Research Article

Analysis of Serum and Salivary Lipid Profile in Control Subjects and Diabetic Patients–A Comparative Study

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ABSTRACT

Patients with diabetes mellitus have a greater risk of dyslipidaemia. Abnormalities in lipid metabolism have been shown to be key risk factors for the development of diabetes-related complications. Lipids are the critical biomolecules for pathological and physiological processes in the human body and their examination is essential for diagnosing health and disease conditions such as diabetes mellitus, atherosclerosis and related complications. The objective of this study was to ascertain the diagnostic potential of saliva in the assessment of lipid profile as compared to that of serum lipid profile. The selected study area of Thiruporur conducted a Diabetic camp to collect the samples from 200 control subjects and 200 Type - II diabetic patients from different age groups. Total Cholesterol (TC), High-Density Lipoprotein (HDL), Triglycerides (TG), Low-Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), and the ratio of Total Cholesterol to High-Density Lipoprotein (TC/HDL) were analysed in the serum and saliva of the diabetic patients and the control subjects. When compared to the control group, patients with Type II diabetes have an abnormal blood lipid profile with elevated TC (246.8253.48mg/dl), high LDL (156.6253.484mg/dl), VLDL (42.36510.99mg/dl), TG (211.833 54.97 mg/dl), and TC/HDL ratio (5.471 1.239), but inadequate HDL (45.2793.199 mg/dl) levels were observed. According to the findings of this study, an increase in serum lipid profile values and corresponding increases in saliva lipid profile values were also observed. As per the outcome of the present study, saliva can be utilised as a non-invasive diagnostic tool for measuring lipid profile in diabetic patients

Keywords: Cholesterol, Diabetes mellitus, Lipid profile, Salivary Lipid profile, Salivary Biomarkers.

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INTRODUCTION

Indian economy is increasing substantially with the increasing diabetic population. Its health-care costs are rising in tandem with a decline in health among its economically productive youth population.[1-4] In 2035, this figure is predicted to rise to 109.0 million.[5] Diabetes was shown to be most prevalent in low-income countries (LIC) and least prevalent in high-income countries (HIC).[6] Diabetes Mellitus (DM) is a set of metabolic illnesses exhibited as by high blood glucose levels caused by insufficient insulin production, insulin action, or both.[7] Excessive metabolism of free fatty acids is caused by insulin insufficiency, which can lead to lipid metabolism-related problems. Insulin has a wide range of effects on mammalian lipid metabolism. It increases fatty acid synthesis in the liver, adipose tissue and gut. Insulin has also been reported to increase cholesterol synthesis.

Cholesterol, despite its conventional image as a powerful adversary of health and longevity, is a necessary chemical that serves in a variety of activities in the body. Cholesterol is needed for the production of bile acids, which are necessary for fat absorption as well as for the

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absorption of several hormones such as testosterone, oestrogen, dihydro epiandrosterone, progesterone and cortisol. In presence of sunlight, cholesterol is essential for the production of vitamin D. Cholesterol is an important component of the cell membrane, where it offers structural support and may also act as an antioxidant. It is necessary for the transmission of nerve impulses, particularly at the synapse level.[8] Cholesterol, play a key role in the pathogenesis of a number of diseases. Kidney disease, arteriosclerosis, hypertension, obesity, diabetes and obstructive jaundice have all been linked to an increased level of lipids in the blood.[9] Diabetes is associated with hyperlipidaemia and disturbed lipid metabolism. The link between elevated serum lipids and vascular complications of diabetes has long been studied since both occur more frequently in people with diabetes than in the general population. Diabetes mellitus is found in association with dyslipidaemia at 95% frequency.[10]

Saliva is used as a diagnostic tool in important fields like dentistry, physiology, internal medicine, paediatrics, endocrinology, immunology, clinical pathology, forensic medicine and sports medicine. In saliva, a number of markers, antibodies and hormones can be readily and consistently monitored for various diseases.[11-13] As a result, saliva is used as an effective diagnostic tool in specific circumstances, instead of body fluid. Salivary steroid hormone tests are widely used and well-validated for fertility issues.[14] Multiple numbers of saliva specimens can be easily collected by the patient for steroid hormone analysis, to monitor fertility cycles, menopausal fluctuation, stress and other diurnal variation.[15]

Due to its origin, composition, roles and relationships with other organ systems, salivary analysis has become an essential resource for the evaluation of salivary problems with physiological and pathological implications, as well as it acts as a mirror that reflects the individual's illness or disease condition. Also, as compared to blood collection, it offers a simple and non-invasive collection method; it is easy to store and also with inexpensive procedure for analysis. The aim of this study is to compare and evaluate the blood and salivary lipid profile in healthy people and diabetic patients, as well as to validate the role of saliva as a non-invasive diagnostic tool for monitoring lipid profiles in a diabetic patients.

MATERIALS AND METHODS

Sample Collection

A diabetic camp was held to collect samples from 200 healthy people and 200 Type –II diabetic patients of different age groups from the study area of Thiruporur, Tamilnadu. The members of the Institutional Ethical Committee gave their approval to conduct the study. Pregnancy, alcoholism, smoking, any chronic conditions, and a recent history of diabetes were all exclusion factors for the control group. All subjects were requested not to eat or drink (except water) for an overnight period prior to the collection of unstimulated whole saliva and a fasting blood sample after being informed and giving their informed consent.

Blood sample

After collection of whole blood, allow the blood to clot by leaving it undisturbed at room temperature for 15–30 min. Clots were removed by centrifugation process at 1,000 – 2,000 rpm for 10 min. Following centrifugation immediately transferred the liquid component (serum) into a clean polypropylene tube using a micropipette and stored at - 20°C for further analysis.

Saliva sample

A systematic approach was used to collect the saliva. Fasting salivary sample collection took place between 7.30 and 10.00 a.m., with study participants sitting upright in a comfortable position in a quiet, isolated room. Before saliva collection the study participants were informed not to eat or drink anything and denture wearers were instructed to remove their dentures. The subjects washed their mouths with water before taking part in the study. Initially, respondents were instructed to spit out or swallow any saliva existing in their mouths, with the samples obtained for the first 30 sec being discarded. Saliva samples were obtained using the spitting method in ice-cold tubes fitted with a funnel. Saliva was collected for at least five minutes.[16-21] Collected samples were, centrifuged at 4000 rpm for 15 min to eliminate any particle material, and the supernatant was immediately transferred to a sterile container, kept and frozen at - 20°C for later investigation.

Estimation of Lipid Profile

Estimation of cholesterol was done by CHOD / PAP method. The principle of this method is cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol is oxidised to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red-coloured quinonelimine dye complex. Intensity of the colour formed, is directly proportional to the amount of cholesterol present in the sample. This was measured at 500 nm by spectrophotometry.

Cholesterol esters + H_2O **Cholesterol Esterase** Cholesterol + Fatty acids

Cholesterol + O_2 **Cholesterol Oxidase** Cholestenone + H_2O_2

H_2O_2+ 4 - Aminoantipyrine + Phenol Peroxidase Red Quinoneimine + H_2O

Estimation of HDL Cholesterol (PEG Method)

This method is based on the principle of Very Low Density (VLDL) and Low Density (LDL) Lipoproteins from serum or plasma precipitation by phosphotungstate in the presence of magnesium ions. After removal by centrifugation, the clear supernatant containing High Density Lipoprotein (HDL) is used for the determination of HDL cholesterol.

Estimation of Triglycerides (GPO /PAP Method)

Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol is formed with ATP in the presence of glycerol kinase to form Glycerol 3 phosphate which is oxidised by the enzyme Glycerol phosphate oxidase to form Hydrogen peroxide. The Hydrogen peroxide further reacts with phenolic compound and 4- aminoantipyrine by the catalytic action of peroxidase to form a red-coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of triglycerides present in the sample. The absorbance was measured at 505 nm.

Estimation of VLDL Cholesterol

Indirect measurement of VLDL was done using the Friedewald equation in serum and saliva samples. VLDL Cholesterol can be calculated by

VLDL= Triglycerides/5

Estimation of LDL Cholesterol

Indirect measurement of LDL was done using the Friedewald equation in serum and saliva. LDL cholesterol can be calculated by

LDL = Total Cholesterol – (HDL + VLDL) mg/dl.

Estimation of Total Cholesterol TC / HDL Ratio (TC / HDL Ratio)

Indirect measurement of Total Cholesterol / HDL Cholesterol ratio in blood and saliva was calculated by

TC / HDL Ratio = Total Cholesterol / HDL Cholesterol

Statistical Analysis

Graph Pad was used to do the statistical analysis. The mean and standard deviations of each study variables were examined using descriptive statistics. The findings of the analysis were expressed as Mean SD, with $p < 0.0001$ as the level of significance.

RESULTS

Lipids play a crucial role in human health; they are important macromolecules in identifying pathological and physiological conditions. In the serum of healthy and diabetic patients, Total Cholesterol (TC), Triglycerides (TGL), High-Density Lipoprotein-Cholesterol (HDLC), Low-Density Lipoprotein-Cholesterol (LDLC), and Very-Low-Density Lipoprotein-Cholesterol (VLDLC) tests are performed (Lipid profile).

From the present study, it was observed that patients with Type -II diabetes have an abnormal blood lipid profile, consisting of elevated blood Total cholesterol (246.82±53.48mg/dl), high level of LDL (156.62±53.484mg/dl), VLDL (42.365±10.99mg/dl), TG (211.833 ±54.97 mg/dl), and TC/ HDL ratio (5.471 ± 1.239) but the inadequate level of HDL (45.279±3.199 mg/dl) as compared with the control subjects (Table 1, Figure 1).

Similar biochemical tests can also be done with other biological fluids (in saliva) because lipids are secreted in saliva also. Increased serum lipid concentration increases the salivary lipid level. The salivary lipid profile of control and diabetic patients were comparatively studied. The high concentrations of lipid fractions in saliva

Table 1: Comparison of Lipid Profile in Blood Sample of Control Subjects and Diabetic Patients (Mean / SD).

Sl. No.	Parameters	Blood (In serum)		*p*- value (Between control and diabetic)
		Control People (n-200)	Diabetic patients (n-200)	
1	Total cholesterol TC (mg/dl)	169.19 ±13.183	246.82±53.48	0.0001 ***
2	Low Density Lipoprotein LDL (mg/dl)	90.792 ± 12.999	156.62±53.484	0.0001 ***
3	High Density Lipoprotein HDL (mg/dl)	54.458 ±2.510	45.279±3.199	0.0001 ***
4	Very Low Density lipoprotein VLDL (mg/dl)	23.918 ±1.788	42.365±10.99	0.0001 ***
5	Triglycerides TG (mg/dl)	119.51±8.898	211.833 ±54.97	0.0001 ***
6	Total cholesterol / High Density Lipoprotein TC / HDL ratio	3.111 ± 0.246	5.471 ± 1.239	0.0001 ***

* - Not Significant, ** - Significant, *** - Highly statistically significant.

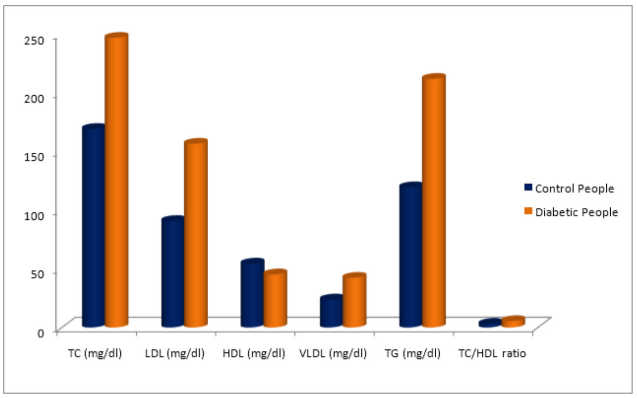

Figure 1: Lipid profile in blood sample of Control, Diabetic patient.

were recorded in diabetic patient, like Total cholesterol (33.765± 7.056 mg/dl), LDL (16.579 ± 6.911 mg/dl), VLDL (11.861 ± 1.262mg/dl) TG (59.319 ± 6.314mg/dl) and TC / HDL ratio (6.591 ± 2.441) respectively when compared with control salivary lipid profile (TC- 19.627 ± 2.573, LDL 4.871 ± 2.705, VLDL 5.764 ± 1.017, TG 28.881 ± 5.072 mg/dl and TC/ HDL ratio 2.244 ± 0.560). The observed results are represented as Mean ±SD, with their level of significance in Table 2 and Figure 2. But reverse conditions of a significantly higher level of HDL Cholesterol were observed in control (9.108 ± 1.834 mg/dl) than in diabetic (5.512 ± 1.431mg/dl) patients. Similar to blood, results of all the lipid profile parameters in saliva (TC, LDL, VLDL, TG and TC /HDL ration) except HDL were observed to be highly significant at $p < 0.0001$.

DISCUSSION

Malgorzata *et al*.[22] in their study emphasized the diagnostic utility of lipids and their relationship to systemic disorders. Their findings also demonstrated the increased serum and salivary lipid concentrations in diabetic patients. This difference in the lipid composition of saliva aids in the detection of bodily abnormalities. The current research also focused on a comparison of lipid profiles in blood and saliva, with notable findings in salivary lipid profiles, which can predict body fluctuations non-invasively.

Yang *et al*.[23] in their study found a two-fold rise in all serum lipid fractions except HDL-cholesterol in diabetic patients as compared to the control people. Salivary glands have been shown to be a target organ in diabetics due to the occurrence of various compositional alterations in saliva. In current investigation also observed that higher concentration of lipid profile when compared to the control group. Hypertriglyceridemia and hypercholesterolemia have been linked to oxidative modification of LDL-C, protein glycation and glucose autooxidation, resulting in an excess of lipid peroxidation products and an increase in oxidative stress in hyperlipidemic individuals.

According to Gheena *et al*.,[24] fat metabolic dysfunction causes abnormalities in glucose metabolism, insulin production and insulin action in diabetics. When compared to healthy people, diabetic patients with Type I and Type II diabetes had higher lipid content in their blood and saliva. The study of Al-Rawi[25] also discovered a favourable association between blood and saliva lipids of diabetic patients. This positive connection might be

Table 2: Comparison of Lipid Profile in Saliva Sample of Control Subjects and Diabetic Patient (Mean / SD).

Sl. No.	Parameters	Saliva		*p*- value (Between control and diabetic)
		Control People (n-200)	Diabetic patients (n-200)	
1	Total cholesterol TC (mg/dl)	19.627 ±2.573	33.765± 7.056	0.0001 ***
2	Low Density Lipoprotein LDL (mg/dl)	4.871 ± 2.705	16.579±6.911	0.0001 ***
3	High Density Lipoprotein HDL (mg/dl)	9.108 ± 1.834	5.512±1.431	0.0001 ***
4	Very Low Density lipoprotein VLDL (mg/dl)	5.764 ± 1.017	11.861±1.262	0.0001 ***
5	Triglycerides TG (mg/dl)	28.881±5.072	59.319 ± 6.314	0.0001 ***
6	Total cholesterol / High Density Lipoprotein TC / HDL ratio	2.244 ± 0.560	6.591 ± 2.441	0.0001 ***

* - Not Significant, ** - Significant, *** - Highly statistically significant.

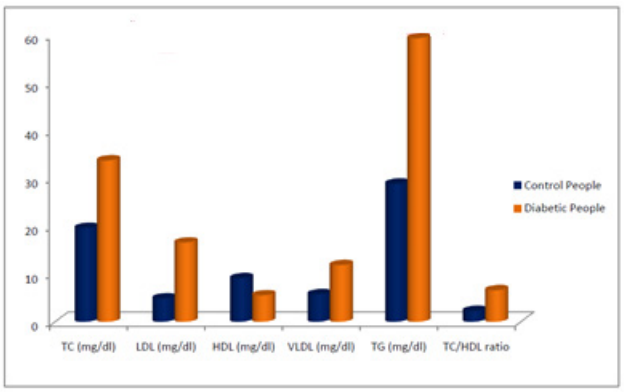

Figure 2: Lipid Profile in saliva Sample of Control, Diabetic patient.

utilised to identify people with high serum cholesterol levels in healthy people.[26]

Cardiovascular morbidity is a substantial burden for Type II diabetes, with endothelial dysfunction being an early indicator of diabetic vascular disease. Diabetics have a faster progression of atherosclerosis than non-diabetics. According to Petitti *et al.*[27] and Abdel-Gayoum,[28] both Type I and Type II diabetes mellitus are related to impaired lipid metabolism. Samatha *et al.*[29] reported in their research work, that diabetics had higher levels of total cholesterol, triglycerides and low-density lipids than healthy people, indicating that dyslipidaemia is more common in diabetes patients and can lead to cardiovascular problems.

Rajeshwari *et al.*[30] investigated the relationship between high and low-density lipids in plasma, MDA and total thiols in the saliva of diabetic patients and concluded that saliva is the best sample for detecting and treating various pathological conditions.

A high level of lipid profile in Type –II diabetic patients was observed as a significant risk factor for cardiovascular diseases.[31] Jain *et al.*[32] and Singh *et al.*[33] conducted a comparative study on the saliva and serum lipid profile of the healthy and diabetic patients to correlate the function of saliva as a non-invasive diagnostic medium, along with HbA1c level to predict diabetes and CVD. Significant results were also found in the current investigation.

CONCLUSION

The current study observed a strong association between serum and saliva lipid profiles in diabetic and control groups. Although the comparison of serum and saliva lipid profile values in the present study group was found statistically significant. Smoking, periodontal disease, calculus, dietary state and drugs, in addition to other things, could impact the lipid profile estimation in saliva. When all of these criteria are included, a better estimation of saliva lipid profile values among high-risk people can be found. It means that if blood lipid profile levels increases, saliva lipid profile values are likely to increase as well, or vice versa. Saliva might be used as a screening tool for monitoring lipid profiles at regular intervals without the need for time-consuming and unpleasant clinical procedures, as well as the pleasure of avoiding some of the needle pricks.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TC: Total Cholesterol; **HDL:** High-Density Lipoprotein; **TG:** Triglycerides; **LDL:** Low-Density Lipoprotein; **VLDL:** Very Low-Density Lipoprotein.

Relevance of the Study

Diabetes has always been regarded as a challenge to the health professionals due to its continuous monitoring requirements. Salivary diagnosis is considered to be useful in cases, with a repeated sample of body fluid collection is regarded impractical, unethical or both. Hence precise, reliable and the best biomarker to unravel the incidence of diabetes through the establishment of a scientific criterion and clinical validation is necessary to make highly exact usable technology in comparison with blood to reach a definitive evaluation, by way of using saliva as a diagnostic tool.

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