Original Article

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Chronic Periodontitis in Type 2 Diabetes Mellitus: Oxidative Stress as a Common Factor in Periodontal Tissue Injury

Biochemistry Section

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ABSTRACT

Introduction: The prevalence of periodontitis is significantly higher among people with poorly controlled diabetes mellitus. Majority of tissue destruction in periodontitis is considered to be the result of an aberrant inflammatory/immune response to microbial plaque and involve prolonged release of reactive oxygen species (ROS). There is increased evidence for compromised antioxidant capacity in periodontal tissues and fluids which may be an added factor for tissue damage in periodontitis.

Aim: To study the possible role of Reactive oxygen species (ROS) and antioxidant status in blood among chronic periodontitis patients with and without Type 2 Diabetes mellitus.

Materials and Methods: The study comprised of total 100 subjects among which 25 were normal healthy controls, 25 were gingivitis patients, 25 were chronic periodontitis patients (CP) and 25 were having chronic periodontitis with type 2 diabetes (CP with DM). ROS levels were determined as MDA (Malondialdehyde) and antioxidant status as plasma total antioxidant capacity (TAC), vitamin C and erythrocyte Superoxide dismutase (SOD) and catalase activity.

Results: There was significant increase in MDA levels in all the patient groups compared with healthy controls ($p<0.05$). The decrease in TAC, Vitamin C and SOD levels among CP with DM patients as compared to controls was highly significant ($p<0.01$). There was a positive correlation between the probing pocket depth and MDA levels among periodontitis patients with diabetes ($r=0.566$, $p=0.003$).

Conclusion: There is increased oxidative stress in chronic periodontitis with and without type 2 diabetes indicating a common factor involvement in tissue damage. More severe tissue destruction in periodontitis is associated with excessive ROS generation which is positively correlated in type 2 diabetic subjects.

Keywords: Antioxidants, Free radicals, Lipid peroxidation, Reactive oxygen species, Total antioxidant capacity, Vitamin C

INTRODUCTION

Periodontal diseases are among the widespread chronic conditions affecting populations worldwide. Gingivitis, characterized by inflammation of the gums, affects around 50% of the adult population [1]. More severe destructive periodontitis associated with gum recession, loss of gingival tissue and underlying alveolar bone, affects 10-15% of the world population, and is the major cause of tooth loss in adults [2]. A study from Southern India showed high prevalence of periodontitis among diabetics (87.2%) and 52.1% had destructive periodontitis with teeth mobility [3].

The microbial plaque induces an aberrant inflammatory response with release of reactive oxygen species (ROS) and this may result in tissue destruction in periodontitis [4]. This ROS generation on the plaque biofilm can also lead to alveolar bone destruction and deepening of periodontal pockets. Chronic inflammation affecting the periodontium results in release of cytokines, prostaglandins, bacterial toxins and enzymes from host and bacterial cells, which have additional damaging effects on the tissues [5].

ROS lead to peroxidation of polyunsaturated fatty acids in plasma membrane forming carbon centered radical (PUFA radical) or lipid peroxide radical and leads to loss of membrane functions. Lipid peroxidation and consequent degradation products such as malondialdehyde (MDA) are seen in biological fluids which indicate the pro oxidant status. ROS, in overwhelming concentrations could damage the cellular macromolecules causing destruction of membranes, loss of cellular homeostasis and consequently cell death. However, antioxidant systems protect against ROS by detoxifying them to less reactive species [6]. But, there is increased evidence for compromised antioxidant capacity in periodontal tissues and fluids which may be an added factor for tissue damage in periodontitis.

Type 2 diabetes mellitus is a complex systemic disease with oral complications as well. The diabetics are at increased risk of infections and oral cavity is a continuous source of infectious agents that could serve as an important risk factor for deterioration of diabetes [7]. The poorly controlled diabetics have a high prevalence of periodontitis, to the extent that periodontitis has been called the sixth complication of diabetes [8]. Conversely, periodontitis has been recognized as a risk factor for systemic diseases where there is low grade inflammation like type 2 diabetes, cardiovascular disease and cerebrovascular disease. Growing evidence entails oxidative stress in the pathobiology of chronic periodontitis and also type 2 diabetes [9]. The pro-oxidative state in periodontitis could lead to decrease in insulin sensitivity and contribute to a significant systemic impact with damage to organ systems distant from the focus of inflammation [5].

AIM

The aim of this study was to assess the levels of free radicals and antioxidants among patients in different phases of periodontal diseases (gingivitis and periodontitis) with and without type 2 diabetes mellitus.

MATERIALS AND METHODS

In this hospital based cross-sectional comparative study the samples were collected from the Department of Periodontology, SDM College of Dental Sciences and Hospital, Dharwad, and study was conducted in Department of Biochemistry, S.D.M. College of Medical sciences and Hospital, Dharwad over a period of one year.

The study included a total of 100 subjects who were categorized as follows:

- Group 1: 25 Controls - Systemically healthy subjects without periodontal disease for atleast 6 months.
- Group 2: 25 patients with gingivitis.
- Group 3: 25 patients with chronic periodontitis (CP) without type 2 diabetes mellitus.
- Group 4: 25 patients with chronic periodontitis with newly diagnosed type 2 diabetes mellitus (CP with DM).

Based on our pilot study, a sample of 25 subjects for each group was estimated to achieve a 95% power at α error of 5% in each of the groups at a gross precision rate of 20%.

Inclusion Criteria

Group 1 – Age and sex matched controls were taken in group 1.

Group 2 – Clinically, gingivitis can be diagnosed using certain subjective (colour and presence of edema and ulcerations) and objective (presence of bleeding on probing) criteria. Besides having the advantages of being an objective criterion, bleeding on probing is considered to be one of the first signs of gingivitis [10]. Sulcus Bleeding Index (SBI) score of ≥2 was used to diagnose gingivitis [11]. The reason for using the Sulcus Bleeding Index (SBI) was that it had a greater scoring range (0-5), and could be considered more sensitive. This index assesses the presence or absence of bleeding from the gingival tissues along with changes in the colour and consistency of the gingival tissues.

Gingival bleeding could be a result of haematological disorders, use of anticoagulant medications, local trauma and hormonal changes. Thus, the subjects included in Group 2 were systemically healthy individuals, non pregnant/lactating women, with no current use of any medications. They were diagnosed with plaque induced gingivitis.

Group 3- Chronic periodontitis was diagnosed as the presence of probing pocket depth (PPD) of ≥5mm along with the presence of attachment loss of ≥2mm with at least 3 teeth assessed at four sites per tooth, using a UNC-15 periodontal probe [12].

Group 4- Freshly diagnosed type 2 diabetes mellitus subjects who had periodontitis were selected in group 4. Uncontrolled diabetes mellitus is considered to be a risk factor for periodontal diseases [13] and at the same time, subjects with well-controlled diabetes have reported to show no significant increase in the risk for periodontitis [14]. Hence, freshly diagnosed diabetics were included who were not on any antidiabetic treatment. The diagnosis of type 2 diabetes mellitus was done based on fasting blood sugar more than 126mg/dl and postprandial blood sugar of more than 200mg/dl for three consecutive times. They had not started taking any antidiabetic medication at the time of sample collection for our study.

Exclusion Criteria

Absence of any other chronic systemic illness, any other source of infection, and intake of any medication affecting immune/inflammatory reactions or any vitamin supplementations. A detailed patient history was taken to rule out any diagnosed medical condition. Smokers were also excluded from the study. Only never smokers were included in the study and the smoking status was ascertained by detailed history.

All the patients signed for informed consent prior to the study. Approval for the study was taken from the institutional ethical committee.

Sample collection and processing: A 5 ml of fasting venous blood was collected aseptically in EDTA bulb. The sample was centrifuged at 3000 rpm for 15 min. Plasma and Red blood corpuscles (RBCs) were separated and the samples were analysed for all the parameters immediately. RBCs were washed in 0.9% NaCl for three times. For MDA analysis, 1:1 RBC haemolysate was used and for determination of superoxide dismutase (SOD) and catalase activity, the haemolysate was further diluted 1:50 with 0.9% normal saline. Total antioxidant capacity and Vitamin C were analysed in plasma. Analysis was performed using UV Visible spectrophotometer (Shimadzu).

PROCEDURE

Free radical level was analysed as MDA (Malondialdehyde) or TBARS (Thiobarbituric acid reacting substances) in RBC haemolysate.

Antioxidant status was analysed by

— Plasma TAC (Total antioxidant capacity)

— Plasma Vitamin C

— RBC – SOD

— RBC – Catalase

MDA levels in erythrocytes were measured by the method of Ohkawa et al., [15]. MDA is estimated as thiobarbituric acid reacting substances (TBARS). 0.75ml of 1:1 RBC haemolysate was added to 3ml of MDA reagent (75mg Thiobarbituric acid and 15g Trichloroacetic acid in 2.08ml of 0.2N HCl made upto 100ml with distilled water) and kept in boiling water bath for 20 min, cooled and centrifuged at 3000 rpm for 10 min. Optical density (O.D) of supernatant was read against reagent blank. MDA reacts with thiobarbituric acid at 100°C in acidic medium to form a pink coloured complex which was measured at 535 nm. MDA concentration was calculated using the molar extinction coefficient of MDA-TBA complex (1.5×10^5L/mol/cm). The result is expressed as nmol/g haemoglobin.

Total Antioxidant Capacity (TAC) was measured by method of Koracevic et al., [16]. The assay measured the capacity of biological fluids to inhibit the production of TBARS from sodium benzoate under the influence of free oxygen radicals derived from Fenton's reaction. A solution of 1 mmol/L uric acid was used as standard.

Vitamin C was measured by Dinitro phenyl hydrazine (DNPH) method [17]. Ascorbic acid is initially oxidized by cupric ions to dehydroascorbate. This reacts with 2,4-Dintrophenyl hydrazine to form a red bishydrazone, and the intensity of colour developed is measured at 520 nm.

SOD was measured in RBC haemolysate using the method of Marklund and Marklund [18]. A 50µl of 1:100 haemolysate was added to 2.85ml of 0.05M Tris buffer (pH 8.5) and 0.1ml of 20mM pyrogallol. The principle involves the inhibition of autoxidation of pyrogallol by SOD which was measured as increase in absorbance at 420 nm for 3 min. One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation and results were expressed as U/g haemoglobin.

Catalase activity was determined by the method of Aebi [19]. A 10µl of 1:100 RBC haemolysate was added to 3ml of 0.05M phosphate buffer and 1ml of 30mM H_2O_2. The decomposition of H_2O_2 by catalase was measured at 240 nm as decrease in absorbance for 3 min. One unit of catalase activity is expressed as mM of H_2O_2 decomposition/mg haemoglobin/min. The molar extinction coefficient of H_2O_2 is 71 L/mol/cm.

STATISTICAL ANALYSIS

All the findings are expressed as Mean ± Standard deviation. The p-value of <0.05 was considered statistically significant, $p<0.01$ as highly significant and $p<0.001$ as very highly significant. Analysis was performed by various statistical tests like ANOVA, Tukey's post-hoc tests, Pearson's correlation using IBM®-SPSS®-statistics-version 20.0.

RESULTS

[Table/Fig-1] shows, the results of one-way ANOVA for comparison of means of biomarkers between overall groups under study. The mean values of free radical marker MDA was highly significant

Parameters	Healthy Controls	Gingivitis	Chronic Periodontitis (CP)	Chronic Periodontitis with Type 2 DM	F	p-value	Sig*
N	25	25	25	25			
MDA (nmol/g Hb)	70.11±26.43 (37.69%)	88.3±25.19 (28.53%)	94.13±23.17 (24.61%)	89.81±20.01 (22.28%)	4.957	0.003	<0.01
TAC (nmol/L)	1.93±0.97 (50.26%)	1.72±0.9 (52.33%)	1.78±0.98 (55.06%)	1.17±0.39 (33.33%)	3.743	0.014	<0.05
Vitamin C (mg/dl)	0.722±0.36 (49.86%)	0.496±0.39 (78.63%)	0.475±0.34 (71.58%)	0.376±0.28 (74.47%)	4.344	0.006	<0.01
SOD (U/g Hb)	103.5±69.24 (66.89%)	80.58±18.3 (22.71%)	79.04±26.35 (33.33%)	61.87±28.63 (46.27%)	4.395	0.006	<0.01
Catalase (mM/g Hb)	1.64±0.43 (26.22%)	1.79±0.37 (20.67%)	1.80±0.43 (23.88%)	1.47±0.36 (24.49%)	3.611	0.016	<0.05

[Table/Fig-1]: One-way ANOVA for comparison of means of biomarkers between different study groups.
All values are expressed in Mean±Standard deviation; Coefficient of variation expressed in percentage in brackets (%)
*Significance levels: p<0.05 is statistically significant, p<0.01 is highly significant, p<0.001 is very highly significant

Parameters	Group comparison	Mean difference ± Std Error	p-value	95% CI
MDA	Healthy controls vs Periodontitis with DM	19.70±6.73	0.022	2.07 to 37.32
MDA	Healthy controls vs Periodontitis	24.02±6.73	0.003	6.39 to 41.64
MDA	Healthy controls vs Gingivitis	18.19±6.73	0.040	0.57 to 35.81
TAC	Healthy controls vs Periodontitis with DM	0.75±0.24	0.012	0.12 to 1.38
Vit C	Healthy controls vs Periodontitis with DM	0.34±0.09	0.004	0.086 to 0.604
SOD	Healthy controls vs Periodontitis with DM	41.63±11.53	0.003	11.49 to 71.77
Catalase	Gingivitis vs Periodontitis with DM	0.31±0.11	0.034	0.016 to 0.614
Catalase	Periodontitis vs Periodontitis with DM	0.33±0.11	0.029	0.029 to 0.626

[Table/Fig-2]: Tukey's Post-hoc tests for comparison of means of biomarkers between individual study groups.

	PPD – CP (n=25)		PPD – CP with DM (n=25)	
	r	p	r	p-value
MDA#	0.07	0.739	0.566	0.003
TAC	0.181	0.386	-0.186	0.374
Vitamin C	-0.062	0.768	-0.146	0.487
SOD	-0.018	0.931	-0.216	0.301
Catalase	0.317	0.123	0.086	0.682

[Table/Fig-3]: Pearson's Correlation between biochemical parameters analysed and PPD among Chronic periodontitis (CP) with and without diabetes (DM).
#MDA (n=50) r=0.275, p=0.05 (with overall PPD of both groups- CP and CP with DM).

between all the groups (p<0.01). The difference in the mean values of total antioxidant capacity and the catalase were statistically significant between all groups (p<0.05). Plasma antioxidant vitamin C and erythrocyte SOD levels showed highly significant difference between all the study groups (p<0.01).

[Table/Fig-2] shows, the Tukey's Post-hoc analysis results for comparison of means of biomarkers between individual groups. The MDA levels were significantly increased among patients with gingivitis (p=0.04), chronic periodontitis (p=0.003) and periodontitis with type 2 diabetes (p=0.022) compared to the healthy control group. TAC was significantly decreased among periodontitis with type 2 diabetes in comparison with healthy controls (p=0.012). Decreased plasma vitamin C levels and erythrocyte SOD levels found in periodontitis with type 2 diabetes as compared to controls were highly significant (p=0.004 and 0.003 respectively). Catalase levels showed significantly low levels among periodontitis patients with type 2 diabetes in comparison with gingivitis and chronic periodontitis patients without diabetes (p=0.034 and 0.029 respectively).

We performed Pearson's correlation between all the analysed parameters with the probing pocket depth (PPD) which indicated the severity of the disease [Table/Fig-3]. Overall MDA values among both periodontitis group with and without type 2 diabetes together (n=50) were positively correlated with PPD (r=0.275, p=0.05). Whereas, there was a very significant positive correlation between the MDA values and PPD among periodontitis patients with diabetes mellitus (r=0.566, p=0.003).

DISCUSSION

Lipid peroxidation causes profound alteration in the structural integrity and function of cell membranes. The extent of tissue damage could be assessed by measuring the concentrations of lipid peroxidation products and antioxidants in erythrocytes and plasma as the lipid peroxidation products leak from the site of inflammation into the plasma [20]. In our study, erythrocyte MDA levels were significantly increased in all the patient groups when compared to healthy controls as observed in previous studies [21,22]. ROS causes periodontal tissue destruction either by degrading the ground substance or by release of collagenases or by release of various inflammatory mediators [23].

The occurrence of periodontitis in Type 2 diabetes commonly suggests that both the conditions have common elements of pathogenesis as both have inflammation in common. This is supported by the fact that Type2 diabetes is a significant risk factor for the development of periodontal disease and aggravates the severity of periodontal infections [24]. Our results show excessive free radical activity (MDA) among diabetics as observed in previous study [25] and decrease in antioxidant capacity (TAC, Vitamin C, SOD) which is highly significant among diabetic periodontitis compared to healthy controls. MDA levels correlated positively with probing pocket depth in all periodontitis patients. But there was a strong positive correlation between excessive ROS generation and the periodontal pocket depth in diabetic periodontitis subjects indicating that there is more tissue destruction in diabetics with excessive generation of ROS. Periodontitis is a low grade inflammatory condition associated with systemic inflammation supported by the association of C reactive protein levels. Studies have shown that the C reactive protein levels mediate the association between probing pocket depths and oxidative stress [26]. ROS compromises the body's ability to fight infection and repair the tissue in periodontal disease. Hence, worst periodontal health status is associated with greater oxidative injury [27]. Since the final result of lipid peroxidation, MDA is considered a biomarker of oxidative stress, the hypothesis of oxidative stress implication in pathogenesis of periodontal disease in diabetic patients can be supported by elevated MDA levels [28].

Increased destruction of periodontal tissue among those with diabetes may be due to altered host susceptibility to periodontal pathogens mediated by accumulation of advanced glycation end

products (AGEs). The inflammatory cells such as monocytes and macrophages have receptors for AGEs. Hence, accumulation of AGEs in diabetic gingiva increases the intensity of the immune-inflammatory response to periodontal pathogens. Interactions between AGEs and their receptors on inflammatory cells result in increased production of proinflammatory cytokines leading to enhanced oxidant stress and accelerated tissue damage [29]. The periodontal tissues are highly sensitive to the products derived from oxidative damage supported by the presence of a high RAGE (receptor for AGEs) expression in periodontal tissues [30]. The interaction between AGE formation, increased ROS by both diacylglycerol and protein kinase C pathway and activation of polyol pathway provides insight to exaggerated periodontal destruction among diabetics.

Exaggeration in innate immune response; impaired polymorphonuclear leukocyte function facilitate bacterial persistence in tissues; impaired bone healing due to accumulation of AGEs; reduction in collagen synthesis and increase in collagenase activity may collectively lead to excessive periodontal tissue breakdown in diabetes [31].

Inadequate total antioxidant defense enhances tissue injury due to excessive free radical production in individuals with periodontal disease [32]. Significantly decreased Total antioxidant capacity and SOD activity was observed in periodontitis patients with diabetes; although lower mean levels were observed in patients with gingivitis and periodontitis as compared to healthy controls. Both cross-sectional and longitudinal experimental studies have also shown a reduction in TAC and SOD both within the local periodontal tissues and in plasma compared to controls [9,33,34]. Reduced plasma total antioxidant defense could result from low grade inflammation induced by the host response to periodontal bacteria [34]. The disturbed antioxidant status may be explained on the basis of increased ROS production by phagocytic cells in periodontitis [35, 36].

The mean vitamin C levels in our study decreased in a graded manner from group1 to group 4 study subjects but significant decrease was noted in diabetic periodontitis group as compared with healthy controls. Low serum vitamin C levels were reported in periodontitis patients with diabetes by Thomas B et al., [37]. Patients with periodontitis tend to have a low level of plasma ascorbate due to gingival bleeding [38]. A 30-40% reduction in plasma vitamin C levels among periodontitis patients were reported by Panjamurthy et al., [21]. Vitamin C deficiency increases susceptibility to infection, impairs the function of neutrophils and macrophages, and also depletes its role as a radical scavenger. Vitamin C is also known to reduce the CRP mediated inflammatory/ immune responses and hence decrease likelihood of tissue damage [9].

SOD and catalase respectively protect the cell against ROS by scavenging superoxide radical and H_2O_2, which cause damage to the structure and function of membrane. The increased mean catalase levels in the gingivitis and periodontitis groups may be explained on the basis that increased free radical production may enhance the antioxidant defense system which counterbalances the pro-oxidant environment [21, 25, 39]. Significantly decreased catalase levels among periodontitis patients with type 2 diabetes in comparison with other patient groups may be indicative of a stage of excessive free radical generation and consumption of antioxidant species which could be mediated by severe hyperglycemia and systemic inflammation [40]. The results of similar studies published in literature in the recent past have been summarized in [Table/Fig-4].

The present study had a few limitations like the group of only Type 2 Diabetic individuals without periodontal disease for comparison was not included.

Strict control of blood sugar levels may decrease the severity of periodontitis in uncontrolled diabetics since tissue destruction is more with excessive oxidative stress in these patients. This can be a starting point for further research on the efficiency of different antioxidant agents for prevention and treatment of periodontal disease in diabetic patient.

Sl. No.	First author	Year	Increase in Oxidative stress parameters	Decrease in Antioxidant parameters
1.	Trivedi S et al., [22]	2014	MDA in plasma and saliva	SOD, Glutathione reductase, Catalase in RBC lysate and saliva
2.	Kumari S et al., [35]	2014		TAC in serum
3.	Monea A et al., [28]	2014	MDA in periodontal tissue	GSH in periodontal tissue
4.	Thomas B et al., [40]	2013		Glutathione, Catalase, and Selenium in serum
5.	Dhotre PS et al., [36]	2012	Lipid peroxide, NO in serum	SOD, GSH, TAC in serum
6.	Panjamurthy K et al., [21]	2005	TBARS in blood and tissue	SOD, Catalase, GSH, Vitamin C, E in blood and tissue
7.	Brock GR et al., [34]	2004		TAC in saliva and blood
8.	Chapple IL et al., [9]	2002		TAC and GSH in GCF and plasma
	Our study		MDA in RBC lysate	SOD, Catalase in RBC lysate, Vitamin C, TAC in plasma

[Table/Fig-4]: Results of similar studies published in literature.

CONCLUSION

Excessive ROS associated with decreased antioxidant defense mechanisms results in tissue destruction in periodontal diseases. The severity of tissue destruction due to excessive ROS is more when periodontal disease is associated with type 2 DM, indicating that oxidative stress is a common factor involved in tissue destruction. Several more controlled interventional studies are warranted to understand the possible role of antioxidant therapy in either preventing the disease or in controlling the progression of disease.

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REFERENCES

[1] Ridgeway EE. Periodontal disease: Diagnosis and management. *J Am Acad Nurse Pract*. 2000;12:79-83.
[2] Brown LJ, Loe H. Prevalence, extent, severity and progression of periodontal disease. *Periodontol*. 2000;2:57-71.
[3] Vinitha R, Khalid G, Kumar V, et al. Periodontal diseases in Asian Indian diabetics: A study from southern India. *Int J Diab Dev Countries*. 2001;21:129-31.
[4] Pendyala G, Thomas B, Kumari S. The challenge of antioxidants to free radicals in periodontitis. *Journal of Indian Society of Periodontology*. 2008;12(3):79-83.
[5] Soory M. Oxidative stress induced mechanisms in the progression of periodontal diseases and cancer: A common approach to redox homeostasis? *Cancers*. 2010;2:670-92.
[6] Omeh YS, Uzoegwu PN. Oxidative stress marker in periodontal disease patients. *Nigerian Journal of Biochemistry and Molecular Biology*. 2010;25(1):50-4.
[7] Gurav A and Jadhav V. Periodontitis and risk of diabetes mellitus. *Journal of Diabetes*. 2011;3:21-22.
[8] Loe H. Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes Care*. 1993;16(1):329-34.
[9] Chapple ILC, Milward MR, Dietrich T. The prevalence of inflammatory periodontitis is negatively associated with serum antioxidant concentrations. *J Nutr*. 2007;137:657-64.
[10] Greenstein G. The role of bleeding upon probing in the diagnosis of periodontal disease: a literature review. *J Periodontol*. 1984;55:684.
[11] Muhlemann HR, Son S. Gingival sulcus bleeding – a leading symptom in initial gingivitis. *Helvetica Odontologica Acta*. 1971;15:107–10.
[12] Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*. 1999;4:1-6.
[13] Mealey BL. World workshop in clinical periodontics. Periodontal implications: medically compromised patients. *Ann Periodontol*. 1996;1:256–321.

[14] Tsai C, Hayes C, Taylor GW. Glycaemic control of type 2 diabetes and severe periodontal disease in the US adult population. *Community Dent Oral Epidemiol*. 2002;30:182–92.
[15] Ohkawa H, Ohishi N, Yaga K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95:351-58.
[16] Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for measurement of antioxidant activity in human fluids. *J Clin Pathol*. 2001;54:356-61.
[17] McCormick DB, Greene HL. 'Vitamins' In: Burtis CA, Ashwood ER (Eds) Tietz textbook of clinical chemistry, 3rdedition; WBsaunders company, USA; Harcourt Brace and company Asia PTE. Ltd, India: 1999; pp.1024-25.
[18] Marklund S, Marklund E. Assay of SOD activity in animal tissue. *J Biochem*. 1988;13(3):305-15.
[19] Aebi H. 'Catalase' In: Methods in enzymatic analysis. Ed. Bergmeyer HU, Vol 3, Academic press, Newyork,1983; pp-276-86.
[20] Kwiatkowska S, Piasecka G, Zieba M, et al. Increased serum concentration of conjugated dienes and malondialdehyde in patients with pulmonary tuberculosis. *Respir Med*. 1999;93:272-76.
[21] Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. *Cell Mol Biol Lett*. 2005;10:255-64.
[22] Trivedi S, Lal N, Mahdi AA, Mittal M, Singh B, Pandey S. Evaluation of antioxidant enzymes activity and malondialdehyde levels in patients with chronic periodontitis and diabetes mellitus. *J Periodontol*. 2014;85(5):713-20.
[23] Sharma A, Sharma S. Reactive oxygen species and antioxidants in periodontics: A review. *International journal of Dental clinics*. 2011;3(2):44-47.
[24] Kim JB, Jung MH, Cho JY, et al. The influence of type 2 diabetes mellitus on the expression of inflammatory mediators and tissue inhibitor of metalloproteinases-2 in human chronic periodontitis. *J Periodontal Implant Sci*. 2011;41:109-16.
[25] Al-Rawi NH. Oxidative stress, antioxidant status and lipid profile in the saliva of type 2 diabetics. *Diabetes and vascular disease research*. 2011;8(1):22-28.
[26] D'Aiuto F, Nibali L, Parkar M, Patel K, Suvan J, Donos N. Oxidative stress, systemic inflammation, and severe periodontitis. *J Dent Res*. 2010;89(11): 1241-46.
[27] Sculley DV, Langley-Evans SC. Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. *Clinical Science*. 2003;105:167-72.
[28] Monea A, Mezei T, Popsor S, Monea M. Oxidative Stress: A link between diabetes mellitus and periodontal disease. *International Journal of Endocrinology*. 2014:1-4.
[29] Schmidt AM, Weidman E, Lalla E, et al. Advanced glycation end products (AGEs) induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. *Journal of Periodontal Research*.1996;31(7):508-15.
[30] Katz J, Bhattacharyya I, Farkhondeh-Kish F, Perez FM, Caudle RM, Heft MW. Expression of the receptor for advanced glycation end products in gingival tissues of type 2 diabetic patients with chronic periodontal disease: a study utilising immunohistochemistry and RT-PCR. *J Clin Periodontol*. 2005;32:40-44.
[31] Um YJ, Jung UW, Kim CS, et al. The influence of diabetes mellitus on periodontal tissues: a pilot study. *J Periodontal Implant Sci*. 2010;40:49-55.
[32] Chapple ILC, Brock G, Eftimiadi C, Matthews JB. Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. *J Clin Pathol: Mol Pathol*. 2002;55:367-73.
[33] Baltacioglu E, Akalin FA, Alver A, Balaban F, Unsal M, Karabulut E. Total antioxidant capacity and superoxide dismutase activity levels in serum and GCF in postmenopausal women with periodontitis. *J Clin Periodontol*. 2006;33:385-92.
[34] Brock GR, Butterworth CJ, Matthews JB, Chapple ILC. Local and systemic total antioxidant capacity in periodontitis and health. *Clin Periodontol*. 2004;31(7): 515-21.
[35] Kumari S, Prasad BR, Rao A, Thomas B. Serum levels of antioxidants and superoxide dismutase in periodontitis patients with diabetes type 2. *Journal of Indian Society of Periodontology*. 2014;18(4):451-55 .
[36] Dhotre P, Suryakar AN, Bhogade RB. Oxidative Stress in Periodontitis. *Eur J Gen Med*. 2012;9(2):81-84 .
[37] Thomas B, Kumari S, Ramita K, et al. Comparative evaluation of micronutrient status in the serum of diabetes mellitus patients and healthy individuals with periodontitis. *Journal of Indian Society of Periodontology*. 2010;14(1):46-49.
[38] Pussinen PJ, Laatikainen T, Alfthan G, Asikainen S, Jousilahti P. Periodontitis is associated with a low concentration of vitamin C in plasma. *Clinical and Diagnostic Laboratory Immunology*. 2003;10(5):897-902.
[39] Liu Z, Liu Y, Song Y, Zhang X, Wang S, Wang Z. Systemic oxidative stress biomarkers in chronic periodontitis: a meta-analysis. *Disease Markers*. 2014;1-10.
[40] Thomas B, Ramesh A, Suresh S, Prasad BR. A comparative evaluation of antioxidant enzymes and selenium in the serum of periodontitis patients with diabetes mellitus type2. *Contemp Clin Dent*. 2013;4(2):176–80.

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