Original Research Article

Saliva – A Diagnostic Tool in Assessment of Lipid Profile

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Abstract: Cholesterol, a crucial substance that performs innumerable vital functions in the body, has also been considered as a risk factor for various pathological states including atherosclerosis. Saliva cholesterol concentration reflects serum concentration to some extent. 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. A total number of 50 subjects were included in the study. The study group included 30 subjects with high risk for having higher serum lipid profile values with history of diabetes mellitus, hypertension or coronary heart disease and the control group included 20 age matched healthy subjects. The subjects selected randomly from the Department of Oral Medicine and Radiology, D.A.P.M R.V. Dental College and Hospital, Bangalore were subjected to a thorough general and oral examination which was recorded. The Lipid profile estimation, which included Total Cholesterol (TC), High Density Lipoprotein (HDL), Triglycerides (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and ratio of Total Cholesterol with High Density Lipoprotein (TC/HDL), was done for the study group and control group subjects for serum and saliva. There was no significant difference in the saliva lipid profile values between the study group and the control group and also between the subjects in the risk groups and the control group. There was a good correlation between serum and saliva lipid profile values in the study group and the control group. The results of the present study showed that an increase in the serum lipid profile values leads to a corresponding increase in the saliva lipid profile values.
Keywords: Serum, Saliva, Cholesterol, Lipoproteins, Triglycerides, Diabetes Mellitus, Hypertension

INTRODUCTION

Cholesterol, contrary to its popular image as a potent enemy of health and longevity, is actually a crucial substance that performs innumerable vital functions in the body.

Cholesterol is required for the synthesis of bile acids, which are essential for the absorption of fats, and of many hormones such as testosterone, estrogen, dihydroepiandrosterone, progesterone, and cortisol. Cholesterol is required to produce vitamin D, in the presence of sunlight. Cholesterol is an essential element of cell membranes, where it provides structural support and may even serve as a protective antioxidant. It is essential for conducting nerve impulses, especially at the level of the synapse[1].

Cholesterol has however also been considered as an active participant in the mechanism of various pathological conditions. Increased amounts in blood have been associated with kidney disorders, arteriosclerosis, hypertension, obesity, diabetes, and obstructive jaundice[2].

LDL cholesterol forms fatty deposits in arterial walls, which become plaques that grow, rupture, and stimulate the formation of artery-blocking blood clots[1]. The belief that low-density lipoprotein (LDL) cholesterol causes atherosclerosis and subsequent heart disease is a fundamental precept of modern medicine[1].

Saliva as a diagnostic aid is increasingly being used to evaluate various systemic conditions. It has been well validated in diagnosing, monitoring systemic disease status and predicting disease progression[3] .

Studies have shown significant differences in the lipid profile values in the high risk subjects as compared to healthy subjects both in the serum and the saliva[3,4]. The positive correlation of serum and saliva cholesterol values further supports the concept that at

least a part of the salivary cholesterol originates from serum. The assay of salivary cholesterol may be useful in identifying individuals with high serum cholesterol levels[5].

This study was intended to ascertain diagnostic potential of saliva in assessment of lipid profile as compared to that of serum lipid profile.

MATERIALS AND METHODS

An informed consent was obtained from all the subjects prior to their inclusion in the study.

A total number of 50 subjects were included in the study. The subjects selected included 23 males and 27 females within the age group of 38 years to 74 years. These subjects were then divided into study group and control Group.

These subjects were divided into two groups. Study Group: This included 30 subjects with high risk for having higher serum lipid profile values with a history of diabetes mellitus, hypertension or coronary heart disease and Control

Group: This included 20 age matched healthy subjects from the general population with no history of any systemic conditions which could alter the lipid profile values.

Patients excluded from the study were patients with hyperlipidemia, liver and kidney disease, Cushing's disease, Hypothyroidism, patients receiving lipid-lowering drugs and pregnant and lactating women.

All the individuals were subjected to general and oral examination. 3 ml of blood was drawn from the antecubital vein with minimal trauma and under aseptic precautions and whole saliva was collected for 5 minutes by spitting method. Lipid profile estimation of both serum and saliva were carried out for all the study and control group subjects with the help of test kits. The parameters evaluated were Total Cholesterol (TC), High Density Lipoprotein (HDL), Triglycerides (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and ratio of Total Cholesterol with High Density Lipoprotein (TC/HDL).

Blood sampling

All subjects included in the study were asked to refrain from eating 12 hours prior to blood collection. 3 ml of blood was drawn from the antecubital vein with minimal trauma and under aseptic precautions. After allowing the blood to stand for 15 to 20 minutes it was centrifuged at 3000 rpm for 15 to 20 min. The supernatant serum was aspirated and stored in a fresh vial at -20°C until it was analyzed.

Saliva sampling

The study subjects were made to sit comfortably on the dental chair in a restful and quiet environment and were asked to flush his/her mouth with tap water. The whole saliva was collected for 5 minutes by asking the subjects to lean forward and spit saliva into the sterile plastic containers which were precooled at -20°C. The containers were then kept in the ice box which was transported immediately where further sampling was done. The saliva sample was transferred to a microfuge vial and cold centrifuged at 3000 rpm at 4°C for 15 minutes. The supernatant was aspirated and transferred to fresh chilled vials and stored at -20°C and was later analyzed.

Estimation of lipid profile

Lipid profile estimation of both serum and saliva were carried using the test kits by 'Span Diagnostics' out for all the study and control group subjects. The parameters evaluated were Total Cholesterol (TC), High Density Lipoprotein (HDL), Triglycerides (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and ratio of Total Cholesterol with High Density Lipoprotein (TC/HDL).

The Excel and SPSS (SPSS Inc, Chicago) software packages were used for data entry and analysis by using Student 't' test, one way Analysis of Variance (Anova) and Correlation Co-efficient.

RESULTS

The study group included 17 males and 13 females (Table 1, Graph 1). Among the study group, 13 subjects gave a history of diabetes mellitus, of which 2 subjects also had a history of CHD. 13 subjects gave a history of hypertension among which 1 subject also had a history of CHD and 4 subjects gave a history of both diabetes mellitus and hypertension. The control group included 6 males and 14 females (Table 1, Graph 1). In the study group subjects, the age ranged between 38 to 74 years. In the control group subjects, age ranged between 39 to 74 years.

The lipid profile was estimated in the study group and control group in both, the serum and the saliva. Evaluation of Lipid profile values consisted of estimation of Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL), Triglycerides (TG), Low Density Lipoprotein Cholesterol (LDL), Very Low Density Lipoprotein Cholesterol (VLDL) and ratio of Total Cholesterol with High Density Lipoprotein (TC/HDL).

The values of serum lipid profile were compared between the study group and the control group:

In serum of the study group, the mean TC was 158.89 ± 26.43; the mean HDL was 55.62 ± 14.42; the mean TG was 95.53 ± 42.97; the mean LDL was 102.08 ± 33.22; the mean VLDL was 49.35 ± 14.57; the mean TC/HDL was 2.90 ± 0.66.

In the serum of the control group, the mean TC was 142.05 ± 24.11; the mean HDL was 47.62 ± 12.17; the mean TG was 86.37 ± 28.89; the mean LDL was 79.43 ± 25.64; the mean VLDL was 41.30 ± 12.90; the mean TC/HDL was 2.88 ± 0.46.

It was observed that the mean serum TC and LDL were higher in study group compared to control group and the difference was statistically significant ($P<0.05$). The mean serum HDL and VLDL values were high in the study group as compared to the control group, the difference was borderline statistically significant (p value was very close to 0.05). The mean serum TG was higher in the study group as compared to the control group and the difference was not statistically significant. The serum TC/HDL ratio was almost similar in both the groups and the difference was not statistically significant (Table 2, Graph 2).

The values of saliva lipid profile were compared between the study group and the control group:

In the saliva of the study group, the mean TC was 4.38 ± 2.16; the mean HDL was 1.93 ± 0.75; the mean TG was 3.53 ± 2.68; the mean of LDL was 3.55 ± 3.27; the mean of VLDL was 2.47 ± 1.23; the mean TC/HDL was 2.47 ± 1.22.

In the saliva of the control group, the mean TC was 4.52 ± 1.68; the mean HDL was 1.92 ± 0.87; the mean TG was 4.30 ± 2.62; the mean of LDL was 3.23 ± 3.81; the mean of VLDL was 2.49 ± 1.24; the mean TC/HDL was 2.80 ± 1.47.

It was observed that the values were almost similar between the study and the control group and no statistically significant difference was found with respect to mean saliva TC, HDL, LDL, VLDL, TG & TC/HDL ratio ($P>0.05$) (Table 3, Graph 3).

In the study group the correlation between the values of the serum and saliva lipid profile was analyzed and it was observed that there was strong correlation between TC levels (0.60) and VLDL levels (0.60) which was statistically significant ($P<0.01$).

There was moderate correlation between HDL levels ($r=0.53$) and LDL levels ($r=0.56$) which was statistically significant ($P<0.01$). There was weak correlation between TG levels ($r=0.33$) which was not statistically significant ($P>0.05$). There was very weak correlation between TC/HDL ratio ($r=0.173$) which was not statistically significant ($P>0.05$) (Table 4, Graph 4).

In the control group the correlation between the values of the serum and saliva lipid profile was analyzed and it was observed that there was a very strong correlation between TG levels($r=0.83$) and strong correlation between TC levels ($r=0.63$) which was statistically significant ($P<0.001$). There was moderate correlation between LDL levels ($r=0.51$) and VLDL levels ($r=0.53$) which was statistically significant ($P<0.05$). There was moderate correlation between TC/HDL Ratio ($r=0.46$) which was not statistically significant ($P>0.05$), there was weak correlation between HDL levels ($r=0.36$) which was not statistically significant ($P>0.05$). (Table 5, Graph 5).

The correlation between the serum and saliva lipid profile values was evaluated with individual risk groups:

In the study group, the correlation between the serum and saliva lipid profile was evaluated in the risk group positive for DM and it was observed that there was a strong correlation between TC levels ($r=0.77$), LDL levels ($r=0.71$), VLDL levels ($r=0.69$) which was statistically significant ($P<0.01$). There was weak correlation between HDL levels ($r=0.25$) and TG levels ($r=0.26$) which was not statistically significant ($P>0.05$). There was very weak correlation between TC/HDL Ratio ($r=0.13$) which was not statistically significant ($P>0.05$) (Table 6, Graph 6).

In the study group, the correlation between the serum and saliva lipid profile was evaluated in the risk group positive for HTN and it was observed that there was very strong correlation between HDL levels ($r=0.80$) and VLDL levels ($r=0.83$) which was statistically significant ($P<0.01$). There was strong correlation between LDL levels ($r=0.64$) and TG levels ($r=0.66$) which was statistically significant ($P<0.05$). There was moderate correlation between TC levels ($r=0.44$) which was not statistically significant ($P>0.05$). There was weak correlation between TC/HDL Ratio ($r=0.23$) which was not statistically significant ($P>0.05$) (Table 7, Graph 7).

Table 1: Gender distribution in the study group and the control group (Graph 1)

GROUP	GENDER		TOTAL SUBJECTS
	Males	Females	
STUDY GROUP	17	13	30
CONTROL GROUP	6	14	20

Table 2: Comparison of serum lipid profile values between the study group and the control group (Graph 6)

Lipid Profile	Group	n	Mean	Std dev	t - value	p-value
Serum - TC	Study group	28	158.89	26.43	2.256	0.029*
	Control group	20	142.05	24.11		
Serum - HDL	Study group	30	55.62	14.42	2.043	0.047
	Control group	20	47.62	12.17		
Serum - LDL	Study group	30	102.08	33.22	2.577	0.013*
	Control group	20	79.43	25.64		
Serum - VLDL	Study group	30	49.35	14.57	2.002	0.051
	Control group	20	41.30	12.90		
Serum - TG	Study group	30	95.53	42.97	0.834	0.408
	Control group	20	86.37	28.89		
Serum - TC/HDL Ratio	Study group	27	2.90	0.66	0.126	0.900
	Control group	18	2.88	0.46		

* Indicates statistically significant

Table 3: Comparison of the saliva lipid profile values between the study group and the control group (Graph 9)

Lipid Profile	Group	n	Mean	Std dev	t - value	p-value
Saliva - TC	Study group	29	4.38	2.16	-0.239	0.812
	Control group	20	4.52	1.68		
Saliva - HDL	Study group	30	1.93	0.75	0.042	0.967
	Control group	20	1.92	0.87		
Saliva - LDL	Study group	30	3.55	3.27	0.314	0.755
	Control group	20	3.23	3.81		
Saliva - VLDL	Study group	29	2.47	1.23	-0.054	0.957
	Control group	19	2.49	1.24		
Saliva - TG	Study group	30	3.53	2.68	-1.013	0.316
	Control group	20	4.30	2.62		
Saliva - TC/HDL Ratio	Study group	30	2.47	1.22	-0.844	0.403
	Control group	20	2.80	1.47		

Table 4 - Correlation between serum and saliva lipid profile values in the study group (Graph 12)

Parameter	r	p-value
TC	0.595	0.001*
HDL	0.530	0.003*
LDL	0.564	0.001*
VLDL	0.598	0.001*
TG	0.330	0.075
TC/HDL RATIO	0.173	0.387

* Indicates statistically significant

Table 5 - Correlation between serum and saliva lipid profile values in the control group (Graph 13)

Parameter	r	p-value
TC	0.638	0.002*
HDL	0.356	0.124
LDL	0.515	0.020*
VLDL	0.531	0.019*
TG	0.830	<0.001*
TC/HDL RATIO	0.459	0.055

* Indicates statistically significant

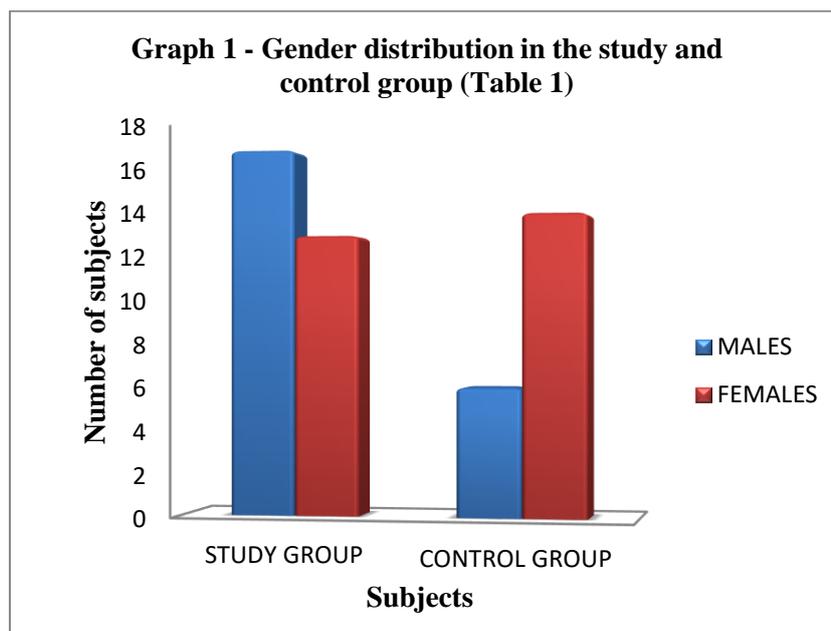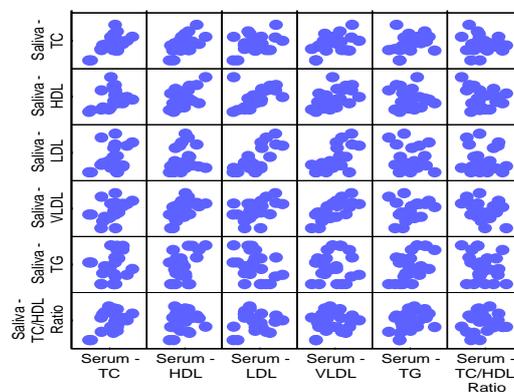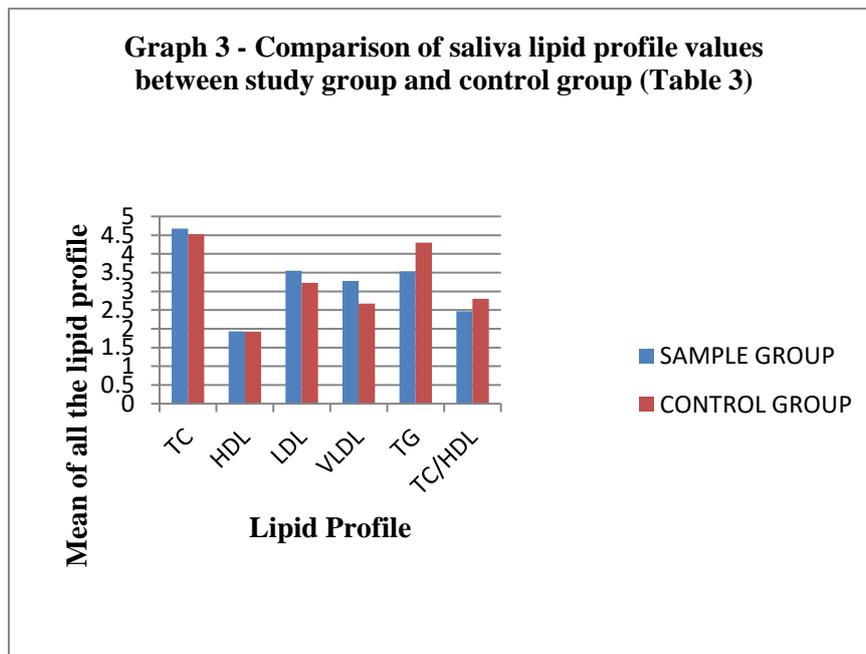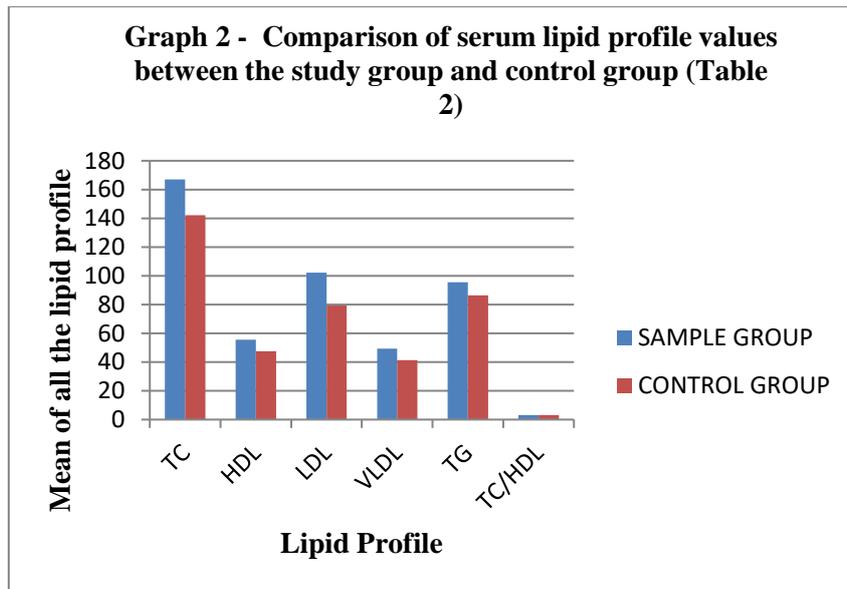**Table 6 - Correlation between serum and saliva lipid profile values in the DM group (Graph 14)**

Parameter	r	p-value
TC	0.772	0.003*
HDL	0.257	0.397
LDL	0.706	0.007*
VLDL	0.692	0.009*
TG	0.262	0.387
TC/HDL RATIO	0.134	0.694

* Indicates statistically significant

Table 7 - Correlation between serum and saliva lipid profile values in the HTN group (Graph 15)

| Parameter	r	p-value
TC	0.441	0.131
HDL	0.795	0.001*
LDL	0.643	0.018*
VLDL	0.828	0.001*
TG	0.662	0.014*
TC/HDL RATIO	0.231	0.470

* Indicates statistically significant

Graph 1 - Gender distribution in the study and control group (Table 1)

Graph 2 - Comparison of serum lipid profile values between the study group and control group (Table 2)

Graph 3 - Comparison of saliva lipid profile values between study group and control group (Table 3)
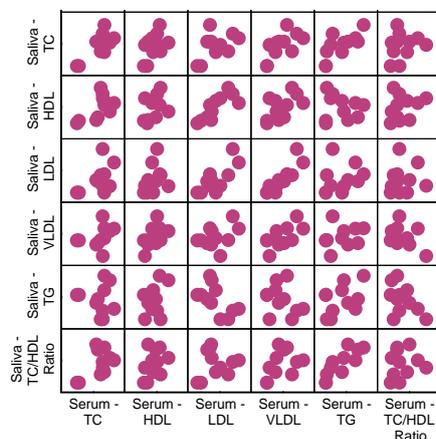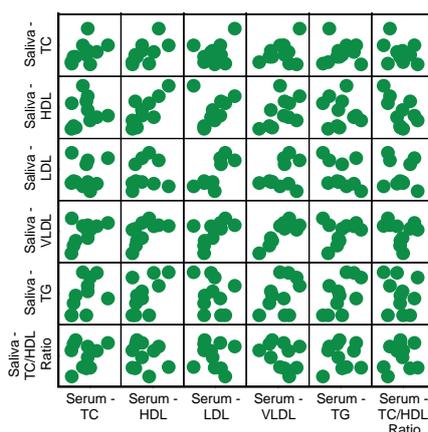

Graph 4 - Correlation between saliva and serum lipid profile values recorded in the study group (Table 4)
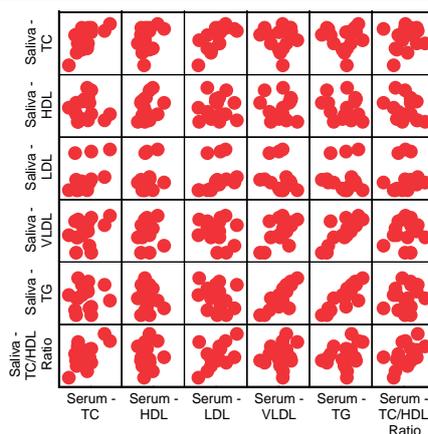
Graph 5 - Correlation between saliva and serum lipid profile values recorded in the control group (Table 5)

Graph 6 - Correlation between saliva and serum lipid profile values recorded in the DM group (Table 6)

Graph 7 - Correlation between saliva and serum lipid profile values recorded in the HTN group (Table 7)

DISCUSSION

The term 'Lipid' refers to substances with poor water solubility which includes biologically important materials such as sterols, including cholesterol, and glycerides, such as triglycerides (TG) and phospholipids. Despite their poor water solubility they are assimilated from the diet and transported within the body by getting incorporated within lipoproteins[6].

Plasma lipoprotein levels are major modifiable risk factors for cardiovascular disease. Increased levels of atherogenic lipoproteins, especially Low density lipoprotein (LDL), contribute to the development of atherosclerosis. Cholesterol laden cells release cholesterol to High density lipoprotein (HDL) for reverse cholesterol transport to the liver for excretion[6].

Studies of the development of diseases of the cardiovascular system such as atherosclerosis, arteriosclerosis, coronary heart disease (CHD) and hypertension (HTN) have demonstrated that age, physique (especially obesity), elevated blood glucose, triglycerides (TG) and serum cholesterol are all interrelated[7]. Plasma cholesterol and TG are clinically important because they are major treatable risk factors for cardiovascular disease. Determination of these components is included as a part of serum lipid profile in order to screen the risk factors for arteriosclerotic cardiovascular disease. Consequently low HDL cholesterol levels predispose to atherosclerosis. Elevated TG, which is common in obesity, diabetes and insulin resistance, is frequently associated with low HDL and increased 'small, dense' LDL[6].

Abnormalities of lipid metabolism most commonly are found following routine blood investigations. Levels of TC, TG, HDL-C should be obtained after a 12 hour fast to permit the calculation of LDL-C[6].

In the recent years, much attention has been paid to the biochemical importance of saliva. Saliva is a complex fluid produced by a number of specialized glands which discharge into the oral cavity. The easy non-invasive, stress-free nature of saliva collection makes it one of the most accessible body fluids to obtain[8].

As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be collected by individuals with modest training and provides a cost-effective approach for the screening of large populations. Whole saliva is most frequently used for diagnosis of systemic diseases, since it is readily collected and contains serum constituents. These constituents are derived from the local vasculature of the salivary glands and also reach the oral cavity via the flow of gingival fluid. Analysis of saliva may be useful for the diagnosis of diseases and assessment of level of drugs[9].

Whole saliva contains about 10 - 100-μg/ml lipids. The most frequent lipids in the saliva are glycolipids, neutral lipids like free fatty acids, cholesteryl ester, triglycerides, cholesterol and a somewhat lower portion of phospholipids. Salivary lipids are mostly of glandular origin, but some lipids such as cholesterol are believed to diffuse directly from serum.

Salivary lipids may play a role in the acquired pellicle, dental plaque, calculus, sialolith, and caries formation[10]. The correlation of serum and salivary cholesterol concentrations and the role of salivary lipids in oral health have been poorly characterized. Cholesteryl esters, cholesterol, triglycerides, diglycerides, monoglycerides and free fatty acids accounted for 96–99% of the total salivary lipids and they exist in a different state of aggregation from lipids in blood or lymph[11].

The reliability and accuracy of measuring lipid profile concentration in saliva of patients with ischemic stroke and patients with stroke-related diseases has been studied and it was reported that lipid profile can be assessed in saliva and can be used as a simple, monitoring tool in stroke-prone individuals with reasonable accuracy[3].

In the study group subjects, the age ranged between 38 to 74 years. In the control group subjects, age ranged between 39 to 74 years. Studies have suggested that approximately 25% of the adult population aged 20 and older have blood cholesterol levels that are considered high. Reports also suggest that lipoprotein levels remain a significant risk factor for coronary artery disease (CAD) in the elderly and that treatment of dyslipidemia in the elderly may have a greater impact on CAD mortality than in younger age group because the total attributable risk from dyslipidemia is greater in the older age group[12]. To obtain a better representation of the high risk population, an age group 38 years and above was selected for the study group and a comparable age group was selected for the control group.

In the present study, comparison was made of the serum and saliva lipid profile values between study and control group.

It was observed that the serum TC and LDL were significantly higher, serum HDL and VLDL were moderately higher and serum TG was minimally higher in study group as compared to the control group. The serum TC/HDL ratio was almost similar in the study group as compared to the control group.

The prevalence of increased total blood cholesterol in the developed and developing nations remains high and has increased in adults, children and adolescents[13]. Abnormal plasma cholesterol levels are frequently present in individuals with Type 2 diabetes[14]. Epidemiological studies have shown that diabetics have 2 - 4 times higher risk of developing

cardiovascular diseases[15]. Patients with hypertension also frequently have higher levels of triglycerides. Hypertension occurs more frequently in hypercholesterolemic subjects, as compared to normolipid men and women[13].

Studies have shown that diabetic individuals demonstrated high prevalence of dyslipidemia among type 2 diabetics and significant association of triglycerides and HDL-cholesterol with obesity, physical inactivity and inadequate glycemic control[14]. The present study is in accordance with this, as the study group had significantly higher serum values of TC and LDL and moderately higher serum values of HDL and VLDL as compared to the control group. The reasons for the higher values of cholesterol may be attributed to obesity, physical inactivity and inadequate glycemic control; these parameters were not assessed in the present study.

A study has shown that, the serum TG levels were lower in the diabetic individuals than in the control subjects though not significant[15]. The present study was in accordance with the above study where serum TG and TC/HDL were not significantly higher in the study group as compared to the control group. This could be due to the better nutritional control such as caloric control, supplementation with monosaturated fats and regime of the management of diabetes by pharmacological agents[16] which could lead to better lipid profiles in the diabetic patients in the present study.

According to a study, the association between dyslipidemia with hypertension may be due to the genetic predisposition, fatty food consumption, saturated fat and cholesterol in the food[17]. In the present study this could be the reason for the serum TC and LDL to be significantly higher and the serum HDL and VLDL to be moderately higher in the study group as compared to the control group. The possibility of antihypertensive drugs adversely affecting other risk factors, such as plasma lipids and lipoproteins have been studied which showed that individuals with untreated high blood pressure probably had a plasma lipid or lipoprotein cholesterol abnormality that may be aggravated by antihypertensive therapy[18]. In the present study, the study group included the subjects with HTN under medication for the same; this could be the reason for serum TC and LDL to be significantly higher and the serum HDL and VLDL to be moderately higher in the study group as compared to the control group. However these positive results could also be affected due to factors like nutritional, physiological, or pathological conditions such as diabetes, which may be concomitantly present and may thus make the subjects conducive to lipemia[19].

The values of saliva lipid profile were compared between the study group and the control group. In the saliva, it was observed that all the lipid profile values were almost similar between the study group and the control group.

Various studies suggested that lipids in saliva exist in a different state of aggregation from lipids in blood or lymph. This could be responsible for the statistically insignificant difference between the saliva lipid profile of the study group and the control group in the present study. The results of the present study may be altered due to the presence or absence of calculus in the subjects as reported by a previous study that stated that the subjects with saliva of reduced calculus deposits contained about 50 per cent less lipids than that subjects with increased deposits of calculus[20]. In the present study the periodontal status and calculus levels were not considered. Another study has reported a tendency to increased cholesterol in saliva (especially when acidic) of caries and erosion susceptible individuals[2]. This could be the cause of statistically insignificant difference between the study group and the control group with respect to all the saliva lipid profile values in the present study.

In the present study, in the study group and the control group the correlation between the values of serum and saliva lipid profile were analyzed, and further, in the DM group and the HTN group the correlation between the values of serum and saliva lipid profile were analyzed.

A study reported that, in healthy adults, saliva cholesterol concentration reflected serum concentration to some extent and could be used to select individuals with high serum cholesterol levels[5]. Yet another study stated that all the lipid values found in saliva followed similar values in the serum but in smaller values[3]. In accordance with the above studies, the present study showed that in the study group there was a strong correlation of TC values and VLDL values and moderate correlation of HDL and LDL values. In the control group there was a very strong correlation of TG values, a strong correlation of TC values, moderate correlation of LDL values and VLDL values. In the DM group there was strong correlation of TC values, LDL values, and VLDL values. In the HTN group there was a very strong correlation of HDL and VLDL and a strong correlation of LDL and TG.

The results of a previous study have reported that a weak correlation between serum and salivary cholesterol concentrations[5] in contrary to the results of the present study. There was also a weak correlation of TG values and a very weak correlation of TC/HDL in the study group between serum and saliva lipid profile

values, moderate correlation of TC/HDL ratio and a weak correlation of HDL in the control group between serum and saliva lipid profile values, a weak correlation of HDL values and TG values and very weak correlation of TC/HDL ratio in the DM group between serum and saliva lipid profile values, and moderate correlation of TC and a weak correlation of TC/HDL ratio in the HTN group between serum and saliva lipid profile values.

CONCLUSION

To summarize, the results of the present study showed a good correlation mainly between serum and saliva LDL and VLDL values in the study group and the control group. Thus according to the present study, though the comparison of the serum and saliva lipid profile values in the study group was not statistically significant, there was a good correlation between the serum lipid profile and the saliva lipid profile. This indicates that as lipid profile values increase in the serum, there was a likely increase in the saliva lipid profile values or vice versa.

However, various factors can affect the lipid profile estimation in the saliva such as smoking, periodontal status, calculus, nutritional status and medications. A better assessment of saliva lipid profile values in the high risk subjects can be made when all these factors are taken into account.

Saliva may thus find its place as a screening tool for monitoring of the lipid profile, at frequent intervals without time consuming and discomforting clinical procedures along with the delight of saving some of the many needle pricks.

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