A STUDY OF SERUM SIALIC ACID LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND ITS RELATION TO LIPID PARAMETERS

Bindu Gopinathan Nair[1], Fathima Beevi Osman[2]

[1]Associate Professor, Department of Biochemistry, Government Medical College, Thiruvananthapuram.
[2]Associate Professor, Department of Biochemistry, Government Medical College, Thiruvananthapuram.

ABSTRACT

BACKGROUND

Sialic acid, an acetylated derivative of neuraminic acid, is reported to be a marker of acute phase response. Elevated serum total sialic acid concentration may reflect the existence or activity of an atherosclerotic process.

The aims and objectives of the current study is to evaluate serum total sialic acid and various lipid parameters in patients with type 2 diabetes mellitus and to examine the relationship between serum total sialic acid with serum lipids which strongly influence the occurrence and advance of atherosclerosis.

MATERIALS AND METHODS

The study group consisted of 40 type 2 diabetic patients and 40 healthy controls between the age group of 20 and 60 years. The serum total sialic acids were analysed by the thiobarbituric acid assay of Warren. Fasting plasma glucose and fasting lipid profile were measured by commercially procured kits in a fully autoanalyser. Statistical analysis was done using SPSS version 22.0. Quantitative data was expressed as mean and SD and quantitative data for the two groups were compared with unpaired 't' test. Association between total sialic acid and lipid parameters was assessed by Pearson correlation. $p<0.05$ is considered significant.

RESULTS

Type 2 diabetic mellitus subjects had a higher total sialic acid levels compared with the controls. The mean serum total sialic level in non-diabetic controls was 55±4.47 mg/dL while in diabetic patients it was found to be 67.68 ± 6.64 mg/dL. Mean total sialic acid was found to be significantly higher than that of the control group ($p <0.001$). Also there was a significant difference in fasting blood sugar and lipid parameters between the two groups ($p<0.05$). Serum Total Sialic Acid correlated significantly with fasting blood sugar, total cholesterol, triglycerides, systolic and diastolic BP ($p<0.001$).

CONCLUSION

In this study, serum total sialic acid which is an inflammatory marker was increased in type 2 diabetes mellitus and significantly correlated with various lipid cardiovascular risk factors. Thus, it contributes to the cardiovascular risk.

KEYWORDS

Type 2 diabetes Mellitus (Type 2 DM), Total Sialic Acid, Coronary Artery Disease (CAD), Acute Phase Response.

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BACKGROUND

Atherosclerosis is the major cause of morbidity and mortality all over the world and its most common manifestation is Coronary Artery Disease (CAD) or Coronary Heart Disease (CHD). Diabetes mellitus patients have two times the incidence of CAD as compared to non-diabetics. Both type 1 and type 2 diabetes mellitus are powerful and independent risk factors for CAD, stroke and peripheral arterial disease. Atherosclerosis accounts for virtually 80% of all deaths among diabetic patients. Prolonged exposure to hyperglycaemia is recognised as a major risk factor in the pathogenesis of atherosclerosis in diabetes.[1]

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Corresponding Author:
Dr. Bindu Gopinathan Nair,
Associate Professor,
Department of Biochemistry
Government Medical College,
Thiruvananthapuram.
E-mail: drbindu2014@gmail.com
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Total serum sialic acid concentration has recently been shown to be a strong predictor of cardiovascular mortality in the general population.[2] Because death from cerebrovascular and coronary heart disease is about two to four times as high in diabetes as in non-diabetic subjects,[3] there is a strong case for examining serum sialic acid levels in people with diabetes and comparing these with concentration in normal subjects. Sialic acid is a generic term for the N- or O-substituted derivatives of Neuraminic acid, a monosaccharide with nine carbon backbone. Sialic acid presents in the terminal sugar moiety of oligosaccharide side chains of various tissue glycoproteins and glycolipids, which act as cofactors of various cell surface receptors and these are found to be increased in various pathological states such as diabetes mellitus. Asian Indians are reported to have increased levels of acute phase proteins in the diabetic state itself even before the occurrence of any specific vascular complications.[4] Sialic acid is a constituent of acute phase reactants and several acute phase proteins are elevated in diabetes mellitus.[5] In human plasma a large quantity of sialic acid is found in orosomucoid, alpha antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, complement proteins and transferrin. Some of these sialylated glycoproteins are acute phase reactants and such substances rapidly increase in concentration after the onset of an inflammation reaction or injury.[6] Since sialic acid

is negatively charged it stabilises glycoprotein conformation in cell surface receptor to increase cell rigidity. This enables signal recognition and adhesion to ligands, antibodies, enzymes and microbes. Since sialic acid is a component of cell membrane elevated levels of sialic acid may indicate excessive cell membrane damage to the cells of vascular tissue.

A patient with diabetes mellitus invariably presents with jeopardy in lipid metabolism which results in elevation of free fatty acid and triglycerides in blood. Several population based studies conducted across the world have clearly demonstrated that an elevated serum sialic acid level is linked directly to various cardiovascular risk factors like increased body mass index (BMI) and lipid profile levels in diabetic subjects suggesting the potential use of this parameter as a marker for cardiovascular disease in diabetes mellitus.[7] The present concept is that atherosclerosis is an inflammatory disease and several population studies have pointed to serum sialic acid as a marker of inflammation in atherosclerosis. Since endothelial activation is in part mediated by dyslipidaemia it is possible that there may be an association between dyslipidaemia and serum sialic acid. The objective of this study is to evaluate serum total sialic acid and various lipid parameters in non-insulin dependent diabetes mellitus patients and to examine the relationship between sialic acid and serum lipids which may strongly influence the occurrence and advance of atherosclerosis.

MATERIALS AND METHODS

A case control study was conducted during June 2012 to September 2013. The study group was selected from the patients attending the Diabetic Clinic and Medical OP in Government Medical College Hospital, Thiruvananthapuram. The study group consisted of 80 individuals which included 40 diabetic patients and 40 healthy controls between the age group of 20 and 60 years. The diabetic group consisted of 23 males and 17 females.

The classification of subjects to Diabetic and Non-Diabetic groups was based on American Diabetic Association Criteria 2011. Pregnant ladies, patients on insulin, smokers, alcoholics, tobacco users, patients with hypertension, type 1 diabetes and other systemic illness were excluded from the study. This study was undertaken after approval by the institutional ethical committee according to the provisions of Helsinki declaration. A written informed consent was obtained from all participants. Blood pressure was recorded in the sitting posture using sphygmomanometer, from the right arm of the individual. Height was measured with the individual wearing no footwear. Weight was measured using Tanita fat monitor. Body mass Index (BMI) was calculated using the formula, BMI= weight in kilograms divided by the square of height in metres (Kg/m^2).

Collection of Blood Samples

6 mL of venous blood was drawn after 12 hours fasting and collected into two tubes. One tube containing sodium chloride and potassium oxalate for plasma glucose estimation and the other was a plain tube.

Plasma was separated from the first tube and used for measuring plasma glucose with a commercial Kit by GOD-POD method on a fully autoanalyser by Transasia Biomed.

The blood in the plain tube was allowed to clot to separate serum. The serum was used to measure fasting lipid profile and serum total sialic acid using commercially procured kits in a fully autoanalyser by Transasia Biomed. High density lipoprotein (HDL) cholesterol was measured after precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol with phosphotungstic acid and magnesium. LDL Cholesterol was calculated by Friedewald formula.

LDL Cholesterol = Total Cholesterol - (HDL cholesterol + Triglycerides/5)

Sialic acids were analysed by Thiobarbituric acid assay of Warren.[8] Periodate oxidation of deoxy sugars was carried out in strong acid solution and a final coloured product formed by coupling of the product of oxidation and thiobarbituric acid. The periodate oxidation product was β-formyl pyruvic acid. This coloured compound was extracted in cyclohexanone and optical density was read in a spectrophotometer (Systronics) at wavelength 532 nm and 549 nm.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0. Quantitative data were expressed as mean and standard deviation. Qualitative data were expressed as proportion. Quantitative data for the two groups were compared with unpaired t test. One way ANOVA was used to compare the quantitative data for more than two groups. Association between two quantitative variables was analysed by Pearson Correlation. p value <0.05 was considered as statistically significant.

RESULTS

The findings in diabetic patients and control subjects are summarised in Table 1. The mean age of the type 2 diabetic subjects is 47.55±5.09 years and that of control group is 48.35±4.59 years. Mean serum total sialic acid level in nondiabetic controls was 55±4.47 mg/dL while in diabetic patients it was found to be 67.68±6.64 mg/dL. Increase in total sialic acid was found to be highly significant ($p<0.001$) (Figure 1). The mean fasting blood glucose level in non-diabetic controls is 80.88±11.95 mg/dL while in diabetic subjects it is 143.58±37.45 mg/dL. The difference is statically significant $p<0.001$. There was also a significant difference in the total cholesterol, triglycerides and LDL cholesterol and both systolic and diastolic blood pressure between the two groups ($p<0.001$).

HDL cholesterol was lower in the diabetic group compared to the control group. Of the 40 diabetic subjects, 10 (25%) were treated by dietary adjustment alone, 13 (32.5%) were treated by oral hypoglycaemic agents and 17 subjects (42.5%) were treated with insulin (Table 2 and Figure 1). It was found that the difference in serum total sialic acid was not statistically significant.

Serum total sialic acid correlated significantly with fasting blood sugar, total cholesterol, triglycerides, systolic and diastolic BP ($p<0.001$) and LDL cholesterol ($p<0.05$). Serum TSA negatively correlated with HDL cholesterol ($p<0.05$) (Table 3).

Para-meters	Case (N=40)		Control (N=40)		T	p
	Mean	SD	Mean	SD		
Age in years	47.55	5.09	48.35	4.59	-.738	.463
Body Mass Index (Kg/m²)	24.95	2.48	24.55	2.73	.686	.495
Total Sialic Acid (mg%)	67.68	6.64	55.00	4.47	10.019	<0.001
Fasting Blood sugar (mg%)	143.58	37.45	80.88	11.95	10.087	<0.001
Total Cholesterol (mg%)	216.72	38.79	179.93	326.97	4.924	<0.001
HDL Cholesterol (mg%)	45.75	8.39	49.53	8.68	-1.978	.051
Triglycerides (mg%)	160.28	81.68	99.23	26.36	4.499	<0.001
LDL Cholesterol (mg%)	139.03	35.65	110.90	28.98	3.872	<0.001
Systolic BP (mmHg)	127.98	10.13	116.75	11.83	4.557	<0.001
Diastolic BP (mmHg)	82.85	6.39	77.80	5.90	3.673	<0.001

Table 1. Summary of Data in Diabetic and Control Subjects

Treatment Groups	N (%)	Total Sialic Acid (mg%)		F	P
		Mean	SD		
Diet	10 (25%)	67.80	5.613	3.127	0.056
Oral hypoglycaemic	13(32.5%)	70.92	8.371		
Insulin	17 (42.5%)	65.12	4.649		
Total	**40 (100%)**	**67.68**	**6.639**		

Table 2. Comparison of Total Sialic Acid levels in Different Groups of Treatment

Correlation of TSA (mg/dL) with other Parameters	Pearson Correlation R	P
Age	.006	.958
Body mass index	.081	.474
Duration of illness in years	-.100	.614
Fasting Blood sugar (mg%)	.647**	<0.001
Total Cholesterol (mg%)	.424**	<0.001
HDL Cholesterol (mg%)	-.225*	.045
Triglycerides (mg%)	.404**	<0.001
LDL cholesterol (mg%)	.338**	.002
Systolic BP (mmHg)	.479**	<0.001
Diastolic BP (mmHg)	.382**	<0.001

Table 3. Correlation of TSA (mg/dL) with Other Parameters

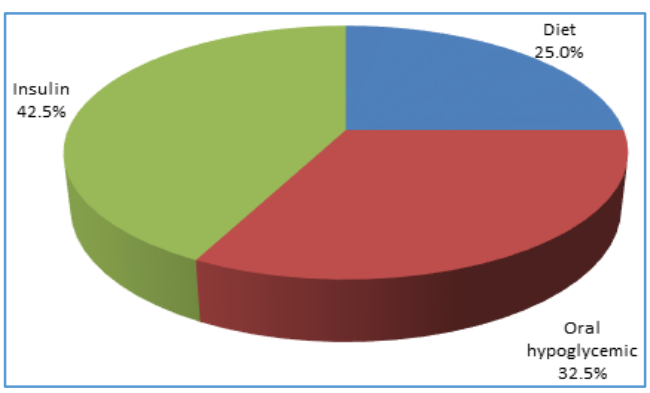


Figure 1. Distribution of Treatment Groups

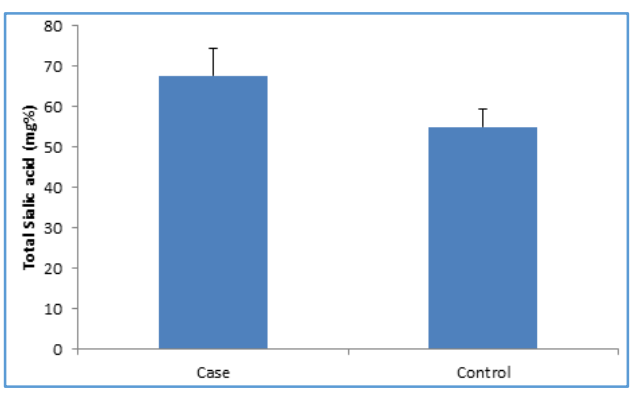
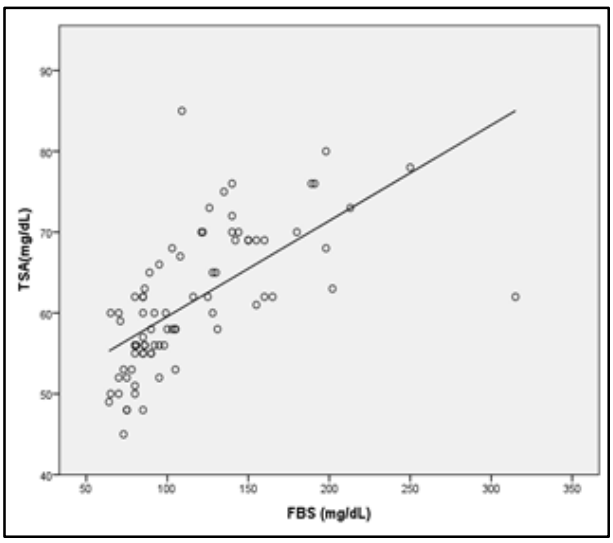


Figure 2. Comparison of Total Sialic Acid between Cases and Controls



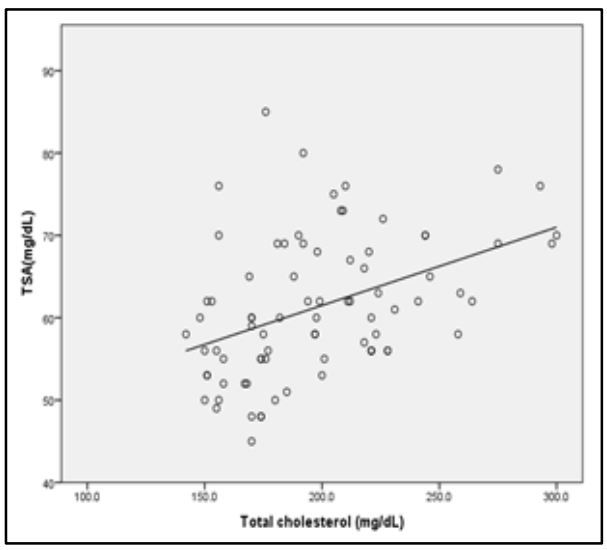
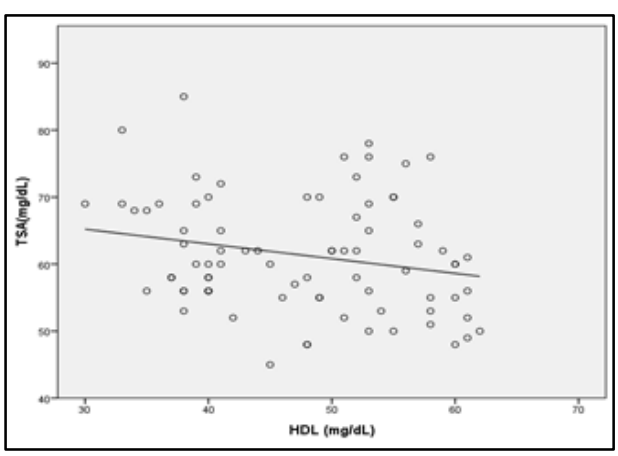
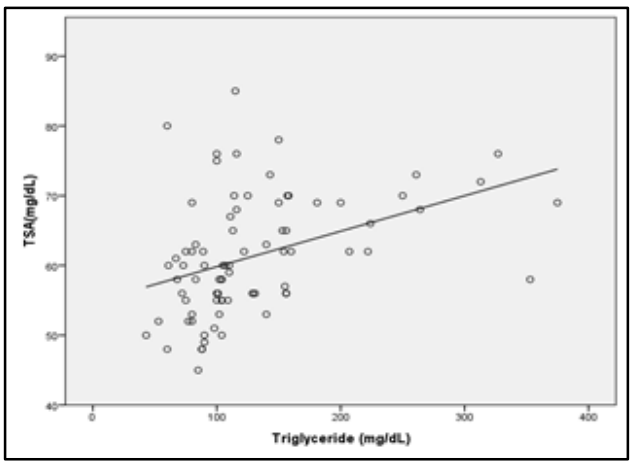


Figure 3. Correlation of Total sialic acid with other Parameters





TSA – Total Sialic acid

DISCUSSION

Coronary artery disease (CAD) remains a major health problem in every country. CAD is assuming epidemic proportions in the Indian sub-continent. Individuals with diabetes have greatly increased CAD risk compared with non-diabetic individuals.[9] In this study, various lipid and non-lipid parameters were studied in diabetes which is a high risk group of CAD. The lipid parameters studied were total cholesterol, triglycerides, LDL and HDL cholesterol. The non-lipid parameters studied were total sialic acid, fasting blood sugar, body mass index (BMI) and BP. The mean Total Sialic Acid in the control group was 55.0±4.47 mg% which is similar to the results of Singh and Ramraj, 1967. Their study showed serum total sialic acid level to be 56±5 mg% in normal Indian adults by Thio Barbituric Acid method.[10]

Serum total sialic acid was significantly increased in the diabetic group compared to the control group ($p<0.001$). This is similar to the earlier studies by Balu Mahendran et al, 2013; Ekin et al, 2003; Melidonis et al, 1988; Crook et al, 1993.[11 - 13] Elevated level of serum sialic acid is a risk factor for overall mortality in type 2 diabetes mellitus patients. Serum sialic acid is a marker of acute phase response.[14, 15] There is an association between type 2 DM and increased total sialic acid, which is possibly due to generalised endothelial dysfunction or macrovascular disease. This is either through loss of sialic acid containing glycoproteins from vascular cells into the blood stream or through an acute phase response. Type 2 DM may be considered as acute phase disease because the serum levels of acute phase proteins have increased without tissue complications.

In our study, we found higher sialic acid levels in diabetic patients treated with oral hypoglycaemic agents compared to those treated with diet alone or insulin, Table 2. This is similar to study by other workers. However, ours was also a study of a relatively small group of patients. So it would be unjustified to draw conclusions about the effects of type of treatment on sialic acid concentrations. Therefore, study involving a larger group of type 2 DM subjects is required. Furthermore, a good correlation was observed between sialic acid and important cardiovascular risk factors such as cholesterol and triglyceride ($p<0.001$) and LDL ($p<0.05$). This is similar to the study by other workers.[16] The correlation between total sialic acid and serum lipids may be due in part to sialylation of lipoproteins.[17] The dyslipidaemia, common in

type 2 DM, is also a feature of natural and experimental acute phase reactions.[18] A study by Lindberg et al, 1993 proves that serum sialic acid, which can be considered as an integrated marker of the levels of several acute phase proteins, predicts CHD.[19]

Russel Ross 1999 has described that the lesion of atherosclerosis represents a series of highly specific cellular and molecular responses that can best be described in aggregate as an inflammatory disease.[20] Activation of innate immunity (Low grade inflammation) is a risk factor for cardiovascular disease mortality in type 2 DM independently of other known risk factors.

Activation of the innate immune system predicts type 2 DM, it may be a common antecedent of type 2 DM and Cardiovascular disease.[21] Thus, chronic activation of the innate immune system which produces the acute phase response has been postulated to lead to insulin resistance, abnormalities in glucose intolerance and lipid metabolism as well as to endothelial activation, resulting in increased vascular tone and atherosclerosis.[18]

CONCLUSION

Since inflammation is involved in the pathogenesis of diabetes and atherosclerosis, measurement of inflammation sensitive markers may be useful for assessment of the cardiovascular risk in diabetic patients. In this study, serum total sialic acid which is an inflammatory marker is increased in diabetes and is significantly correlated with various lipid cardiovascular risk factors. In conclusion, this preliminary study shows that serum sialic acid may be important in diabetes and should be studied in larger groups of diabetic patients.

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