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A Study to co-relate Salivary and Plasma glucose with Lipid Profile in Type 2 Diabetes Mellitus Patients.

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ABSTRACT
Introduction:Type 2 diabetes mellitus (T2DM) is a syndrome of abnormal carbohydrate, fat and protein metabolism. T2DM is caused by absolute or relative lack of insulin and is major public health concern.
Aim: To study the comparison between salivary and plasma glucose level and correlation with lipid profile in type 2 diabetes mellitus patients.
Materials and Methods: 50 patients with type 2 diabetes mellitus will be recruited from medicine OPD of Rama medical college hospital, age and sex matched healthy controls will be included in the study.
Result: Clinical data is studied to find out the Gender and age distributionof case and control. The observation of the predominantly female population which comprises 50% in case and 22% in control and the ratio of male over female is 1:1(1:2 approximately), and 1:4.6 respectively.
Conclusion: Effect of salivary and plasma glucose is associated with lipid disorders that are characterized by increased TC, TG, LDL-C, VLDL-C and decreased HDL-C levels.

Keywords:
Type 2 Diabetes Mellitus
Lipid profile
Salivary glucose
Plasma glucose

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a syndrome of abnormal carbohydrate, fat and protein metabolism. T2DM is caused by absolute or relative lack of insulin and is a major public health concern. [1]

According to the last International Diabetes Federation (IDF) report, the rate of diabetic patients will reach to 438 million patients worldwide in 2030. [2]

Since 1965 World Health Organization (WHO) has periodically updated and published guidance on how to classify diabetes mellitus. This document provides an update on the guidance last published in 1999. The main feature of Diabetes Mellitus (DM) is chronic hyperglycemia resulting from either a defect in insulin secretion from pancreas or resistance of body's cells to produce insulin or both. The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia and unexpected weight loss. [3]

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 year ago. In 1936, the distinction between type 1 and type 2 DM was clearly made. Type 2 DM was first described as a component of metabolic syndrome in 1988. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. Type 2 DM results from interaction between genetic, environmental and behavioral risk factors.[4-9]

The biological fluid with numerous functions within the oral cavity, mainly facilitating the maintenance of oral health and creating the suitable ecological balance in the mouth is saliva. Human saliva mirrors the body's health and approximately 20-30% of proteins found in human blood are also present in human saliva, highlighting the diagnostic potential of the saliva.[10]

The results based on their literature survey reported that lipids present in the saliva are important elements. Increased serum lipid concentration increases salivary lipid level. In the course of certain systemic disease saliva undergoes to change in their constituents. In the diagnosis of certain diseases, changes in the salivary lipid profile helps to find out the fluctuations in the body non-invasively.[11]

A study between diabetic patients and healthy controls to compare the level of lipid profile. They concluded that diabetic patients had elevated levels of total cholesterol, triglycerides and low density lipids. This indicates that dyslipidaemia, is more common in diabetic patients, and may cause cardiovascular disorders.[12]

Correlation between high density lipid and low density lipids in plasma with MDA and total

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SUBJECT		CASE	CONTROL	TOTAL

thiols in diabetic patient's saliva. They concluded that results acquired by the correlation between plasma lipid profiles with the salivary markers of oxidative stress show the ability of saliva as a non-invasive diagnostic tool in the diagnosis and prognosis of diabetes. This indicates that saliva can be used as a choice of sample in the detection and treatment of different pathological diseases.[13]

II. MATERIALS AND METHODS

This study will be conducted in Department of Biochemistry, Rama Medical College Hospital & Research Centre Kanpur.
Sample will be collected from Rama medical college hospital.
Study subjects
50 Patients with type 2 diabetes mellitus will be recruited from medicine OPD of Rama medical college hospital, age and sex matched healthy controls will be included in the study.
Case: 50 patients of type 2 diabetes mellitus.
Control: 50 normal healthy age and sex matched persons will be included.
Study Design
This was a case control study.
Case: 50 patients of type 2 diabetes mellitus.
Control: 50 normal healthy age and sex matched persons will be included.

Inclusion criteria:

1. Patients diagnosed with type 2 diabetes mellitus in age range 35-75 years.
2. Age and gender matched healthy individuals.
3. Duration of diabetes more than 4 years.

Exclusion criteria:

1. Age below 35 years and above 75 years.
2. Patients having any other systemic disease and on regular medication for the same.
3. Pregnant woman, mentally compromised, radiotherapy for head and neck cancer, oral mucosal or salivary gland disorders, antibiotic or corticosteroid therapy for preceding 3 months.
4. Patients with habits of tobacco or alcohol and smoking.

Specimen collection:
5ml of fasting blood sample will be collected from antecubital vein in which 2ml collected in fluoride vial for plasma glucose estimation and 3ml collected in plain vial for lipid profile estimation.
3ml salivary sample will be taken in graduated container for salivary glucose estimation.
Specimen Processing:
Blood and salivary sample will be separated by centrifugation at 4000 rpm (rotation per minute) for 5 minutes in the biochemistry department and analysis will be conducted. The serum and salivary sample will be stored at -20^{o}C until assayed.
Glucose estimation
Serum Glucoses was estimated on Erba CHEM – 5 plus semi-auto analyzer by Trinder's Method.
Total Cholesterol (TC) estimation
Serum Total Cholesterol was estimated on Erba CHEM – 5 plus semi-auto analyser by CHOD-PAPend point method.
Triglycerides (TG)
Serum Triglycerides was estimated on Erba CHEM – 5 plus semi-auto analyser byGPO Trinder method.

High Density Lipoprotein – Cholesterol (HDL–C)
Serum HDL – C was estimated on Erba CHEM – 7 plus semi-auto analyser by Phosphotungstic acid method.
Very-Low Density Lipoprotein cholesterol (VLDL–C)
Friedewald's equation.
Low Density Lipoprotein cholesterol (LDL–C)
Friedewald's equation
Table 3.1: The gender and age distribution of the total Subjects (type 2 DM patients and healthy indivisuals) included in the study.

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		n	%	n	%		
GENDER	MALE	25	50	11	22	36	100
	FEMALE	25	50	49	98	74	
AGE (YEARS)	35-45	21	42	25	50	46	100
	46-55	19	38	11	22	30	
	56-65	10	20	05	10	15	
	66-75	0	0	09	18	09	
TOTAL		50		50			
	100						

n= frequency of subjects.

Clinical data is studied to find out the Gender and age distribution of case and control. The observations of the Table 3-1evince predominantly female population which comprises 50% in case and 22% in control and the ratio of male over female is 1:1 (1:2 approximately), and 1:4.6 respectively. There is a trend toward a higher prevalence of co-relate salivary and plasma glucose with lipid profile in type 2 diabetes mellitus patients in the age group 46-55 years in case; majority (38%) of subjects in control are in 56-65 years age group (Table 3-1).

The serum levels of Salivary glucose and FBG, Lipid Profile (TC, HDL-C, LDL-C, VLDL-C and TG) obtained on analyzing specimens collected from study subjects are tabulated.

The mean values and standard deviation of these parameters have been calculated for case control study of patients of co-relate salivary and plasma glucose lipid profile in type 2 diabetes mellitus patients (case) and Healthy Individuals (control).

Table 3.2: Analysis of variance among case and control.

Parameter	Case Mean±SD	Control Mean±SD	'p'Value
Salivary glucose	38.84±4.22	20.55±5.97	.000
FBG	227.00±20.75	86.44±8.42	.000
TC	279.29±14.79	116.66±12.50	.000
HDL-C	27.52±5.83	39.32±5.47	.000

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LDL-C	**173.10±16.41**	**106.36±16.33**	**.000**
VLDL-C	**49.16±5.12**	**22.85±2.18**	**.000**
TG	**337.17±64.24**	**114.45±10.86**	**.000**

Table 3.2 shows higher Salivary glucose [38.84±4.22] is recorded in case compared to control [20.55±5.97]. The variance in mean Salivary glucose among the case and control is found to be highly significant with 'p' value 0.000.
Higher mean FBG [227.00±20.75] is recorded in case compared to control [86.44±8.42]. The variance in mean BP among the case and control is found to be highly significant with 'p' value 0.000.
Table 3.2 shows that mean serum TC level in case is higher [279.29±14.79] compared to control [116.66±12.50]. The variance in mean TC among the case and control is found to be highly significant with 'p' value 0.000.
Lower mean HDL-C [27.52±5.83] is recorded in case compared to control [39.32±5.47]. The variance in mean HDL-C among the case and control is found to be highly significant with 'p' value 0.000.
Higher mean LDL-C [173.10±16.41] is recorded in case compared to control [106.36±16.33]. The variance in mean LDL-C among the case and control is found to be highly significant with 'p' value 0.000.
Table 3.2 shows that mean VLDL-C level in case is higher [49.16±5.12] compared to control [22.85±2.18]. The variance in mean VLDL-C among the case and control is found to be highly significant with 'p' value 0.000.
Table 3.2 shows that mean TG level in case is higher [337.17±64.24] compared to control [114.45±10.86]. The variance in mean TGamong the case and control is found to be highly significant with 'p' value 0.000.

III. DISCUSSION

The present study was conducted at Rama Medical College, Hospital & Research Centre, Kanpur, Uttar Pradesh, India with the objective a study to co-relate salivary and plasma glucose with lipid profile in type 2 diabetes mellitus patients and compare it with matched healthy indivisuals in the population.The primary task of health management in Type 2 diabetes mellitus (T2DM) patients is to prevent diabetes-related complications. Previous studies have shown that good control of lipid profiles and glycemic levels can effectively prevent complications such as cardiovascular disease, diabetic nephropathy and diabetic retinopathy. Lipid profiles referred to lipids in plasma, generally including triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) clinically. For patients with cardiovascular disease and T2DM, lipid profiles should be strictly controlled to reduce mortality and complications. [14-17]

Good control of lipid profiles was one of the important factors influencing glycemic control in patients with T2DM. For example, infusion therapy of HDL increased plasma high density lipoprotein cholesterol (HDL-C) levels and reduced plasma glucose levels in T2DM patients by increasing plasma insulin and activating AMP-activated protein kinase in skeletal muscles. On the other hand, good glycemic control contributed to control of lipid profiles for patients with T2DM. Fujita Y, et al. reported that short-term intensive glycemic control could significantly decrease levels of TC by improving lipid metabolism. Therefore, good control of glycemic levels and lipid profiles are very important as well as being complicated in patients with T2DM. [18-20]

T2D patients had an increased risk of death and disability due to associated complications, suggesting the significance of good control for lipid profiles and glycemic level. [21] Therefore, the rates of FPG control and lipid profile control were evaluated, and the relationships between FPG control and lipid profiles were analyzed for T2D patients in this study. The results showed that the rates of FPG control and lipid profiles control were low, and that FPG control was significantly associated with HDL and TC. In this study, the control rates of FPG, TG, TC, HDL-C, and LDL-C for all T2D patients were 27.50%, 73.10%, 28.10%, 64.20%, and 44.80% respectively, with especially lower control rates for FPG and TC in T2D patients. A study from Beijing, China, showed a glycemic control rate of 30%. [22]

Daniel and Philip have determined the effect of lipid profile on type 2 diabetes mellitus. Patients with type 2 diabetes influences lipids, thus disclosing them to cardiovascular disease. They concluded that patients with type 2 diabetes mellitus had increased levels of triglycerides, decreased levels of high density lipid with either normal or increased levels of low density lipid. This indicates that type 2 diabetes influences on patients abnormal lipid profile along with increased risk of cardio vascular disease. Thus analysis of lipid profile in type 2 diabetics is very important in the clinical reviews and treatment. [23]

Popa et al., have studied that all the subjects with type 2 diabetes are at a higher risk of cardiovascular disease (CVD). Their Current interest is in identification and development of novel biomarkers which are specifically designed for individuals with diabetes. [24]

Karjalainen et al. assessed the salivary cholesterol in healthy adults and they concluded that serum concentration is reflected by salivary concentration levels to some extent. [25] For the premature mineralization of the dental plaque, salivary lipids play a major role in calculus formation. Hence mouth plays an important role in monitoring the systemic disease and oral health. For the evaluation of systemic disorders whole saliva is used for the salivary analysis. [26] Lipids present in saliva do not float like blood plasma lipoproteins. Therefore, their aggregation state is different from lipids in blood or lymph. Lipase activity of plasma lipoproteins was not found in parotid or submandibular saliva. The free fatty acids and partial glycerides level was high in saliva. [27]

The mean salivary glucose levels (1.380 ± 0.516 mg/dl) in non-diabetic subjects were higher in the present study compared to other studies. [28]which could be attributed to the carbohydrate-rich dietary pattern of the Indian population. Salivary glucose levels were significantly higher in diabetic subjects (group I>group II) than in non-diabetic subjects (group III). Salivary glucose levels showed significant positive correlations with serum glucose levels in all the study groups. However a previous study reported such positive correlation only in the group with uncontrolled diabetes.[29]

Various investigators have reported decreased salivary flow rate in diabetic subjects.[30-31] In our study salivary flow rates were significantly decreased in diabetic subjects compared to nondiabetic subjects. Previously lower salivary flow rate is reported in type 2 diabetic patients compared with healthy individuals.[32] A statistically significant correlation was observed between salivary flow rate and the

The study by Abikshyeet et al. revealed the mean FSG as 4.22 ± 3.59 mg/dl for diabetic group14and Panchbhai et al. in 2010, recorded a mean FSG of 7.64 ± 6.44 mg/dl.[34]Panchbhai again did a study in 2012 and found FBG as 6.83 mmol/dl.10 Another study conducted by Ravindran et al. observed mean of FSG as 6.567 ± 3.04 mg/dl for the diabetic group.[35]

In obesity, the low plasma HDL-C levels have been attributed to increased fractional clearance of HDL secondary to depletion of its cholesterol.[36]

Schmitt et al reported LDL uptake by fibroblasts may be impaired in type 2 diabetes and this leads to increase in LDL: HDL ratio in type 2 diabetics.[37]Patients with type-2 diabetes have increased risk of cardiovascular disease associated with atherogenic dyslipidemia and coronary artery disease, especially myocardial infarction is the leading cause of morbidity and mortality worldwide.[38]

In our study is limited by the limited number of patients attending only Rama Medical College Hospital and Research Centre, Kanpur U.P and also limited period. Additionally, the list of potential confounders for above enzyme assay disturbances is long which need to be studied in details the requires large population to reflect the correlation correctly.

IV. CONCLUSION

Biochemical screening for Salivary and plasma glucose is of paramount importance in all Type 2 diabetes mellitus patients, as well as in all patients with unexpected worsening of their lipid profile or vice-versa because our data statistically suggest that the effect of salivary and plasma glucose is associated with lipid disorders that are characterized by increased TC, Triglycerides, LDL-C, VLDL-C and decreased HDL-C levels.

From this study, it can be concluded that fasting salivary glucose level can be used as a non-invasive diagnostic, as well as a monitoring tool to assess the glycemic status of Type 2 diabetes mellitus patients.

From this study, it can be concluded that type 2 diabetes is most common in middle aged subjects. So, clinicians should remain highly suspicious in middle aged subjects with lipid profile for increase in atherogenic parameters which may enhance the risk for atherosclerosis leading to coronary artery disease.

Therefore, treatment and follow-up of type 2 diabetes mellitus patients should include the

monitoring of lipid profile parameters in order to decrease the possible effect of changing in the level of these parameters on the risk of cardiovascular diseases in the patients of T2DM.

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Conflicts of Interest
None.

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