Original article:

Lipid peroxidation marker in saliva of Type 2 Diabetes Mellitus with oral manifestations – A Clinical and biochemical study.

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Abstract:

Background: Type 2 Diabetes progresses gradually and in a stepwise order. It commences with insulin resistance and progresses slowly with time until the body fails to maintain glucose homeostasis. These alterations are accompanied with changes in lipid peroxidation. The determination of the oxidative stress requires sometimes invasive techniques. Exploring saliva for oxidative stress has great clinical interest***Objective:*** The present study was undertaken to estimate, compare and correlate the levels of malondialdehyde (MDA) in the serum and saliva of patients with type 2 diabetes mellitus with oral manifestations and healthy controls. ***Materials and Methods:*** Serum and salivary Malondialdehyde levels were estimated in 45 healthy subjects (Group I) and 45 patients with Type 2 Diabetes with oral manifestations (Group II). Estimation of Random blood sugar levels were done by GOD-PAP methodology. The data obtained from the present study was analyzed using SPSS software. Independent T test was used to compare the levels in the study and control group. Pearson's correlation coefficient was used to correlate the changes in serum and saliva. P< 0.05 was considered significant. ***Results:***The mean serum Malondialdehyde levels in Group I was 0.958 µM/l, while the mean serum Malondialdehyde levels of Group II was 2.828 µM/l. The mean salivary Malondialdehyde levels in Group I was 0.217 µM/l, while the mean salivary Malondialdehyde levels of Group II was 0.688µM/l. The mean serum and salivary Malondialdehyde levels were significantly increased in subjects with Type 2 Diabetes with oral manifestations in comparison to the healthy subjects. Fair positive correlation was observed between serum and salivary Malondialdehyde levels in Group I (r = 0.341) and very good positive correlation was observed between serum and salivary Malondialdehyde levels in Group II (r = 0.613). ***Conclusion:*** Serum and salivary Malondialdehyde was significantly higher in subjects with Type 2 Diabetes Mellitus with oral manifestations when compared to healthy controls. The increase in serum and salivary levels of MDA, shows that free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation, which may play an important role in the development of complications in diabetic patients. Fair positive correlation was found between serum and salivary Malondialdehyde in healthy subjects and very good positive correlation was observed between serum and salivary Malondialdehyde in subjects with Type 2 Diabetes Mellitus with oral manifestations. This study highlights that type 2 diabetic patients undergo abnormally high levels of oxidative stress. Hence exploring saliva for oxidative stress is of great importance. Thus saliva could be used as a reliable, non-invasive tool in the assessment of oxidative status.

Keywords: lipid peroxidation, malondialdehyde, oxidative stress, Type 2 Diabetes Mellitus.

Bangladesh Journal of Medical Science Vol. 17 No. 04 October'18. Page : 644-651
DOI: http://dx.doi.org/10.3329/bjms.v17i4.38330

Introduction:

Type 2 diabetes mellitus is a familiar endocrine and metabolic condition. It has touched epidemic magnitudes globally and symbolizes a grave community health concern. It is projected that it will be affecting roughly three hundred sixty six million people by 2030[1,2]. This rampant disorder is increasing at anextra ordinary rate in youngsters, with the major

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escalation in developing nations[1].

Diabetes mellitus is categorized by hyperglycemia. Biochemically there are variations in glucose levels and lipid peroxidation. Lipid peroxidation is a process connected to free radicals. It is an uninhibited, self-augmenting process that leads to disruption of membranes, lipids and other cell constituents[3].The occurrence of lipid peroxidation induced by free radicals cause considerable alterations in the cell membrane.

The lipid peroxidation of the membranes is induced by the reactive oxygen species (ROS). The destructiveness of the fatty acids peroxides that have been produced are the prime cause of failure of cellular functions. The most comprehensively used assay for lipid peroxidation is the measuring malondialdehyde (MDA). Therefore, the lipid peroxide in the blood offers evidence for the prognosis of diabetes.

The assessment of the oxidative stress comprises procedures like blood sample collection that are quite invasive in nature. Whole saliva is a dynamic fluid which contains enormously complex constituents. Variable measures of blood and serum products are existing in whole saliva. Discovering saliva for oxidative stress has abundant clinical importance [4].

Diabetes can be suspected on the basis of a multitude of systemic and oral signs and symptoms, including gingivitis and periodontitis, persistent oral fungal infections and compromised wound healingcapacity[5].

Oral health is a mirror image of patients' general health. The capacity of saliva to be utilized to observe a patient's status of health and illness is a exceeding lydesired objective for promotion of health and research in the field of health sciences[6].

Thus the present study is undertaken to estimate and compare the levels of malondialdehyde (MDA) in the serum and saliva of patients with type 2 diabetes mellitus with oral manifestations and healthy controls and to correlate the changes in the saliva and serum.

Materials and methods:

The present study was conducted on subjects who reported to Department of Oral Medicine and Radiology at a dental college in South India during 2013-2016. After obtaining the Institutional ethical clearance, the nature of the study and the purpose were explained to the participants in detail, and informed written consent was attained from the participants who were included in the study. A detailed case history of each subject was recorded with thorough examination of the oral cavity.

Sample size of 90 subjects was divided into 2 equal groups of 45 patients each.

Control Group (Group I) : 45 healthy subjects without any oral and systemic diseases.

Study Group (Group II): 45 subjects diagnosed clinically with type 2 diabetes mellitus with laboratory investigations for confirmation and who had oral manifestations of diabetes. Study group included subjects who were already diagnosed with type 2 diabetes mellitus for more than 5 years and under oral hypoglycaemic drugs (Example: biguanides like metformin, sulphonylureas like glimepiride, thiozolidinediones like pioglitazone, insulin and alpha glucosidase inhibitors like miglitol).

Convenient sampling technique was employed to recruit subjects under both the Group I and Group II. Strict inclusion criteria's were followed for both control group and study group. Healthy subjects in the age group of 30 - 60 years and without any history of oral and systemic diseases were taken as controls. Random blood sugar levels were estimated to rule out undiagnosed type 2 diabetes mellitus.

Subjects included in the Group I were not on any medications and did not have adverse oral habits like smoking, tobacco chewing and alcohol consumption.

Study group consisted of subjects clinically diagnosed with type 2 diabetes mellitus and confirmed with laboratory investigations. It included patients who are diagnosed with type 2 diabetes mellitus for more than 5 years and under oral hypoglycemic drugs. Subjects with oral manifestations of diabetes mellitus like burning mouth syndrome, candidiasis, dental caries, gingivitis, glossodynia, lichen planus, neurosensory dysesthesias, periodontitis, salivary dysfunction, taste dysfunction, xerostomia were included in the study group.

Subjects with history of any systemic diseases (other than type 2 diabetes mellitus) were excluded from the study. Pregnant and lactating women, subjects diagnosed with any malignancies, who are on any medications other than oral hypoglycemic drugs, subjects with any other oral mucosal lesions other than those stated in inclusion criteria and those patients who have adverse oral habits like smoking, tobacco chewing and alcohol consumption were excluded from the study.

Each patient was thoroughly examined both intra orally and extra orally under artificial light.

Armamentarium used for clinical examination were:

1. Physiological dental chair with artificial illumination, water jet, and compressed air facility.
2. Sterile disposable gloves and mouth mask.
3. Sterile two plain mouth mirrors (No.5)
4. Sterile single ended straight probe (No.9)

5. Sterile explorers (No.17)
6. Sterile tweezers
7. Sterile kidney trays
8. Sterile gauze pieces and cotton pellets
9. Williams Graduated Periodontal probe

Periodontal examination:

Each participant was examined using a mouth mirror and Williams's periodontal probe under artificial light.

The severity of inflammation was assessed by using Gingival index and pocket probing depth.

Gingival Index as given by Loe and Sillness, in the year 1963.

Method: Tissues surrounding each tooth are divided into 4 scoring units – distal facial papilla, facial margin, mesial facial papilla, entire lingual margin. The severity of gingivitis was scored for all the teeth present. The following scores indicate -

0 Absence of inflammation
1 Mild inflammation- slight / change in colour, slight edema, no bleeding on probing
2 Moderate inflammation – moderate glazing, redness, edema and hypertrophy, bleeding on probing.
3 Severe inflammation – marked redness and hypertrophy ulceration. Tendency to spontaneous bleeding.

Pocket depth was measured in millimeters using the probe at 4 sites per tooth. Clinical attachment loss was calculated using Williams graduated periodontal probe. Patients with periodontal disease having clinical loss of attachment more than or equal to 4mm were considered for study group (Group II). Patients should have maintained good oral hygiene with gingival index score of less than 2.0 for control group (Group I). The oral cavity of the subjects was also examined for any other oral manifestations of diabetes as mentioned in the inclusion criteria for Group II.

Method of collection of data:

Sample collection:

Informed consent was taken from the patients included in the study. Ethical clearance was obtained from the Institutional Ethical Committee (Ethical Certificate Number: ABSM/EC/41/2012).

Saliva collection:

Samples of saliva were collected from participants two hours after intake of food using spit technique. The patient was made to sit on the dental chair with head slanting forward and asked not to speak or swallow saliva. Then the patient was asked to spit into a sterile container every minute for five to eight minutes. Salivary sample represents whole mouth fluid. The collected sample was centrifuged at 3000 rpm for ten minutes and the supernatant is collected and stored at -20^{0}C.

Blood collection:

5ml of venous blood was collected from the antecubital vein with syringe and placed in vials. Serum was then extracted and stored at temperature of -20^{0}C in glass vials.

Estimation of Random Blood Sugar (R.B.S) levels by GOD-PAP methodology[7]**:**

Random blood glucose level was determined by using glucose oxidase-peroxidase (GOD-POD) method. In this method the preliminary enzymatic oxidation of glucose is caused by the enzyme glucose oxidase (GOD). The calorimetric indicator is Quinone. It is produced by hydrogen peroxide from 4 aminoantipyrine and phenol because of the catalytic action of peroxidase (POD) (Trinder's reaction). 3 test tubes each labelled as 'Blank', 'Standard' and 'Test' were taken. Then a pipette was used to transfer 1000 µl of reagent solution to each of these test tubes. 10 µl of standard was added to the test tube marked as 'Standard'. This was followed by addition of 10 µl of test sample to the 'Test' test tube. The sample was mixed and incubated for 10 minutes at 37^0 C. Within 60 minutes absorbance was measured at 505 nm against reagent blank using Roche automated clinical chemistry analyser.

Estimation of Malondialdehyde[8]**:**

Principle:

MDA reacts with thiobarbituric acid (TBA) to give a pink colour. This was read at 535nm. Both serum and saliva samples were analysed.

Sample volume:

Serum: 100µl

Saliva: 250µl

Chemicals that were used:

1) Tri chloro acetic acid (TCA)-(CH3COOCl3)
2) 2-thiobarbituric acid (TBA)-(C4H4N2O2S)
3) Hydrochloric acid (HCl)
4) Malonaldehyde bis (dimethyl acetal)-(C7H16O4)

Preparation of the reagent:

TCA-TBA-HCI reagent:

- 0.25N HCI: 2.21ml of cone. HCI is made upto 100ml with distilled water.
- 15% TCA and 0.375% TBA - 15g TCA and 0.375g of TBA is dissolved in 100ml of O.25N HCI the reaction mixture was warmed to dissolve the contents and stored at 4°C.

MDA Standard (Stock-164flg/ml):

- 16.4l of the standard malonaldehyde solution was taken and made up to 100ml with distilled water.

MDA Standard (Working-1.64flg/ml):

- 100µl of the stock was made up to 10ml with distilled water

Estimation of malondialdehyde in sample:

Sample preparation:

- Serum-100µl serum was diluted to 500µl with distilled water.
- Saliva- 250µl of the saliva was diluted to 500µl with distilled water.

Treatment:

To the diluted sample one ml of TCA-TBA-HCl reagent was added. The samples were kept in boiling water bath for a period of fifteen minutes. The reaction mixture was cooled and centrifuged. The supernatant was taken and the optical density of the pink colour formed was read at 535nm. The concentration of malondialdehyde in the sample was got by plotting the obtained absorbance against the standard graph. The optical density of the pink colour formed was directly proportional to the concentration of malondialdehyde in the given sample.

Calculation:

The optical densities of the test samples were calculated by the plotting against the standard graph and multiplied by the respective dilution factors and the final concentration was expressed as µM/l.

Method of analysis

The data obtained from the present study was analysed using SPSS version 17.0 software. Independent t test was used to compare the levels in the study and control group. Pearson's correlation coefficient was used to correlate the changes in serum and saliva. $p < 0.05$ was considered as significant.

Results:

Demographic data analysis of the groups:

Demographic data analysis of Control Group (Group I):

The mean age in this group was 40.58 years. Females comprised of 48.9% (22/45) while males comprised of 51.1% (23/45) [Table 1,2].

Demographic data analysis of Study Group (Group II):

The mean age in this group was 47.91 years. Females comprised of 60.0% (27/45) while males comprised of 40.0% (18/45) [Table 1,2].

Table 1: age distribution in healthy subjects and subjects with type 2 diabetes mellitus

	GROUP	N	Mean	Std. Deviation
Age	Control	45	40.58	7.56
	Diabetes with oral manifestations	45	47.91	7.633

Table 2: gender distribution in healthy subjects and subjects with type 2 diabetes mellitus

			Gender		Total
			F	M	
Group	Control	Count	22	23	45
		% within Group	48.9%	51.1%	100.0%
	Diabetes with oral manifestations	Count	27	18	45
		% within Group	60.0%	40.0%	100.0%
Total		Count	49	41	90
		% within Group	54.4%	45.6%	100.0%

In the study group among 45 patients with Type 2 diabetes Mellitus, 42 patients had Dental caries, 39 patients had Chronic periodontitis, 5 patients had Gingivitis, 2 patients had Candidiasis and 1 patient had Denture stomatitis. Most of the patients showed combinations of two oral manifestations.

The mean Random Blood Sugar levels of Control Group (Group I) was 102.71 mg/dl and the mean Random Blood Sugar levels of Study Group (Group II) was 210.91 mg/dl. In the Study Group (Group II) 28/45 patients were poorly controlled diabetics and 17/45 were well controlled diabetics. The mean duration of Type 2 diabetes in Study group (Group II) was 8.73 years.

Analysis of mean serum and salivary Malondialdehyde levels in Controls and Type 2 Diabetics with oral manifestations:

Mean Serum Malondialdehyde levels:

The mean serum Malondialdehyde levels in Group I was 0.958 µM/l, while the mean serum Malondialdehyde levels of Group II was 2.828 µM/l[Table 3].

Mean Salivary Malondialdehyde levels:

The mean salivary Malondialdehyde levels in

Group I was 0.217 µM/l, while the mean salivary Malondialdehyde levels of Group II was 0.688 µM/l [Table 3].

Analysis of Statistical Significance:

Serum Malondialdehyde levels:

When serum levels of Malondialdehyde were compared between Group I (0.958 µM/l) and Group II (2.828 µM/l), the difference was statistically highly significant (p<0.001) [Table 3].

Salivary Malondialdehyde levels:

On comparing salivary Malondialdehyde levels of Group I (0.217 µM/l) and Group II (0.688 µM/l), the difference was highly significant (p<0.001) [Table 3].

Table 3: comparison of serum and saliva levels of malondialdehyde using independent t test

	Group	N	Mean	Std. Deviation	T	Df	P Value
Serum Malondialdehyde (Mda) In Mm/L	Control	45	0.958222	0.225129	-14.387	50.255	**≤0.001**
	Diabetes With Oral Manifestations	45	2.828	0.842277			
Saliva Malondialdehyde (Mda) In Mm/L	Control	45	0.217778	0.070126	-14.67	54.317	**≤0.001**
	Diabetes With Oral Manifestations	45	0.688222	0.203373			

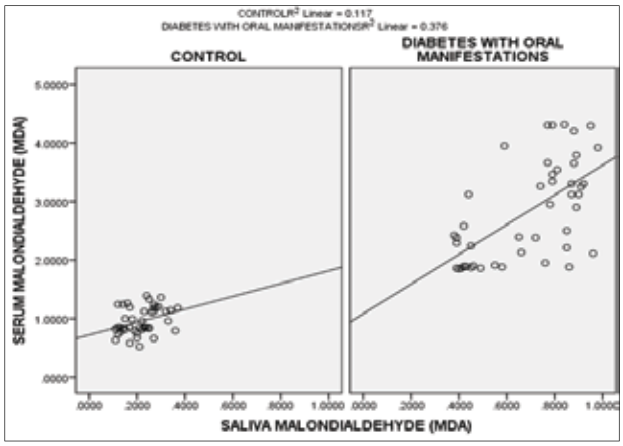
Correlation of Serum Malondialdehyde with Salivary Malondialdehyde levels among the two groups:

Group 1: Fair positive correlation was observed between serum and salivary Malondialdehyde levels. (r = 0.341) [Table 4 Graph 1].

Group 2: Very good positive correlation was observed between serum and salivary Malondialdehyde levels. (r = 0.613) [Table 4 Graph 1].

Table 4: correlation of salivary and serum levels using pearsons correlation

Group			Saliva Malondialdehyde (Mda)
Control	Serum Malondialdehyde (Mda)	Pearson Correlation	.341
		Sig. (2-Tailed)	**.022**
		N	45
Diabetes With Oral Manifestations	Serum Malondialdehyde (Mda)	Pearson Correlation	.613
		Sig. (2-Tailed)	**≤0.001**
		N	45



Graph 1: correlation of salivary and serum levels using pearsons correlation

Discussion:

Type 2 Diabetes is becoming a majorhealth problem in the developing nations. This long-lasting and multifacetedcondition can badlyimpact both longevity and quality of life[9]. Diabetes Mellitus is one among the earliestailmentsrecognisedby the human race. It was initiallydescribed in Egyptian manuscript about three thousand years ago[10].

Type 2 DM is the outcome of the interactions between hereditary, environmental and behavioural risk factors. Individualsexisting with type 2 DM are more vulnerable to short term and long term complications. The higher morbidity and mortality rate is seen in patients with type 2 DM because of

the commonness of this type of DM, its insidious and deceptive beginning and late recognition[10].

It is projected that the incidence of DM in adult population will see a rise in the next two decades and considerable increase will be seen in the countries that are still developing. Moreover, it is predicted thatthe bulk of these patients would beaged between 45 and 64 years[10].In our study the mean age of the patient in the study group consisting of Type 2 diabetics with oral manifestations was 47.91 years.

Diabetes mellitus can have variable effects on the tissues in the oral cavity. Patients with poor control in their glucose levels are for the most part susceptible to severe persistent and recurrent bacterial and fungal infections[11].Persistent poor glycemic control has been connected to the occurrence and advancement of diabetes as well as its associated complications, including gingivitis, periodontitis and alveolar bone loss. Patients with diabetes mellitus are susceptible to oral sensory, periodontal and salivary disorders. This has the capacity to escalate the risk of fresh as well as recurrent dental caries.Hypofunction of saliva also risesthe oral candidal carriage in adults with diabetes mellitus. Occurrences of burning mouth syndrome, glossodynia, lichen planus, neurosensory dysesthesias, salivary and taste dysfunction and xerostomia are also quite common among diabetics[5].

Free radicals plays huge role in the origin and difficulties of diabetes mellitus[12].Scavenging of reactive oxygen species may be unsuccessful due to the variations and perturbations in the endogenous free radical defence mechanisms. The consequences of this is the oxidative damage injury to the tissues[9].

Malondialdehyde, as TBARS (Thio Barbituric Acid Reacting Substances), is often used to measure the prooxidant/antioxidant equilibrium in type 2 diabetic patients as they are stable as well as easily measurable lipid peroxidation products[13].The present study was carried out with the objective of estimating, comparing and correlating the serum and salivary Malondialdehyde in healthy subjects and subjects with Type 2 Diabetes Mellitus with oral manifestations.

The mean serum Malondialdehyde level in our study among healthy controls was 0.958 µM/l, while in subjects with Type 2 diabetes mellitus with oral manifestations it was increased to 2.828 µM/l. This was in accordance to a study conducted by Collier et al[14], Pasaoglu H et al[15]and Mahboob et al[3] where they stated that free radicals are producedin diabetes mellitus extremely disproportionately because ofdegradation of glucose, glycation of proteinsnon-enzymatically, and the oxidative degradation. The generation of free radicals may lead to lipid peroxidation in diabetes mellitus.

Further the present study is consistent with the studies by Kalaivanam et al[16]and Peerapatditet al[17]where the serum levels of MDA were significantly higher in diabetic patients in comparison to the normal controls. Diabetes Mellitus has been known to be a state where there is surplus generation of free radicals due to numerous mechanisms, including hyperglycaemia and antioxidant status, causing oxidative stress. This oxidative stress exaggerates the progression and development of diabetes mellitus and its complications. Disproportionate production of excessive free radicals and its inadequate elimination results in injury to membrane lipids and nucleic acids and cellular proteins.

In the present study, the mean serum level of MDA in Type 2 diabetic patients was 2.828 µM/l which was significantly higher than the healthy controls, which was in accordance to the studies conducted by Suryawanshiet al[18], Kumari et al[19] and Natheer H Al-Rawi[4]thereby establishing that heightened susceptibility and predisposition of cells to lipid peroxidation and inflammation due to oxidative stress plays a prime role in the pathogenesis of diabetes mellitus and its complications.

Till date salivary studies which have been documented in literature with the goal of estimating the Malondialdehyde levels in Type 2 Diabetics with oral manifestations are very few. In our study we attempted the estimation in saliva. The mean salivary Malondialdehyde levels in healthy controls was 0.217 µM/l, while the mean salivary Malondialdehyde levels in subjects with type 2 diabetes mellitus with oral manifestations was significantly increased to 0.688 µM/l.

The present study is in conformity with the study by Natheer H Al-Rawi[4] where MDA levels were elevated in the salivary samples of diabetic patients. The study stated that the salivary MDA level was significantly increased in the diabetic group which mirrored the high oxidative stress levels. Increased oxidative stress was communicated by an enhanced production of free radicals, peroxidation of lipids and reduction in antioxidant status.

The present study is in accordance to the study conducted by Mahadevan et al[20] where high levels of MDA were observed in diabetics as compared to controls validating the role of oxidation of free radicals in pathogenesis of diabetes mellitus. The results established that salivary MDA is the indicator

of oxidative stress in subjects with diabetes mellitus. Thus, saliva being minimally invasive and easy to collect can be used to assess MDA levels of the patients with diabetes mellitus.

As seen in the present study, Rajeshwari et al[21] reported the increase in the salivary MDA levels and stated that diabetes mellitus can cause dysfunction in the endothelium owing to increased oxidative stress. Thus it could be stated that the overwhelming reaction of the body towards oxidative stress which is reflected in the saliva could be used as a reliable marker.

No studies have documented the correlation between serum and salivary Malondialdehyde in healthy subjects and subjects with Type 2 Diabetes mellitus with oral manifestations.The Pearsons correlation analysis revealed fair positive correlation between serum and salivary Malondialdehyde in healthy subjects (r = 0.341). Very good positive correlation was observed between serum and salivary Malondialdehyde in subjects with Type 2 Diabetes Mellitus with oral manifestations (r = 0.613). Thus, the lipid peroxidation evaluated in saliva of diabetic patients may be valuable in evaluating the activity and severity of the disease. The findings of this study suggests the role of saliva as an adjunctive tool to monitor prognosis of diabetes mellitus.This study suggests that exploring saliva for oxidative stress may have boundless clinical importance. So examining the salivary content of peroxidation of lipids in patients with type 2 diabetes mellitus that accurately reflect the severity of the oxidative stress is worthy.

Conclusion:

In the present study, both serum and salivary Malondialdehyde was significantly higher in subjects with Type 2 diabetes mellitus with oral manifestations when compared to that of the healthy individuals. Moreover, there was a positive correlation between serum and salivary Malondialdehyde in both the healthy subjects as well as subjects with Type 2 diabetes mellitus with oral manifestaions.

The results obtained by comparison of serum and salivary levels of oxidative stress marker shows that the prospects for saliva as a non-invasive medium in screening and in monitoring prognosis of diabetes mellitus is abundant. This implicates a possibility for using saliva as an adjunctive diagnostic tool along with serum in the future.

Further extensive studies are required with larger samples to establish the reliability of Malondialdehyde in saliva as a potential biomarker of oxidative stress in diabetes mellitus and to establish the role of lipid peroxidation in the pathogenesis of diabetes mellitus and its complications.

Acknowledgement:

My sincere thanks to Dr. Srikanth N for compiling the statistics for this study. I sincerely thank Ms. Harshini, Research assistant, Nitte University of Science and Advanced Research who helped me to conduct the biochemical analysis in the Research Laboratory.

Conflict of interest:

The authors have no potential conflicts of interest to declare.

References:

1. Song F, Jia W, Yao Y, Hu Y, Lei l, Lin J, Sun X, Liu l. Oxidative stress, antioxidant status and DNA damage in patients with impaired glucose regulation and newly diagnosed type 2 diabetes. *Clinical Science* 2007;**112**: 599-606.
2. Rathmann W, and Giani G. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27:** 2568–2569.
3. Mahboob M, Rahman MF, Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. *Singapore Med J* 2005;**46:** 322-324.
4. Rawi NHA. Oxidative stress, antioxidant status and lipid profile in the saliva of type 2 diabetics. *Diabetes & Vascular Disease Research* 2011; **8:** 22-28.
5. Ship JA.Diabetes and oral health an overview.*JADA* 2003;**134**:4S-10S.
6. Mandel ID. Salivary diagnosis: more than a lick and a promise.*JADA* 1993;**124**(1):85-7.
7. Trinder P, Ann Cain. *Biochem* 1969;**6**:24.
8. Buege JA and Aust SD.Microsomal lipid peroxidation. *Methods enzymol* 1978;**52**:302-10.
9. Kolhe SM, Khanwelkar CC. Oxidative stress, Antioxidants and Metformin in type 2 diabetes mellitus. *J Med Edu Res* 2013;**3**(2):25-32.
10. Olokoba AB, Obateru OA, Olokoba LB. Type 2 Diabetes Mellitus : A review of current trends. *Oman Med J* 2012; **28**(4):269-273.
11. Manfredi M, McCullough M J, Vescovi P, Al-Kaarawi Z M, Porter S R. Update on diabetes mellitus and related oral diseases. *Oral Diseases* 2004; **10:** 187–200.
12. PJ Hisalkar , AB Patne , MM Fawade. Assessment of plasma antioxidant levels in type 2 diabetes patients. *Int J Biol Med Res* 2012; **3**(2): 1796-1800.
13. Kumari MK, Sankaranarayan T. Evaluation of oxidative stress in type 2 diabetes mellitus patients. *JDMS* 2014;**13**(5):46-50.
14. Collier A, Rumley A, Rumley AG, Paterson JR, Leach JP, Lowe GDO, Small M. Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. *Diabetes* 1992;**41:**909-13.
15. Pasaoglu H, Sancak B, Bukan N. Lipid peroxidation and resistance to oxidation in patients with Type 2 diabetes mellitus. *Tohoku J Exp Med* 2004;**203:**211-218.
16. Kalaivanam KN, Dharmalingam M, Marcus SR. Lipid peroxidation in type 2 diabetes mellitus. *Int J Diab Dev Ctries* 2006;**26(**1):30-32.
17. Peerapatdit T, Patchanans N, Likidlilid A, Poldee S, Sriratanasathavorn C. Plasma lipid peroxidation and antioxidant nutrients in type 2 diabetic patients. *J Med Assoc Thai* 2006; **89** (Suppl 5) : S147-55.
18. Suryawanshi NP, Bhutey AK, Nagdeote AN, Jadhav AA, Manoorkar GS. Study of lipid peroxide and lipid profile in diabetes mellitus. *Indian J Clin Biochem* 2006**, 21** (1) 126-130.
19. Kumari MK, Sankaranarayan T. Evaluation of oxidative stress in type 2 diabetes mellitus patients. *JDMS* 2014;**13**(5):46-50
20. Mahadevan K, Velavan S. Assesment of salivary lipid peroxidation and protein oxidation status in patients with diabetic and oral cancer. *Int J Med Biosci* 2012;**1**(4):66-68.
21. Rajeshwari SG, Choudhry AA, Gururaja A, Prabhu K. Correlation of plasma lipid profile with salivary oxidative stress markers in type 2 diabetes mellitus patients. *J Clin Diagn Res* 2014;**8**(6):8-10.