Correlation of fasting blood, salivary glucose and malondialdehyde in subjects with & without type 2 diabetes

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Oxidative stress is identified as the common pathogenic factor that leads to insulin resistance in diabetics. Malondialdehyde is a product of lipid peroxidation. **Aim:** The aim of this study was to determine the variation in the Salivary malondialdehyde (MDA) among subjects with and without T2DM in comparison to the fasting blood and Salivary glucose. **Methods:** This study involved 29 healthy participants as Controls (group I) and 29 participants with Type 2 Diabetes Mellitus as Cases (group II). Salivary Glucose was analysed by glucose oxidase end-point assay. Thiobarbituric acid (TBA) assay method was considered for estimation of MDA in fasting saliva. Data was Statistically analysed using SPSS20. Parametric test was performed to analyse the data. **Results:** The correlation calculated between FBG with FSG level was found to be highly significant. A positive correlation between MDA levels with FBG was found. The relationship between FBG and FSG (r = 0.7815, p < 0.05), FBG and MDA (r =0.3678, p < 0.05) and FSG and MDA (r = 0.2869, p < 0.05) were found to be positively significant. **Conclusion:** Saliva as a unique body fluid can serve as a medium for biochemical analysis only in standard settings and with multiple measures to be used as a diagnostic tool in par with the gold standard serum. Salivary MDA levels can be considered as one of the oxidative stress markers in Type 2 Diabetic condition.

Keywords: Diabetes mellitus. Glucose oxidase. Malondialdehyde. Oxidative stress. Biomarker.

Introduction

Type 2 Diabetes mellitus is a multifactorial disease with its prevalence increasing at an alarming rate in modern day[1]. Hyperglycaemia, the characteristic feature of diabetes increases the risk for many serious health complications leading to

deteriorated quality of life and expectancy[2]. Elevated levels of free radicals in the plasma and saliva are observed because of the biochemical alterations of glucose and lipid peroxidation[3]. Diabetes Control and Complications Trial (DCCT) has emphasised the need of maintaining glycaemic control in order to delay or reduce the complications of diabetes[4].

Although insulin resistance is the real root cause of Type 2 diabetes it is evidenced that the role of oxidative stress is pivotal in contributing to the devastating effects of the acute and delayed systemic complications. Oxidative stress is identified as the common pathogenic factor that leads to insulin resistance with impaired function of pancreatic beta cells ultimately resulting in type 2 DM (T2DM)[5]. Evidence points at the overwhelming concentrations of reactive oxygen species (ROS) particularly superoxide anion generated through mitochondrial oxidative metabolism among diabetics[6]. Hyperglycaemia generating reactive oxygen species (ROS) is suggested as the "dangerous metabolic route in diabetes" that results in tissue damage by a variety of mechanisms[7]. Exposure to a relatively high concentration of ROS and/or a decrease in antioxidant defence system against ROS leads to Oxidative stress[8]. Estimation of Malondialdehyde (MDA), a product of lipid peroxidation in plasma is documented as a primary biomarker of the level of oxidative stress in clinical situations[9,10].

The percentage of Glycated Haemoglobin (HbA1c) in blood gives an indication of the glycaemic level of an individual. Although practice of HbA1c test as a routine diagnostic test for detection of Diabetes has several limitations, its role as an important marker for risk of microvascular complication in diabetics is well established in the literature[11].

Saliva as a diagnostic tool for the assessment of oxidative stress is widely considered[12]. Routine use of Invasive procedures causes a great deal of mental trauma, discomfort, and anxiety especially in paediatric and old aged patients. Step up in the prevalence of Type 2 diabetes mellitus (T2DM) necessitates an efficient non-invasive screening strategy[13]. Therefore, this study aims at correlating the levels of Fasting Blood and Salivary Glucose, Salivary Malondialdehyde (MDA), levels in subjects with and without T2DM to append the role of Salivary MDA in determining the oxidative stress in T2DM.

Materials and Methods

Study Subjects selection

This study was designed and performed following the Helsinki Declaration of 1975 (revised in the year 2000) after obtaining approval from the Institutional Review

Board (IRB) of Jagadguru Sri Shivarathreeswara Dental College and Hospital, JSS AHER, Mysore, Karnataka, India (IEC Research Protocol No. 34/2019). Study subjects were recruited from the outpatient department of JSS Hospital following attainment of informed consent. Based on our pilot study sample size of 58 was determined with 29 for each amongst the Diabetic and Control group, assuming even group sizes to achieve 80% power and a significance level of 5% for detecting a true difference means between the test and reference group of 2.9 i.e., 5.22 − 2.23 units.

This comparative cross-sectional study had subjects with age group ranging between 30–60 years. Subjects with Blood Glucose levels in the fasting state (FBG) of 126 mg/dl or higher and PPBS (post prandial blood sugar) of 200 mg/dl or higher and HbA1c level more than 6.5% were considered under group II: Cases. Apparently healthy Subjects with no history of Type 2 Diabetes Mellitus who visited the hospital for routine check-up, not on any systemic medication, whose fasting blood Glucose (FBG) less than 100 mg/dl and PPBS less than 140 mg/dl and HbA1c level 5.9 % or less were categorised under group I: Controls. The exclusion criteria for both groups included subjects with chronic systemic illness, infection, on vitamin supplements, Pregnant woman, Uncooperative patients, mentally compromised, completely edentulous patient.

Clinical Evaluation

Participants selected for this study were subjected to oral examination. Demographics along with details of physical dependency, oral habits, history of exposure to medication, daily medication intake were recorded in the study proforma. A unique barcode was assigned for each of the subject record for identification and confidentiality. Age and gender of the subjects recruited were matched.

Collection of Saliva Sample

Clinical evaluation was performed followed by saliva collection. Subjects were provided with prior instruction and guided to rinse their mouth with 10 ml of tap water for 20 second and expectorate. Unstimulated whole saliva was collected using modification of method reported by Navazesh[14] in the literature using 5ml prelabelled sterile sample collection tubes. Subjects were instructed not to perform any kind of oral hygiene measures minimum 1 hour prior to sample collection such as flossing, brushing, using mouthwash etc. Saliva samples collected from the hospital were immediately stored on ice and transferred to the lab where it was stored at −80°C until analysis.

Plasma Glucose and HbA1c estimation: Instructions were given to subjects for overnight fasting (minimum 8 hours) and to visit hospital for fasting blood glucose estimation in the morning. Disposable syringe was used to withdraw 2 ml of peripheral venous blood from the antecubital vein and collected in a sterile tube. Samples collected were immediately transported to the biochemistry laboratory for analysis the same day.

Salivary glucose estimation: Unstimulated whole saliva sample was subjected to centrifugation at 8000 rpm for twenty minutes. When the clear supernatants appeared, it was immediately processed for fasting salivary glucose estimation with glucose oxidase end-point assay[15] and HITACHI 902 Automatic analyser for the estimation.

Oxidative stress estimation: Thiobarbituric acid (TBA) assay method as reported by Baliga et al.[16] was used for estimation of MDA in fasting saliva. Saliva samples were diluted with distilled water (10 times). Each sample (10μl) was mixed with 1ml TBA reagent. The mixture was heated in a boiling water bath at 950C for 60minutes. The test tubes were cooled at room temperature and absorbance was measured at 532nm using UV visible spectrophotometer.

Statistical analysis

Data collected was transferred to a spreadsheet application for statistical analysis using SPSS20 software. Parametric test was performed to analyse the data. Independent t test was performed to measure the variability between test and control groups and Correlations among Blood Glucose, HbA1c level, fasting salivary Glucose and Fasting salivary MDA was analysed with Karl Pearson's correlation coefficient. The statistical significance was set at 5% ($p < 0.05$).

Results

Subjects catering to the inclusion and exclusion criteria were recruited to the study. Study population consisted of a cohort of 23 male (41.66%) and 35 females (58.34%) with a mean age of 50.79±6.69 for Group I (control) and 42.55±10.62 for Group II (Cases).

A descriptive analysis of FBG levels among controls (≤100 mg/dl) and type II diabetic status (>126mg/dl) was then calculated in comparison with salivary levels of MDA (μmol/l), HbA1c (%), and FSG (mg/dl) as depicted in Table 1. Normality of Fasting Blood Glucose level (mg/dl), Fasting salivary Glucose (mg/dl) and MDA and HbA1c were calculated using Kolmogorov Smirnov test. The scores of Fasting Blood and Salivary Glucose level (mg/dl) and MDA, HbA1c, followed a normal distribution as demonstrated Table 2 and therefore parametric tests were applied. Table 3 demonstrates the result of the independent t test. The results showed that mean Fasting blood glucose, HbA1c, salivary MDA and glucose levels significantly higher amongst cases when subjected to comparison against the healthy controls. FBG levels among controls were 92.24±9.32 mg/dl and among cases were 157.21±39.30 mg/dl. FSG levels among controls were 2.89±2.60 mg/dl and among cases were 9.27±6.74 mg/dl. The levels of MDA among controls were 0.41±0.26 μmol/l and among cases were 1.16±1.21 μmol/l. HbA1c levels among the controls were 4.75±0.59 % and among cases were 6.79±1.34 %. These differences were found to be statistically significant.

Table 1. Comparison of T2DM and control groups with FBG, FSG and MDA and HbA1c,

	Test group	%	Control group	%	Total	%	Chi-square	p-value
FBG								
<100mg/dl	0	0.00	25	86.21	25	43.10	49.1110	0.0001*
100-126 mg/dl	5	17.24	4	13.79	9	15.52		
127-153 mg/dl	13	44.83	0	0.00	13	22.41		
154-180 mg/dl	4	13.79	0	0.00	4	6.90		
>180 mg/dl	7	24.14	0	0.00	7	12.07		
FSG								
0.30- 5.00 mg/dl	12	41.38	25	86.21	37	63.79	18.7100	0.0001*
5.01- 9.71 mg/dl	3	10.34	4	13.79	7	12.07		
9.72- 14.42 mg/dl	3	10.34	0	0.00	3	5.17		
> 14.42 mg/dl	11	37.93	0	0.00	11	18.97		
MDA								
0.10- 0.60 µmol/l	10	34.48	20	68.97	30	51.72	15.5830	0.0010*
0.61- 1.11 µmol/l	7	24.14	9	31.03	16	27.59		
1.12- 1.62 µmol/l	7	24.14	0	0.00	7	12.07		
> 1.62 µmol/l	5	17.24	0	0.00	5	8.62		
HbA1c								
<5.9 mg/dl	0	0.00	29	100.00	29	50.00	35.4440	0.0001*
6-7.9 mg/dl	23	79.31	0	0.00	23	39.65		
>8.1 mg/dl	6	20.69	0	0.00	6	10.35		
Total	29	100.00	29	100.00	58	100.00		

Table 2. Normality of Fasting Blood Glucose level (mg/dl), Fasting salivary Glucose and MDA and HbA1c, by Kolmogorov Smirnov test.

Parameters	Test group		Control group	
	Z-value	p-value	Z-value	p-value
Fasting Blood Glucose level (mg/dl)	1.0320	0.2370	1.2910	0.0710
Fasting salivary Glucose	1.2210	0.1010	1.0340	0.2350
Fasting salivary MDA	1.1590	0.1360	1.1600	0.0890
HbA1c	0.3890	0.9980	0.7510	0.6250

Note: The scores of Fasting Blood Glucose level (mg/dl), Fasting salivary Glucose, fasting salivary MDA and HbA1c follow a normal distribution. Therefore, the parametric tests were applied

Table 3. Comparison of T2DM and control groups with mean of FBG, FSG, MDA, HbA1c by independent t test

Variables	Groups	Min	Max	Mean	SD	SE	t-value	p-value
Fasting Blood Glucose level (mg/dl)	Control	74.00	107.00	92.24	9.32	1.73	8.6610	0.0001*
	Test	126.00	268.00	157.21	39.30	7.30		
	Total	74.00	268.00	124.72	43.30	5.69		

Continuation

|								
Fasting salivary Glucose	Control	0.22	9.45	2.89	2.60	0.48	4.7580	0.0001*
	Test	0.31	18.20	9.27	6.74	1.25		
	Total	0.22	18.20	6.08	6.00	0.79		
Fasting salivary MDA	Control	0.13	0.89	0.41	0.26	0.05	3.2340	0.0020*
	Test	0.13	6.41	1.16	1.21	0.22		
	Total	0.13	6.41	0.79	0.94	0.12		
HbA1c (mg/dl)	Test	4.25	10.24	6.79	1.34	0.25	7.4870	0.0001*
	Control	3.56	5.96	4.75	0.59	0.11		
	Total	3.56	10.24	5.77	1.45	0.19		

*$p < 0.05$

Table 4 depicts the degree and direction of the relationship between Fasting Blood Glucose level (mg/dl), HbA1c (mg/dl), Fasting salivary Glucose and Fasting salivary MDA (µmol/l), in test and control groups calculated by Karl Pearson's correlation coefficient. The strong positive correlation ($r = 0.7815$, $p < 0.05$) was found between FBG with FSG level and a moderate positive correlation between MDA levels with FBG ($r = 0.3678$, $p < 0.05$) and FSG and MDA ($r = 0.2869$, $p < 0.05$) inferring that the value of salivary MDA and FSG and FBG were positively correlated to each other and the rise of glycaemic level in blood is reflected in saliva and with increase in FBG there is a moderately significant rise in MDA level in saliva. Figure 1 represents incorporation of these pattern of data on a scattered plot diagram. It showed that even though the points are observed to be somewhat scattered a positive relationship is indicated in a wider band.

Table 4. Correlations among Fasting Blood Glucose (mg/dl), Fasting Salivary Glucose (mg/dl), MDA (µmol/l), HbA1c (%), in test and control groups by Karl Pearson's correlation coefficient

Groups	Variables		FBG	FSG	MDA	HbA1c
Test group	Fasting Blood Glucose level (mg/dl)	r value	--			
		P value	--			
	Fasting salivary Glucose (mg/dl)	r value	0.7815	--		
		P value	0.0001*	--		
	Fasting salivary MDA (µmol/l)	r value	0.3678	0.2869	--	
		P value	0.0500*	0.1310	--	
	HbA1c (%)	r value	0.7785	0.7426	0.0695	--
		P value	0.0001*	0.0001*	0.7200	--

Control group	Fasting Blood Glucose level (mg/dl)	r value	--		
		P value	--		
	Fasting salivary Glucose (mg/dl)	r value	0.4298	--	
		P value	0.0200*	--	
	Fasting salivary MDA (µmol/l)	r value	0.3744	0.1549	--
		P value	0.0450*	0.4220	--
	HbA1c (%)	r value	0.6060	-0.0013	0.3026	--
		P value	0.0001*	0.9950	0.1110	--

*p<0.05
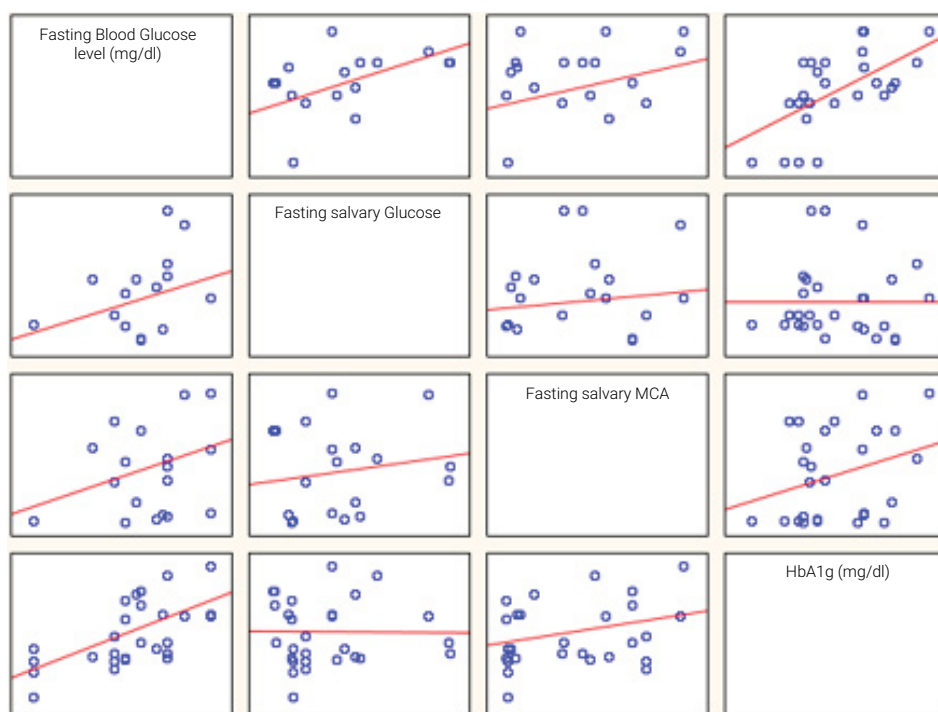
Figure 1. Correlation of FBG, FSG, and Fasting Salivary MDA and HbA1c in Test group

Discussion

Type II diabetes mellitus is a chronic disease associated with impairment of multiple metabolic functions leading to secondary pathophysiological consequences in multiple organ systems eventually resulting in micro- and macrovascular complications. Generation of oxidative stress plays a pivotal role in the pathogenesis of both type 1 and type 2 diabetes which is well established in the literature[5]. Diabetic hyperglycaemia is accompanied with a rise in generation of free radicals in all tissues from auto-oxidation of Glucose molecules and protein glycosylation leads to damage of enzymes, intricate cellular functionality and also increased insulin resistance[17]. Lipid peroxidation is a biochemical process that leads to formation of Malondialdehyde

(MDA) which is considered as a critical biomarker of oxidative stress. A need arises to find out a quick easy and non-invasive method to diagnose this silently growing pandemic. In this regard, this study was planned and constructed to ascertain and compare levels of Malondialdehyde (MDA) and glucose in fasting saliva with fasting plasma glucose[18].

American Diabetes Association recommends the fasting plasma glucose test (FPG) to be the preferred method for diagnosing diabetes owing to its ease of performance, convenience, and cost effectiveness when compared to other tests. Fasting (for at least 8 hours before the test) and 2-hour blood glucose concentrations correlate closely with β-cell function of the pancreas, the impairment of which has been identified as the principal factor responsible for the pathogenesis of type 2 diabetes[19]. The American Diabetes Association also recommends Glycated haemoglobin (HbA1c) level as an alternative method of diagnosing Diabetes. It is considered an important indicator of long-term Glycaemic control as it reflects the cumulative glycaemic status of previous two to three months[20]. It has been documented that in diabetics a significant correlation also exists between blood glucose and salivary glucose and HbA1c levels[21].

In our study subjects with Type 2 Diabetes Mellitus who have been diagnosed for minimal of two years with fasting blood Glucose (FBG) higher than 126 mg/dl and PPBS of 200 mg/dl or higher and HbA1c level more than 6.5% were involved to eliminate other causes of increased blood glucose. It was noticeable that 44.83% of subjects in the T2DM groups had their FBG values in the range of 127-153 mg/dl.

Saliva is a unique kind of bodily fluid, and its innate potential as diagnostic media is being explored and developed. It is a well-known fact that saliva is an ultrafiltrate of blood produced in various salivary glands. Study has shown that glucose value in saliva can vary depending on the fasting duration of the subjects and data showed Glucose levels can also vary depending on the time of day of blood sample is withdrawn, subject's physical activity or intake of alcohol[22]. In this study unstimulated 12hour fasting saliva was collected from the recruited subjects.

Literature has evidenced that Type2 diabetics with and without complication were susceptible to oxidative stress and elevated blood glucose level had a significantly high positive correlation with serum MDA level[23,24]. Further ahead it was observed that salivary MDA level increases in patient with type 2 Diabetes[10,25]. Studies have also concluded that salivary MDA appears to be an indicator and also reflects the value of serum MDA concentration which in turn reflects precisely the severity of the oxidative stress[12,21]. The paucity of studies on the extent of association between FBG and salivary MDA levels in type 2 diabetics directed us to undertake this as one of the objectives. Our findings confirmed the evidence of significant and positive correlation between fasting salivary MDA levels with FBG with Pearson's correlation coefficient of 0.3678 among Diabetics thus concluding that estimation of salivary MDA levels in Type 2 Diabetic condition can be used as an oxidative stress marker and further as an adjuvant diagnostic aid with extended research.

Glucose is one of the many components of blood that is transferrable to salvia in proportion to their blood concentration. As per biochemical investigations it has

been documented in the literature the normal value of salivary glucose in a healthy non diabetic is less than 2 mg/dl[15]. Studies have documented the association with salivary glucose levels and blood glucose levels suggesting that salivary glucose level can be used as a non- invasive tool for monitoring glycaemic level in DM in a dental setup[15,26]. Systematic review and meta-analysis of observational studies on effect of salivary glucose in type 2 DM has concluded that saliva can be a biomarker especially when it is used for screening of type 2 DM in Large scale[18]. Our study findings were in consensus with existing literature to have been able to establish a positive correlation between fasting blood glucose and fasting salivary glucose and Salivary MDA levels among type 2 Diabetics[21,23,24]. This study also found a significant correlation that exists between FBG and FSG with HbA1C levels which adds onto diagnostic potential of saliva for T2DM and requires further exploration. As the study estimates were one time measures it can be concluded that multiple measures are required for conclusive results to indicate consideration of saliva as a diagnostic Parameter in Diabetic condition among different population groups.

Literature has highlighted the application of salivary diagnostic tests in clinical sciences[27]. Therefore it is valuable to integrate salivary diagnostics into clinical practice by advancing dentistry into primary health care[28]. With emerging need for Chair side diagnostics to overcome the inconvenience of invasive procedures to the patients the authors would recommend routine estimation of Salivary MDA to evaluate oxidative stress following further research.

In conclusion, the use of saliva as a "diagnostic tool" offers the advantage over serum as the collection process of saliva is not invasive and it's cost effective. With the limitations of this study, it can be concluded that saliva can indeed be used as a medium for biochemical analysis only in standard settings and with multiple measures to consider saliva as a diagnostic tool in par with the gold standard serum. Salivary MDA levels can be considered as one of the oxidative stress markers in Type 2 Diabetic condition. This further strengthens the inferences in the literature.

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Data Availability

Datasets related to this article will be available upon request to the corresponding author.

Author Contribution

Dr. Sunila Bukanakere Sangappa has mentored this study, involved in Conception, design, analysis and/or interpretation of data. Dr PREETHI.B.P is involved in analysis, interpretation of data and review of manuscript. All the authors of this study have actively participated in the manuscript findings and have revised and approved the final version of the Manuscript.

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