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Salivary and Serum Glucose in relation to HbA1c Levels in Type 2 Diabetes Mellitus.

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Abstract

Diabetes Mellitus (DM) regularly requires blood investigation. Investigation which can efficiently measure the common parameters without painful collection procedure can be beneficial for the patients. The study aimed at measuring and correlating salivary and serum glucose along with serum HbA1C in patients of type 2 DM. The study was an observational case control study carried over a period of one year and four months. It included 42 type 2 DM patients as cases and 41 non diabetic patients as controls, from 35 to 70 years of age. Blood and saliva samples were collected. Serum and salivary glucose with serum HbA1C

levels were measured and analysed using SPSS version 16. 55% of study subjects were < 45 years. M: F ratio was 3:2. The mean FBS, mean salivary glucose and HbA1C in type 2DM was 155.8 mg/dl, 20.4 mg/dl and 8.35 where as in control group they were 88.9 mg/dl, 9.35 mg/dl and 5.5 respectively. The FBS and the salivary glucose correlated significantly ($p<0.001$) and HbA1c levels had strong positive linear correlation to FBS and salivary glucose. Saliva is an effective medium for regular assessment of sugar level in DM.

Key words: Saliva; HbA1c; blood sugar; diabetes mellitus.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from insulin deficiency (type 1 DM) or impaired insulin action, secretion and resistance (type 2 DM). It is a multi-system disorder and has varied complications. Fasting blood sugar (FBS) and glycosylated hemoglobin (HbA1c) are the two basic tests routinely performed for the adequate management of DM. Blood collection is painful and invasive in nature. Saliva which is readily accessible contains an array of analytes that can be used as biomarkers for translational and clinical applications like DM [1]. Thus clinical applications of saliva can range from the forensic field to drug monitoring and diagnosis of systemic and local conditions [2].

Subjects and Methods

The study was an observational case control study carried at the Advance Research Lab of Institute of Technology and Science – Centre for Dental Study and Research (ITS-CDSR) in collaboration with Avantika Hospital, Ghaziabad from January 2014 to April 2015. The protocol was approved by Institutional Ethics Committee and the study included 42 type 2 DM patients as cases and 41 non diabetic persons as controls, from 35 to 70 years of age in both the groups. The subjects were explained about the study and informed consents were taken for obtaining the saliva and blood samples from the subjects. Consecutive random sampling was done on Sunday morning OPD of the hospital from 8.00 am to 10.00 am. The study excluded the subjects with any known hepatic and renal disorders ruled out by measuring serum glutamic-pyruvic transaminase (SGPT) and serum creatinine levels

respectively.

Collection of the blood sample

Blood was collected by venepuncture in the fasting state. The blood sample was collected in fluoride vial for glucose estimation and in plain vials for other parameters.

Collection of the saliva sample

Unstimulated saliva sample was collected in a sterilised container in morning in the fasting state. The subjects were asked to rinse mouth with distilled water and spit 3 times. Then the individual was made to sit comfortably and bend forward. The free flow saliva was collected every 20 seconds for 5 minutes avoiding forcible spitting. The saliva samples collected were centrifuged by L-450™ Benchtop Medical Laboratory Centrifuge for 15 minutes at 3000 rpm and supernatant was taken for the detection of chemical properties.

The glucose estimation in blood and saliva was done using Erba ® Mannheim Chem 7 ™ based on calorimetry principles using enzymatic reagent containing glucose oxidase, peroxidase, 4-amino antipyrine and phenol in phosphate buffer (pH 7.0). The aldehyde group of glucose was oxidised by glucose to produce gluoconic acid and hydrogen peroxide (Glucose + H_2O+ O_2 → Gluconic acid + H_2O_2). The hydrogen peroxide is broken down to water and oxygen by peroxidase (H_2O_2 → H_2O+ O_2). The oxygen reacted with 4-aminoantipyrine in the phenol buffer to form pink coloured compound and intensity was determined at 530 nm.

The HbA1c measurement was done using NycoCard™ HbA1c reader based on the principle of boronate affinity assay. The kit contained prefilled reagents that lysed erythrocytes and also boronic acid conjugate that bonded with the cis-diols of glycated hemoglobin forming a precipitate blue in colour. The colour intensity was measured with the NycoCard ™ reader and thus level of HbA1c was detected which was proportional to both the average glucose concentration and the life span of the hemoglobin in the concentration. The results were obtained and statistical analysis done using SPSS™ version 16. Paired t test was used for significance assessment between the mean values of intragroup serum and

salivary glucose. Correlations were found by using Karl Pearson's correlation coefficient between the glucose levels in serum and saliva with HbA1c. Linear regression curves were plotted to find the association between the values.

Results

Age and sex distribution

The study included 42 previously diagnosed type 2 DM patients as cases and 41 non diabetic persons as controls. The females comprised 39.8% of the total subjects, 40.5% of cases and 39.1 % of controls. More than 60 % of study subjects were < 45 years of age with the mean age in both the groups being 45.6 years (Table 1). The study thus had similar and comparable subjects in the both the groups.

Table 1: Table depicting the age and sex distribution among the study groups.

Profile	Cases (Type 2 DM)	Controls (Non diabetic)	Total	Percentage
Number of the subjects	42	41	83 (100%)	
Age in years				
35-40 years	13	13	26	31.3% *
40-45 years	12	11	23	26.7% *
45-50 years	3	3	6	7.3%
50-55 years	3	3	6	7.3%
55-60 years	5	5	10	12.04%
60-65 years	3	3	6	7.3%
65-70 years	3	3	6	7.3%
Sex				
Females	17/42	16/41	33/83	39.8%
Males	25/42	25/41	50/83	60.2%
*more than 50 % of the study subjects were less than 45 years of age. †40 % of the study subjects were females and hence male: female ratio was 3:2.				

Fasting blood sugar (FBS), Fasting salivary glucose (FSG) and glycosylated hemoglobin (HbA1c) levels (Table 2)

The FBS level ranged from 100 to 335.8 mg/dl in cases and from 67 to 132 mg/dl in control. The mean FBS in cases was 155.84 ± 57.55 mg/dl, which was much higher than the mean level of 88.95 ± 15.43 mg/dl in control group. The FSG level ranged from 10.0 to 30.0 mg/dl in cases and from 6.5 to 10.6 mg/dl in controls. The mean FSG in cases was 20.4286 ± 5.29248, which was higher than the mean level of 9.3488 ± 2.97910 mg/dl in control.Mean FSG value was significantly associated with the mean FBS value in both the groups (t = 16.37, P < 0.001 in cases and t= 33.01, P < 0.001 in controls). The HbA1c level ranged from 7 to 10.8 in cases and from 4.7 to 7.9 in controls. HbA1c mean was 8.55 in cases where as in controls it was 5.5

Table 2: Table depicting FBS, FSG and HbA1c levels in both the groups.

Group	Parameter	Range	Mean	Paired t test Significance	Correlation Significance
Cases (n=42)	FBS	100 – 335.8 mg/dl	155.84 ± 57.55 mg /dl	t=16.37 P<0.001*	Coefficient: r = 0.770 P< 0.001†
	FSG	10-30.0 mg/dl	20.43 ± 5.29 mg/dl		
	HbA1c	7-10.8	8.55	-	-
Controls (n=41)	FBS	67 – 132 mg/dl	88.95 ± 15.43 mg /dl	t= 33.01, P < 0.001*	Coefficient: r=0.107 P=0.507 §
	FSG	6.5 - 10.6 mg/dl	9.35 ± 2.98 mg/dl		
	HbA1c	4.7 - 7.9	5.5	-	-

*****paired t test of FSG and FSG values were significant in both the groups (P<0.001)

†correlation between the FBS and FSG was positive and significant in cases

§ correlation between the FBS and FSG was positive but not significant in controls.

The mean levels of FBS, FSG and HbA1c were significantly higher in the cases in comparison to the controls (p<0..001) (Table 3):

Table 3: Table depicting the means, standard deviation and standard error of mean of parameters in the cases and controls.

Parameter	Group	N	Mean (mg/dl)	Std deviation	Std Error Mean	T (unpaired)	P
Fasting Blood Sugar	Cases Controls	42 41	155.84 88.95	57.56 15.43	8.88 2.41	7.267	<0.001§
Fasting salivary glucose	Cases Controls	42 41	20.43 9.35	5.29 2.97	0.81 0.46	11.788	<0.001§
HbA1c	Cases Controls	42 41	8.55 5.50	1.02 0.78	0.16 0.12	15.126	<0.001§
§ mean values of the FBS, FSG, HbA1c in between the two groups were highly significant.							

Correlations between the values (Table 4):

The FSG values correlated positively and significantly with the FBS levels in cases (r = 0.770 with P< 0.001) and but not so strongly in controls (r = 0.107 with P=0.507). The HbA1c correlated positively and significantly with the FBS levels in both cases (r= 0.868, P<0.001) and controls (r=0.706, P<0.001). HbA1c was correlated with the FSG and it was found that they were positively and significantly associated in cases (r= 0.758, P< 0.001) but mildly negative without any significance in controls (r= -0.120, P= 0.454).

Linear regression curve plot demonstrated positive relationship between FSG and FSG in cases, controls and also in combined data (Figure 1A). HbA1c and FBS again had positive relationship in cases, controls and combined data (Figure 1B). HbA1c and FSG had positive relationship in cases and in combined data but mild inverse relationship in control group (Figure 1C).

Table 4: Table depicting the correlation between the FBS, FSG and HbA1c levels in both the groups.

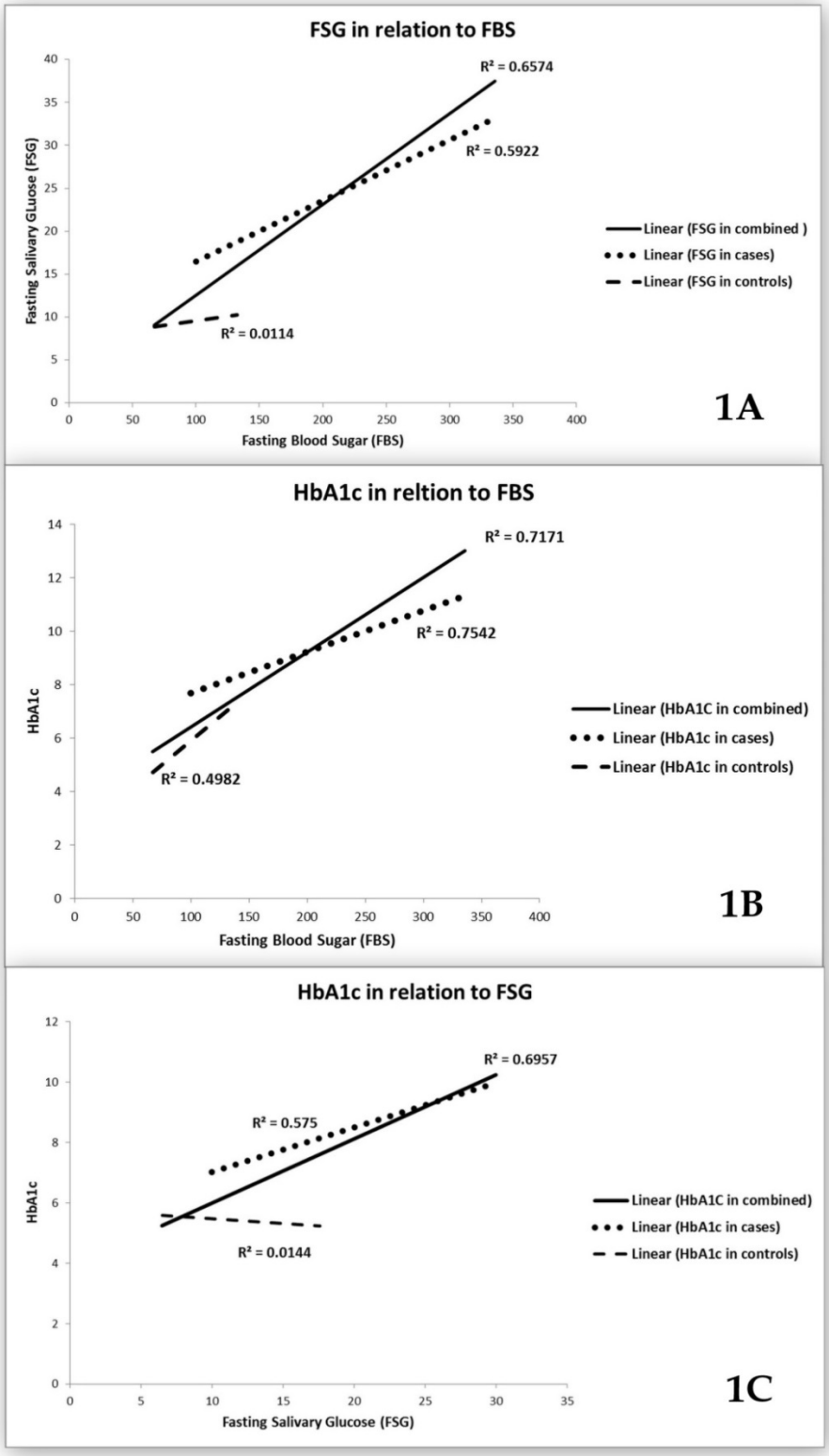
	Parameters	FSG	HbA1c
Cases N=42	FBS Pearson Correlation Sig. (2-tailed)	 0.770 0.000†	 0.868 0.000*
	FSG Pearson Correlation Sig. (2-tailed)	NA	 0.758 0.000‡
Controls N=41	FBS Pearson Correlation Sig. (2-tailed)	 0.107 0.507†	 0.706 0.000*
	FSG Pearson Correlation Sig. (2-tailed)	NA	 -0.120 0.454‡

*correlation between the HbA1c and FBS levels were positive and significant in both the cases and controls.

†correlation between the FSG and FBS was positive in both the cases and controls but significant only in cases.

‡correlation between the HbA1c and FSG was positive and significant in case but was not positive in the controls.

Figure 1: Figure depicting the linear correlation between FBS & FSG, FBS & HbA1c, and FSG & HbA1c. FSG had strong positive linear correlation with the FBS in cases and combined data (1A). HbA1c had strong linear positive correlation to FBS in cases, controls and in combined data (1B). HbA1c had strong linear positive correlation to FSG in cases and combined data but not in control group (1C).



Discussion

Prevalence of diabetes mellitus has been increasing and about 90% of the patients are of type2 DM (T2DM). The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India which is further going to increase to 69.9 million by 2025 [3]. The disease leads to the various systemic complications and timely detection with regular monitoring has been the main focus in managing the disease. The rise in incidence is more for the age above 40 years, but the present study highlights higher incidence (> 50 %) below 45 years. The younger age group and epidemiological transition can be attributed to change in life style, urban migration, fast food culture and sedentarism [4]. Many other studies in India also report younger age of incidence [5]. The prevalence of the disease in male and female sex varies in various studies. The present study had 3:2 male: female ratio. The previous studies have reported equal incidence, male preponderance as well as female preponderance in varied percentages [5]. These unequal distributions can be due to loco regional influences and demographics.

The diagnosis, regular care and control of the diabetes require monitoring of various serological parameters. These serological assessments involve invasive techniques which are painful and stressful. Alternative methods of non-invasive techniques of measuring parameters in urine, breath and saliva have been studied. The present study evaluated saliva for the assessment of glucose levels in diabetics and non-diabetics. Saliva as a body fluid has complex composition and analytes with specific roles. The analysis of biochemical constituents in saliva is of great help in diagnostics and therapeutics of diseases of oral cavity as well as systemic diseases. Unstimulated and stimulated saliva can be collected for the assessment of the analytes. The present study collected unstimulated saliva in the fasting state. The most common and essential assessment in diabetes mellitus is fasting blood sugar. Glucose diffuses through the blood vessel membrane and reaches gingival fluid becoming a constituent of saliva [6].

In the present study the FBS was significantly higher in T2DM than the control (non-diabetics). Similarly the fasting salivary glucose concentration (FSG) and glycosylated hemoglobin levels were also higher in the cases than the controls. Higher levels of the

salivary glucose have been also reported by many studies across the globe [7-11]. The exact value of the salivary glucose differs in the various studies due to different methods of collection and estimation [7]. The levels of salivary glucose vary in stimulated as well as unstimulated saliva [12]. The FSG in the present study was found to be having a significant linear positive relationship with the FBS in the combined data and in the cases (T2DM) but not so strong correlation was found in the controls (non-diabetics), the reason may be the less amount of glucose secretion in normal subjects. Similar findings were found by Jurysta et al [12] and their study demonstrated increase in salivary glucose concentration and glucose excretion rate in diabetic patients, as compared to normal subjects in stimulated as well as unstimulated saliva. The FBS had significant positive linear correlation with the HbA1c in cases, controls as well as in combined data. Linear progression curve also suggested a positive relationship. Blood glucose levels have been found to have positive correlation with the HbA1c. Rohlfing et al [13] demonstrated strong positive and linear relationship between Mean Plasma glucose (MPG) and glycated hemoglobin HbA1c. Monnier et al [14] reported that fasting hyperglycaemia played major contributor to the overall diurnal hyperglycaemia in poorly controlled diabetic patients. However, post prandial and random sugar assessment also do play role in management of sugar control in diabetes. FSG was found to have strong positive linear correlation with the HbA1c in cases and in combined data but not in the control group. The similar finding of correlation between FSG and HbA1c has been reported by Satish et al [15]. But few study like Darwazehet al [16] demonstrated that concentration of salivary glucose was related to blood glucose but there was no relationship existed with HbA1c.

Conclusion

The study thus demonstrates the fasting salivary glucose as the parameter that can be used for the routine regular assessment for sugar control in T2DM. However, the method and timing of estimation needs to be standardized and proper guideline needs to be laid down for the widespread adaptation of the salivary glucose as a routine parameter for the T2DM monitoring.

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