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A Correlative Study Between Serum, Salivary Lipid Profile and Glucose Level in Healthy and Type 2 Diabetic Patients.

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ABSTRACT

Diabetes is a chronic metabolic disorder characterized by increased blood glucose. Since the diabetic patients are at increased risk of cardiovascular diseases (CVD), lipid profiling is essential for monitoring this predisposing factor. This study was performed to correlate salivary and serum lipid profile with a blood glucose level in type 2 diabetes (T2D) by selecting a total of 60 subjects. Lipid profiling and glucose levels in the samples were measured using enzymatic kit methods. Results showed a highly positive correlation between saliva and serum in parameters such as cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein and blood sugar. The results of these studies are based on age, sex, history, lifestyle and study sample sizes. The values are statistically significant in both T2D and non-diabetic group. These results corroborate that saliva can be used as a non-invasive, painless technique for profiling of lipid parameters and assessment of blood glucose in T2D patients.

Keywords: Diabetes; Lipid profile; Saliva; Serum; Glucose

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INTRODUCTION

Diabetes mellitus (DM) is the chronic metabolic disorder characterized by increased levels of blood glucose. This oldest disease arises due to defects in the production or action of insulin. Type 2 diabetes mellitus (T2D) makes up about 90% of DM cases and therefore considered as a major global threat [1]. Family history, obesity, age, ethnicity, genetics, diet, and physical activity are the common risk factors of T2D [2]. There has been an increase in the total number of T2D cases in all the countries, especially in low- and middle-income countries [1]. This raise in the T2D morbidity is also associated with higher mortality rates, the main reasons being the predisposing factors of T2D such as cardiovascular disease (CVD) and stroke [3]. Therefore, it is of vital importance to monitor these factors to decrease the mortality rate.

Regular profiling of lipids is very important for laboratory diagnosis and monitoring of many diseases with cardiovascular complications including DM [4]. More commonly, blood is used as a sample for the analysis of lipid. Since sampling of blood involves repeated invasions, it induces anxiety and discomfort in the patients [5]. Therefore, it is required to develop a non-invasive method for the profiling of lipids. The earlier report has suggested a moderate correlation between salivary and serum levels of many parameters of lipids, including total cholesterol (CHO), triglycerides (TG), high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) [5]. Therefore, use of saliva as a clinical tool for the analysis of lipids is open to exploring.

Saliva is the biological fluid with many physiological functions, which not only facilitates the digestion but also helps in the maintenance of oral health. The activity of saliva was decreased during T2D and the patients are susceptible to dyslipidemia [6]. Since it is easier to collect, store, ship, and handle the saliva, using saliva as a clinical sample, over blood or serum is advantageous [7,8]. It is well known that many lipids are secreted in saliva [5,9,10]. Also, saliva contains most of the serum constituents [8] and therefore, can be used for laboratory analysis of lipids and diagnosis of many systemic diseases.

In this study, we sought to analyse the correlation between salivary and serum lipid profile with a blood glucose level in T2D patients. This study is of higher importance since it highlights on the use of saliva as a clinical tool for the diagnosis and monitoring of the complications of T2D.

METHODS AND MATERIALS

Subjects

Sixty patients (both male and female) aged between 35 to 65 years were selected for the present study. They were classified into two groups of 30 subjects each. Group one consisted of non-diabetic healthy individuals with no history of T2D. The second group includedthe patients with T2D, with a fasting blood glucose level >110 mg/dl. Type 1 diabetic patients, pregnant women, and patients who either smoked or consumed alcohol in the last 24 h were excluded from the study. All the subjects were registered on a convenient sampling basis.

Collection of sample

Detailed information was given to all the individuals about the sampling methods. Both saliva and blood samples were collected from each individual of both the groups after overnight fasting. After rinsing out of the food particles using water, unstimulated saliva was collected up to a period of 5 min, using the standard spitting method. Collected saliva was centrifuged at 3000 rpm for 15 min, and the supernatant was stored at –20°C until used.

Venous blood was collected under aseptic conditions from all the subjects. To prepare serum, blood was allowed to clot at room temperature up to 30 min and centrifuged at 3000 rpm for 15 min. The supernatant was stored at – 20°C until used [11].

Glucose and lipid profiling in both the saliva and serum samples were analyzed on a semi-automated Biochemistry analyzer using kits procured from ERBA diagnostics (Transasia Bio-Medicals Ltd, Germany), which are based on the principle of spectroscopy. Ethical clearance was obtained from the institutional human ethics committee to conduct the present study.

Measurement of glucose

Estimation of glucose was performed by the glucose oxidase method. Briefly, 1 ml of reagent was added to 10 µl of the sample, mixed well and absorbance was read at 505/670 nm following incubation for 10 min at 37°C. The absorbance value was proportional to the glucose concentration in the sample [12-14].

Measurement of total cholesterol (CHO)

The cholesterol oxidase/peroxidase (CHOD-PAP) enzymatic method was used to estimate total-cholesterol in saliva and serum. 1 ml of reagent was incubated at 37°C for 10 min after mixing with 10 µl of the sample. During the reaction, free CHO formed after hydrolysis of esterified cholesterols by cholesterol esterase was oxidized to produce hydrogen peroxide. Thus formed hydrogen peroxide, through the action of peroxidase enzyme, reacts with phenol and 4-aminoantipyrine and forms a red colored quinoneimine product. Spectrophotometric absorbance of quinoneimine was recorded at 505/670 nm, which is proportionate to the concentration of CHO.

Measurement of triglyceride (TG)

Trinder's method (GPO) was employed to quantitate the concentration of TG in saliva and serum samples. Briefly, 1 ml of reagent was added to 10 µl of the sample, mixed well and incubated for 10 min at 37°C. Lipoprotein lipase enzyme present in the reagent hydrolyzes the triglycerides present in the samples into glycerol and free fatty acids. Glycerol, then yields hydrogen peroxide by the action of glycerol kinase and glycerol phosphate oxidase (GPO) enzymes, respectively. The hydrogen peroxide further forms a quinoneimine product as mentioned in cholesterol analysis, which is quantified spectrophotometrically at 546/670 nm.

Measurement of HDL

Quantification of HDL was performed by using the direct HDL method. Initially, LDL, VLDL and chylomicrons present in samples were made inaccessible to cholesterol oxidase (CHOD) and esterase (CHES) enzymes by the action of polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) present in the assay reagent. Further, CHOD and CHES react with HDL present in the samples to form hydrogen peroxide, which was quantified using Tinder's reaction as mentioned earlier. The experiment was performed by mixing 375 µl of reagent 1 with 5 µl of the sample and incubating at 37°C for 5 min. After incubation, 125 µl of reagent 2 was added and incubated again for 5 min at 37°C before reading absorbance at 600/700 nm.

Measurement of LDL and VLDL

The concentration of LDL and VLDL was evaluated using Friedwald equation, by performing lipoprotein analysis [11]. In this assay, the concentration of total CHO, TG, and HDL were initially analysed. Further, the LDL and VLDL concentrations were calculated using the equations [15]:

$$VLDL = Triglycerides/5$$
$$LDL = Total\ cholesterol - (HDL + VLDL)$$

Statistical Analysis

SPSS version 22 software was used for analysis of data. Data were analysed using Mann Whitney test between the non-diabetic and diabetic groups. Wilcoxon Signed Rank test was performed to compare blood and saliva samples of both non-diabetic and diabetic group separately. The Spearman Rank correlation was used to find the relationship between blood and saliva samples. $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Development of a non-invasive method for the profiling of lipids, especially for patients with cardiovascular diseases are of vital importance. Though blood is the most commonly used biological samples for laboratory diagnosis of diseases and their monitoring, there is a requirement for the development of alternative sample for blood. In this regard, saliva is one of the best alternatives for blood, because of its non-

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invasive sample collection method and cost-effectiveness [16]. The earlier report has detected glucose in both saliva and plasma of diabetic and non-diabetic subjects and recommended that saliva can be used as a marker for the diagnosis of diabetes [13].

Thirty diabetic and 30 healthy individuals were used in the present study. Various lipid parameters such as CHO, TG, HDL, and VLDL, along with glucose were analyzed in both serum and saliva samples. Median (interquartile range) values of these parameters, in blood and saliva samples of both the groups, were given in table 1 and 2, respectively. The median values of blood versus saliva in non-diabetic and diabetic groups were given in Table 3 and 5, respectively. The correlation coefficient of test parameters of blood versus saliva was represented in Table 4 (non-diabetic) and Table 6 (diabetic).

Table 1: Median (IQR) values of parameters in blood samples of diabetic and non-diabetic groups

Parameters	Group	Median	Q1	Q3	IQR (Q1,Q3)	P
Sugar	Diabetic	130.50	104.75	164.50	104.75, 164.50	<0.001
	Non-diabetic	96.00	85.75	101.25	85.75, 101.25	
CHO	Diabetic	156.50	132.00	188.50	132.00, 188.50	0.701
	Non-diabetic	167.50	133.00	188.25	133.00, 188.25	
TG	Diabetic	110.00	92.75	164.00	92.75, 164.00	0.037
	Non-diabetic	90.50	65.00	142.25	65.00, 142.25	
HDL	Diabetic	47.00	38.00	53.00	38.00, 53.00	0.662
	Non-diabetic	46.00	39.00	52.00	39.00, 52.00	
LDL	Diabetic	89.50	63.25	125.50	63.25, 125.50	0.496
	Non-diabetic	97.00	73.75	120.25	73.75, 120.25	
VLDL	Diabetic	22.00	18.75	33.00	18.75, 33.00	0.045
	Non-diabetic	18.00	13.00	28.25	13.00, 28.25	

Table 2: Median (IQR) values of parameters in saliva samples of diabetic and non-diabetic groups

Parameters	Group	Median	Q1	Q3	IQR (Q1,Q3)	P
Sugar	Diabetic	5.73	5.09	6.32	5.09, 6.32	<0.001
	Non-diabetic	4.16	4.00	4.58	4.00, 4.58	
CHO	Diabetic	14.00	9.00	16.00	9.00, 16.00	0.342
	Non-diabetic	14.00	11.75	17.00	11.75, 17.00	
TG	Diabetic	8.00	7.00	12.00	7.00, 12.00	0.358
	Non-diabetic	9.00	7.75	13.00	7.75, 13.00	
HDL	Diabetic	5.60	4.70	6.23	4.70, 6.23	0.539
	Non-diabetic	5.75	4.88	6.20	4.88, 6.20	
LDL	Diabetic	6.75	1.58	8.38	1.58, 8.38	0.412
	Non-diabetic	6.65	4.48	8.70	4.48, 8.70	
VLDL	Diabetic	1.60	1.40	2.40	1.40, 2.40	0.358
	Non-diabetic	1.80	1.55	2.60	1.55, 2.60	

Table 3: Blood versus saliva in non-diabetic group showing median value in saliva and blood of non-diabetic group

Parameters		Median	Q1	Q3	IQR (Q1,Q3)	P
Sugar	Blood	85.75	96.00	101.25	96.00, 101.25	0.001
	Saliva	4.00	4.16	4.58	4.16, 4.58	
CHO	Blood	133.00	167.50	188.25	167.50, 188.25	0.001
	Saliva	11.75	14.00	17.00	14.00, 17.00	
TG	Blood	65.00	90.50	142.25	90.50, 142.25	0.001
	Saliva	7.75	9.00	13.00	9.00, 13.00	
HDL	Blood	39.00	46.00	52.00	46.00, 52.00	0.001

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	Saliva	4.88	5.75	6.20	5.75, 6.20	
LDL	Blood	73.75	97.00	120.25	97.00, 120.25	0.001
	Saliva	4.48	6.65	8.70	6.65, 8.70	
VLDL	Blood	13.00	18.00	28.25	18.00, 28.25	0.001
	Saliva	1.55	1.80	2.60	1.80, 2.60	

Table 4: Correlation in non-diabetic group

			Blood Sugar
Spearman's rho	**Salivary Sugar**	Correlation Coefficient	0.951
		P	0.001
		N	30
			Blood CHO
Spearman's rho	**Salivary CHO**	Correlation Coefficient	0.888
		P	0.001
		N	30
			Blood TG
Spearman's rho	**Salivary TG**	Correlation Coefficient	0.793
		P	0.001
		N	30
			BloodHDL
Spearman's rho	**Salivary HDL**	Correlation Coefficient	0.981
		P	0.001
		N	30
			Blood LDL
Spearman's rho	**Salivary LDL**	Correlation Coefficient	0.831
		P	0.001
		N	30
			BloodVLDL
Spearman's rho	**Salivary VLDL**	Correlation Coefficient	0.761
		P	0.001
		N	30

Table 5: Blood versus saliva in diabetic group showing median value in saliva and blood of diabetic group

Parameter	Sample	Median	Q1	Q3	IQR (Q1,Q3)	P
Sugar	Blood	130.50	104.75	164.50	104.75, 164.50	0.001
	Saliva	5.73	5.09	6.32	5.09, 6.32	
CHO	Blood	156.50	132.00	188.50	132.00, 188.50	0.001
	Saliva	14.00	9.00	16.00	9.00, 16.00	
TG	Blood	110.00	92.75	164.00	92.75, 164.00	0.001
	Saliva	8.00	7.00	12.00	7.00, 12.00	
HDL	Blood	47.00	38.00	53.00	38.00, 53.00	0.001
	Saliva	5.60	4.70	6.23	4.70, 6.23	
LDL	Blood	89.50	63.25	125.50	63.25, 125.50	0.001
	Saliva	6.75	1.58	8.38	1.58, 8.38	
VLDL	Blood	22.00	18.75	33.00	18.75, 33.00	0.001
	Saliva	1.60	1.40	2.40	1.40, 2.40	

Table 6: Correlation in diabetic group

	Blood Sugar

Spearman's rho	**Salivary Sugar**	Correlation Coefficient	0.885
		P	0.001
		N	30
			Blood CHO
Spearman's rho	**Salivary CHO**	Correlation Coefficient	0.966
		P	0.001
		N	30
			Blood TG
Spearman's rho	**Salivary TG**	Correlation Coefficient	0.815
		P	0.001
		N	30
			Blood HDL
Spearman's rho	**Salivary HDL**	Correlation Coefficient	0.967
		P	0.001
		N	30
			Blood LDL
Spearman's rho	**Salivary LDL**	Correlation Coefficient	0.929
		P	0.001
		N	30
			Blood VLDL
Spearman's rho	**Salivary VLDL**	Correlation Coefficient	0.812
		P	0.001
		N	30

For blood samples, the median value of sugar in the diabetic group is 130.50 with interquartile range (IQR) of 104.75 and 164.50, whereas in the non-diabetic group is 96 (IQR 85.75, 101.25). In case of TG, median value in the diabetic and the non-diabetic group is 110.0 (IQR 92.75, 164.0) and 90.50 (IQR 65.00, 142.25), respectively. For VLDL, the median value in the diabetic group is 22.00 with IQR of 18.75 and 33.00, and in the non-diabetic group is 18.00 (IQR 13.00, 28.25). Test statistics clearly showed that the median (IQR) values of sugar (P=0.001), TG (P= 0.037) and VLDL (P=0.045) differed significantly in blood samples of both diabetic and non-diabetic groups (Table 1.). Interestingly, no significant differences were observed for CHO, HDL, and LDL.

In case of saliva, only sugar level differed significantly (P=0.001). The median value of sugar in the diabetic group is 5.73 with IQR 5.09, 6.32, while the median value of sugar in the non-diabetic group is 4.16 (IQR 4 and 4.58). This is in agreement with the earlier result, wherein higher glucose concentration was observed in saliva [14]. But, no significant differences were observed for other lipid parameters (Table 2.). Further, the median value of all the parameters of blood and saliva sample also differed significantly (P<0.05) in both non-diabetic (Table 3.) and diabetic (Table 5.) groups.

Correlation of all the parameters between saliva and serum in both non-diabetic and diabetic group was also analyzed. It was observed that, there is a highly positive correlation between saliva and serum for all the parameters in both non-diabetic (Table 4.) and diabetic (Table 6.) groups. These correlations were significant. The similar significant correlation between glucose level of serum and saliva was also observed in earlier studies by Panda *et al* [17].

Comparison of lipid profiling between diabetic and non-diabetic subjects were also studied earlier [12], which confirmed the increased levels of total cholesterol, triglycerides, and LDL in diabetics. This suggested the dyslipidaemia which in turn may lead to cardiovascular diseases [12]. Jain *et al* [18] have also shown that the HbA1c can be used as an indicator for screening diabetes. In this study, we have revealed that the saliva can be one of the potential samples for diagnosis of diabetes and its associated complications especially cardiovascular diseases. Similar concepts of using saliva in diagnosis are supported earlier for detecting tumour markers, hormone levels, infections and autoimmune diseases [7,19,20]. Taken together, our study will help in the development of the non-invasive method for the diagnosis and monitoring of diabetes.

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CONCLUSION

The present attempt was designed to establish a non-invasive procedure to measure glucose level and lipid profile concentration using saliva, which is the most easily obtainable sample. It was found that the median value of sugar in blood and saliva of diabetics and non-diabetics differred significantly. Though no significant differences were observed for lipid parameters in saliva, TG and VLDL differed significantly in blood. A highly significant correlation between saliva and serum of diabetic and non-diabetic groups was observed. This confirmed the possibility to use saliva as a non-invasive, painless technique for the assessment of lipid profile and glucose concentration in T2D patients and healthy controls. The results of these studies are based on age, sex, history, lifestyle and study sample sizes.

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