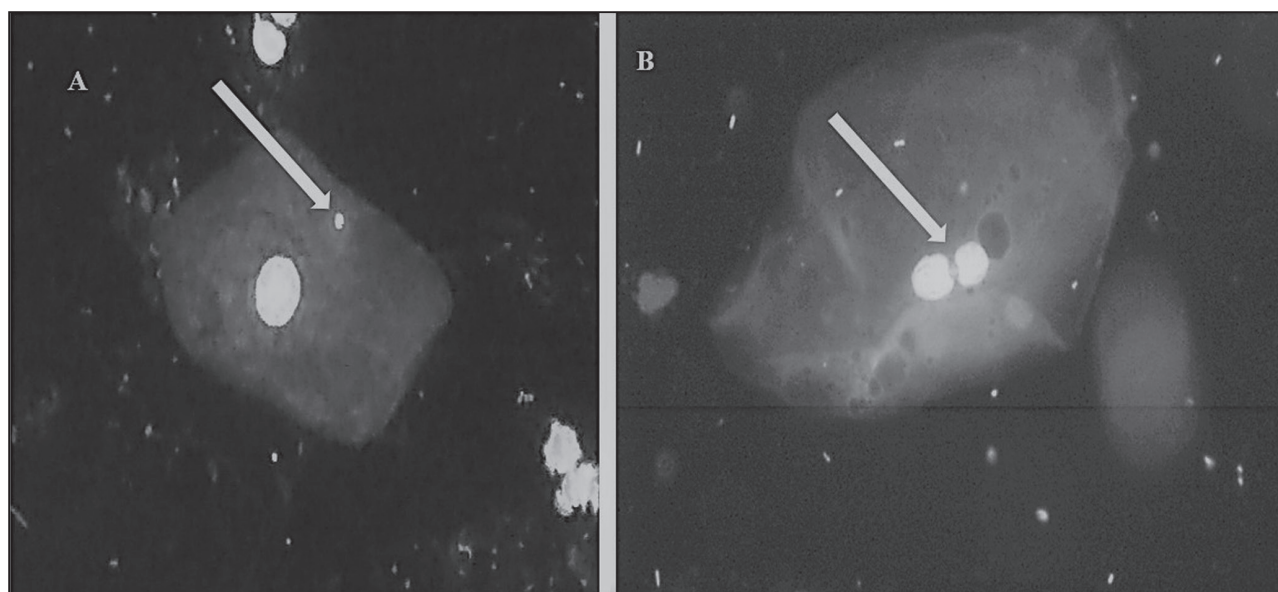
**Figure 2:** Levels of micronuclei between subject categories and control group. The trend shown by micronuclei resembles that of MDA levels, as periodontal disease was not accompanied by an increase in Mn levels, whereas superimposition of DM and TBSCH showed an exponential increase in levels



**Figure 3:** Scatterplot depicting mild positive correlation (Pearson's  $r$  value = 0.161) of malondialdehyde levels and micronucleation

**Table 2: Multinomial logistic regression showing correlation between levels of inflammation and genotoxic markers**

Group		Beta	Std. error	Sig.	Odds ratio
Category 2	Intercept	-1.368	1.137	0.229	
	MDA levels	14.776	10.774	0.170	2612542.014
	Micronucleation	0.174	1.188	0.884	1.190
Category 3	Intercept	-4.070	1.818	0.025	
	MDA levels	26.455	12.848	0.039	308499983384.985
	Micronucleation	2.515	1.360	0.064	12.363
Category 4	Intercept	-5.741	2.010	0.004	
	MDA levels	29.899	12.951	0.021	9663937571294.900
	Micronucleation	3.115	1.460	0.033	22.529
Category 5	Intercept	-7.716	2.278	0.001	
	MDA levels	31.832	12.993	0.014	66774077947375.100
	Micronucleation	3.635	1.496	0.015	37.885



**Figure 4:** Deoxyribonucleic acid damage due to oxidative stress resulting in formation of genotoxic biomarkers observed in mucosal cells in this study: (A) Micronucleation.(white arrow) (B) Binucleation (white arrow) (normal objective  $\times 100$ , acridine orange stain)

status/levels. It may also suggest that MDA levels in saliva may not always be present in significant concentrations to accurately represent the lipoperoxidase (LPO) status. These results are in line with the findings of Wei *et al.*<sup>[7]</sup> who calculated MDA levels in serum, saliva, and Gingival crevicular fluid (GCF) in patients with CP and in a control group. They found that GCF MDA levels differed significantly between the CP group and control subjects,<sup>[7]</sup> but that the serum and saliva MDA concentrations were almost the same in the CP and control groups. The results pertaining to Mn findings are also in line with the findings of Avula *et al.*<sup>[21]</sup> who found that the differences in Mn levels between the control, CP, and generalized aggressive periodontitis (GAP) were not significant, indicating that the cytogenetic damages observed in periodontitis groups

were not significantly different from those in control groups.

The results of this study also indicate that the superimposition of TBSCCH and DM on the disease state is causative of the exponential increase in biomarker levels observed, with maximum damage incurred in Category 5 with the highest levels of MDA (statistically significant,  $P = 0.003$ ) and Mn (closest to P value 0.078, P value is 0.05). Studies have shown that DM,<sup>[10]</sup> characterized by chronic hyperglycemia, is associated with systemic oxidative stress as a result of increased free radical production (mainly superoxide radicals), through nonenzymatic, enzymatic, and mitochondrial respiratory chain processes. Under diabetic hyperglycemic conditions, multiple effects occur on the protein levels and the activity of antioxidant enzymes such as superoxide dismutase, glutathione

peroxidase, and glutathione reductase; as a result,<sup>[11]</sup> there is further augmentation of oxidative stress in patients with diabetes due to suppression of host defense response. Johansen *et al.*<sup>[10]</sup> and Corbi *et al.*<sup>[14]</sup> have elaborated on the role that DM [Figure 5] plays in the formation of oxidative stress—large amounts of ROS are produced because of the oxidative metabolism of glucose under the hyperglycemic condition that occurs in DM. West<sup>[11]</sup> reported increased MDA levels in leukocytes of patients with type 2 DM. By West<sup>[11]</sup> reported increased MDA levels in leukocytes of patients with type 2 DM. They concluded that ROS production was increased significantly in type 1 diabetes, and even more so in patients with type 2 diabetes compared to healthy controls, and that antioxidant defenses, both enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and nonenzymatic (vitamins A, C, E, free radical scavengers, and total radical-trapping antioxidant capacity), were lowered in patients with diabetes compared to control subjects. An increased frequency of Mn in patients with type 2 diabetes was noted by Corbi *et al.*<sup>[14]</sup> who reported a statistically significant increase in Mn levels between controls and patients with poorly controlled DM, dyslipidemia, and periodontitis, but not with controlled diabetics. Our results are in line with those of the aforementioned studies as Categories 4 and 5 showed increased levels of both MDA and Mn compared to Categories 1 and 2, the difference with Category 5 being statistically significant.

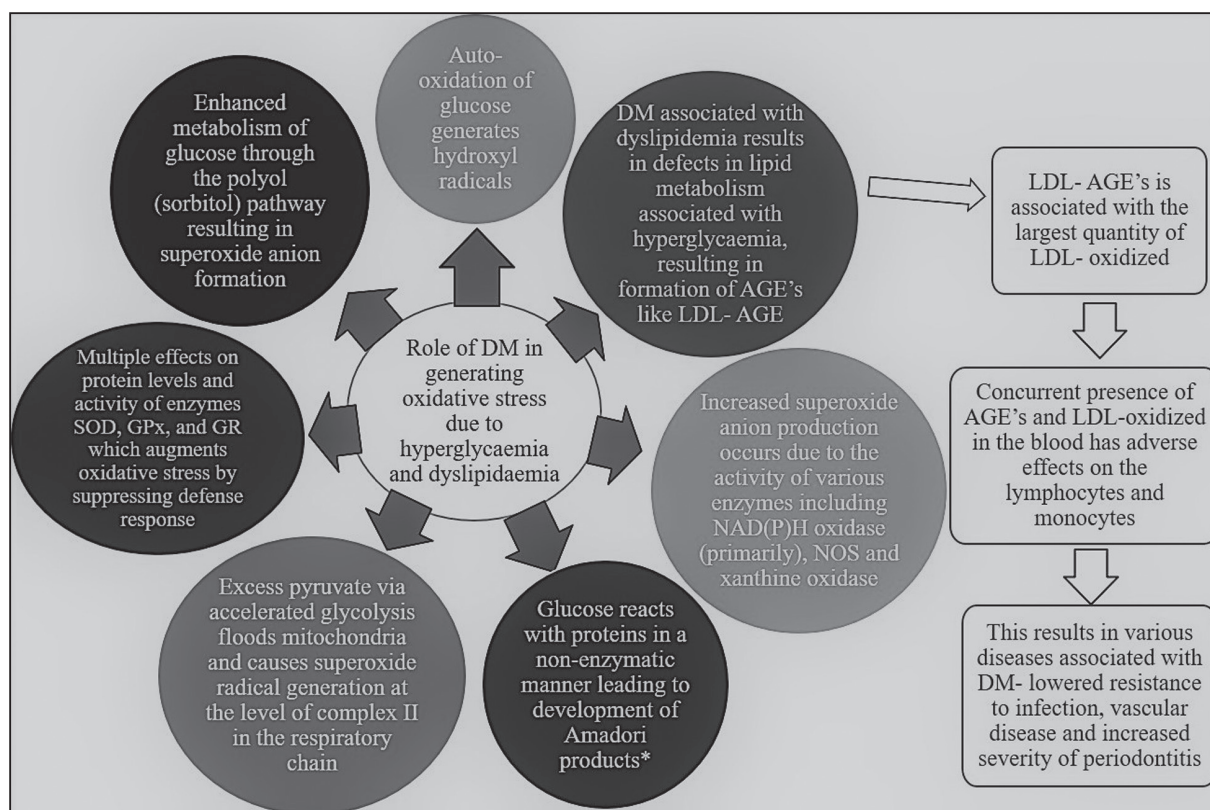
Studies also show that tobacco habits, such as cigarette smoking, cause an increase in systemic oxidative stress in patients engaging in the habit. Cigarette smoke comprises two phases: a tar phase and a gas phase—both of which are rich in FRS and non-radical oxidants. Oxidative damage is caused due to the direct effect of oxidants (such as, superoxide anions, hydroxyl radicals, and peroxy radicals) present in the cigarette smoke,<sup>[9]</sup> and from the activation of the phagocytic cells that generate further amounts of ROS as part of the inflammatory response. Some of the other effects of TBSCCH that contribute to oxidative stress are listed as follows:

- Peroxyl radicals and ROS cause direct damage, stimulating LPO
- Aldehydes can deplete reduced glutathione (which acts as a hydrogen donor for antioxidant enzyme, glutathione peroxidase)
- The tar phase of cigarette smoke contains hydroquinone/quinone complexes, which diffuse across cell membranes, give rise to semiquinones,

and lead to the formation of superoxide radicals and H<sub>2</sub>O<sub>2</sub>.

The resultant oxidative stress is further exaggerated by the suppression of antioxidant levels such as glutathione peroxidase (enzymatic) and vitamin C (nonenzymatic) that are normally responsible for the inactivation of FRS.<sup>[2,8,9]</sup> Morrow *et al.*<sup>[8]</sup> reported that plasma levels of LPO were significantly higher in smokers than those in nonsmokers. Nielsen *et al.*<sup>[2]</sup> reported that daily smokers had a significantly higher average concentration of plasma-MDA than that in nonsmokers, and they concluded that smoking is a prominent risk factor for increased lipid peroxidation. However, the results of this study are in line with the findings of Kamceva *et al.*,<sup>[9]</sup> who found no significant difference in the MDA levels between smoker and nonsmoker groups. Our results concluded that although biomarker levels for Category 3 subjects were higher than those of Category 1 or 2, the difference was not statistically significant.

The biomarkers showed correlation with the disease process but correlated poorly with each other indicating that alternative mechanisms are acting that affect their production independently due to pathological or carcinogenic entities. It is known fact that healing is compromised in periodontitis because of increased oxidative stress and inflammation. However, a periodontal disease coupled with TBSCCH or DM shows a worse prognosis when compared to periodontitis alone due to complete breakdown of immune mechanisms. Therefore, it can be surmised that treatment of periodontitis with underlying conditions would include treatment of the conditions as well. Without the treatment of underlying conditions, the oxidative stress produced in those conditions would serve to propagate the periodontal tissue destruction, hence its implication in the pathogenesis of periodontal disease. In consequence, the treatment of periodontitis would be incomplete or unsuccessful. Furthermore, Mn counts and MDA levels along with clinical changes can be evaluated during various phases of treatment to check for prognosis and response to treatment. A study by Güven *et al.*<sup>[22]</sup> also implicated the role of increased levels of *Fusobacterium nucleatum* and *Porphyromonas gingivalis* in head and neck cancer tissue. However, they did not report on the pathogenic role of these oral microbiomes. Sawhney *et al.*<sup>[17]</sup> also reported an exponential increase in epithelial genotoxicity between gingivitis and periodontitis. Most recently, Tadin *et al.*<sup>[23]</sup> also found that periodontal disease increases the frequency of nuclear morphological changes in gingival epithelial cells.



**Figure 5:** The role of diabetes mellitus in generating oxidative stress due to chronic hyperglycemia and dyslipidemia. DM = diabetes mellitus, AGE = advanced glycation end products, LDL = low-density lipoprotein, [NAD(P)H] = nicotinamide adenine dinucleotide phosphate, NOS = nitric oxide synthase, SOD = superoxide dismutase, GPx = glutathione peroxidase, GR = glutathione reductase. \*Amadori products give rise to Advanced Glycation End-Products (AGE's) – ROS are generated at multiple steps during this process

## CONCLUSION

Within the limitations of this study, it was concluded that periodontitis alone did not significantly increase the levels of oxidative stress compared to healthy controls. The inclusion of TBSCH and DM had an augmented effect on biomarkers levels, indicating an exponential increase in oxidative stress, and the resultant tissue damage. Hence, the treatment of periodontitis with underlying conditions would necessitate treatment of those conditions as well, without which periodontal treatment would fail, as the oxidative stress generated from the conditions would serve to further destroy and deteriorate the periodontal tissue structures. MDA levels and Mn frequency could also be measured along with clinical changes during various phases of periodontal treatment, and they could find use as indicators for prognosis and outcome of treatment. The Mn test could serve as a tool for estimating genotoxic damage in assessing the success of periodontal therapy.

## ACKNOWLEDGMENT

NA.

## FINANCIAL SUPPORT AND SPONSORSHIP

The study was funded by a short-term student research grant from the Indian Dental Association Karnataka State Branch (Ref no.: 225384).

## CONFLICTS OF INTEREST

There are no conflicts of interest.

## AUTHOR CONTRIBUTIONS

F.S. and N.M. were involved in Concepts, Design, Data Acquisition, Manuscript Editing and preparation. S.P. was involved in Concept and Design and Data Acquisition. S.N. was involved in Statistical analysis, Manuscript Editing and Preparation. A.S. and A.M. were involved in Design and Data Acquisition.

## ETHICAL CONSENT AND INSTITUTIONAL REVIEW BOARD STATEMENT

The study was done after obtaining IEC approval (protocol ref no: 15149).

## PATIENTS DECLARATION OF CONSENT

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/



her/their images and other clinical information to be reported in the journal.

#### DATA AVAILABILITY STATEMENT

The data set used in the current study is made available on request from manaktala.nidhi@manipal.edu and srikant.n@manipal.edu.

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