Original article:

A study of serum sialic acid in non insulin dependent diabetes mellitus

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Abstract:

Background and objectives: Diabetes mellitus is the most common endocrine disorder, the prevalence of which is rising alarmingly in India. Serum sialic acid, an acute phase reactant is found to be increased in various conditions like diabetes mellitus, cardiovascular diseases, cancer etc. In diabetes mellitus, acute phase reactants are considered as the indicators of microvascular angiopathy. Microalbuminuria is a predictor of incipient nephropathy and coronary vascular disease in the diabetic patients. Therefore our study was undertaken to understand the association of serum sialic acid levels in incipient diabetic nephropathy patients and to assess the correlation of serum sialic acid with glycemic control.

Methods: Present study involved 90participants of which 60 were non insulin dependent diabetes mellitus (NIDDM) patients studied for their serum sialic acid, fasting blood glucose and serum creatinine levels. 30 non diabetic age and sex matched healthy subjects were taken as a control group. Blood samples were drawn under aseptic precautions from study subjects. The values were tabulated for cases and controls.

Results: serum sialic acid concentrations found to be elevated in NIDDM ,compared to controls(1.798 ±0.24 mmol/l) whereas serum creatinine shows significant increase only in NIDDM

Conclusions: The study concludes that elevated serum sialic acid levels are strongly associated with the progression microvascular complications such as of diabetic nephropathy. Serum sialic acid can be used as a marker of renal dysfunction in various stages of diabetic nephropathy.

Key words: FBS, Creatinine Sialic acid; Diabetes mellitus

Introduction:

Diabetes mellitus is a metabolic disorder of multiple etiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1]

Type2 Diabetes mellitus is the predominant form of diabetes worldwide, accounting for 90%of cases globally[2] It is not a single disease entity but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia.[3] All forms of diabetes, both inherited and acquired, are characterised by hyperglycemia, a relative or absolute lack of insulin, and development of diabetic specific microvascular pathology in retina, renal glomerulus, and peripheral nerve.[4]

International diabetic federation (IDF) estimates the total number of diabetic subjects in India to be around 40.9million and this is further set to raise to 69.9million by the year 2025.[5] Large prospective clinical studies show a strong relationship between glycemiaand diabetic microvascular complications in both type1 diabetes mellitus and type2 diabetes mellitus. There is a continuous, though not linear, relationship between level of glycemia and risk of development and progression of these complications.[4]

It has been proposed that inflammatory process play an important role in the developmentof diabetes and its late complications. Various acute phase reactants are being studied in diabetic process as indicators or predictors of diabetic microvascular complications.[6]

Serum sialic acid is a newly established potent risk factor for the development of micro and macro vascular complications of diabetes.[7] Serum sialic acid is a component of glycoproteinssuch as acute phase proteins and several serum acute phase proteins are elevated indiabetes.[8]

The study was undertaken to correlate serum sialic acid which is the marker of early renal damage to estabilish the role of estimation of sialicacid in NIDDM.

Materials and methods:

Setting:

A Case control study was conducted during march2013-September 2014, in the Department of Clinical Biochemistry, Osmania General Hospital, Hyderabad; Telangana.

Sources of Samples and Data;

- The established NIDDM patients attending the OPD of Osmania General Hospital, and Department of Clinical Biochemistry Osmania General Hospital, Hyderabad.

Control group :

Consists of Age and gender matched healthy controls. None of the patients had a history of Diabetes mellitus, Hypertension, Hepatic, Renal, any other systemic illnesses. Also they were judged to be free of any illness by clinical examination.

Cases:

The diabetic patients:

- Age between 40 to 60 yrs of Both Genders.
- The Classification of Subjects in to Diabeticand Non-Diabetic Groups was based on American Diabetes Association Criteria 2011.

Exclusion Criteria:

- Type 1 diabetic cases.
- Urinary albustic positive cases ofNIDDM
- Cases with inflammatory disorders like eczema,seconderyhyperglycemic states like hypothyroidism,
- Proteinuric conditions like congestive cardiac failure, renal failure, and pregnancy.
- Female patients with menstrual disorder
- Cases with severe combined immune deficiencies.
- Study Grouping
- All the study subjects (60 cases + 30 controls) / participants were explained the nature of the study. Informed consent was obtained from all the 90 subjects.
- The study sample
- In the present study 90 subjects were selected and divided into two age and sex matched type2 diabetic cases and non diabeticcontrol groups.
  Sample collection:

- Participants were in overnight fasting status. They were in supine position for 5 to 10 minutes before venipuncture and 3ml to 4ml venous blood was drawn and collected into three tubes. One containing sodium fluoride and potassium oxalate (grey top), and the other was a plain tube (red top). The blood in plain tube was allowed to clot to separate serum. Serum, plasma was separated within one hour after sample collection. Care was taken to avoid Hemolysis. All icteric, hemolysed samples were ignored .Serum for other parameters was stored at -20ºC.
- Blood samples were analysed for fasting blood glucose, serum creatinine,and serum sialicacid.
- Samples from all subjects were analyzed for the following parameters:
- 1. Plasma fasting Blood Sugar
- 2.serum creatinine
- 3.serum sialic acid

ESTIMATION PROCEDURES FOR ANALYTES:

GLUCOSE: (GOD-POD METHOD)

Method: GLUCOSE OXIDASE PEROXIDASE METHOD.

ESTIMATION OF SERUM CREATININE:

Method: modified jaffe's reaction

ESTIMATION OF SERUM SIALIC ACID:

Method: MODIFIED THIOBARBTURIC ACID ASSAY OF WARREN (Lorentz and Krass)

Type 2 diabetes usually develops in obese patients who are over 40 years old. Its pathogenesis involves a combination of insulin resistance and impairment of insulin secretion. Insulin resistance in several tissues like skeletal muscle, adipose tissue and liver leads to increased insulin secretion from pancreas. This compensatory hyperinsulinemia maintains glucose levels within normal range but individual is at high risk of developing diabetes. Beta cell function eventually declines and leads to development of impaired glucose tolerance and eventually overt diabetes mellitus.[11]

Environmental influences, such as dietary habits and sedentary life styles, clearly have a role which becomes evident when obesity is considered. Genetic factors are even more important in type 2 than in type 1 diabetes.[12]

Obesity and insulin resistance

Insulin resistance is the link between obesity and diabetes. The risk for diabetes increases as the body mass index (a measure of body fat content) increases, suggesting a dose response relationship between insulin resistance and body fat.[12]

Diabetes mellitus is a metabolic disorder of multiple etiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. [1]

Results:

The present study was undertaken in the Department of Biochemistry, Osmania Medical College and Osmania General Hospital, Hyderabad.

The following parameters were analysed.

1. Fasting plasma glucose
2. Serum creatinine
3. Serum sialic acid

The results were expressed in milligrams /deciliterfor Fasting plasma glucose, Serum creatinine, mmol/lit for serum sialicacid.

The data was analysed using GraphPad Prism software version 6.0.

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Descriptive results are expressed as mean and SD of various parameters in different groups.

Student's t-test was used for testing the significance difference in mean scores of various bio-chemical parameters between case and control groups. Results indicated that the mean scores on various biochemical parameters differ significantly between cases and controls.

Pearson correlations were computed to see the association between different biochemical parameters for case and control groups. Significance of the correlations was indicted with (*) for $p < 0.05$ and (**) for $p < 0.01$.

The statistical significance was set at minimum 5 percent ($p < 0.05$). Results were represented in the form of tables and bar diagrams.

Table 1: mean and sd values of parameters in three groups

Parameters	FBS		Sr.creatinine		Serum sialicacid	
	mean	Sd	mean	s.d	mean	s.d.
NIDDM patients	145.4	40.24	1.206	0.2834	2.794	0.2122
Controls	88.90	10.53	0.9445	0.1402	1.798	0.2402

Table 1 showing mean ± sd of fasting plasma glucose, serum creatinine, and serum sialic acid levels in controls and NIDDM patients . All parameters were found to be increased in NIDDM patients

Table 2: Comparision of p values between

Parameter	Controls	NIDDM patients
FBS	< 0.0001	< 0.0001
Serum creatinine	0.2917	0.0759
Serum sialicacid	< 0.0001	< 0.0001

Table 2 shows serum sialic acid levels showed significant difference between controls and NIDDM patients

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Table 3:Pearsons Correlation between different parameters in control group

Parameter	FBS	SR.CREATININE	SERUM SIALICACID
FBS		0.142	0.126
		0.446	0.499
SR.CREATININE	0.142		-0.037
	0.446		0.842
SERUM SIALICACID	0.126	-0.037	
	0.499	0.842	

Table 4 .Pearsons Correlation between different parameters in NIDDM patients

Parameter	FBS	SR.CREATININE	SERUM SIALICACID
FBS		0.111	0.219
		0.559	0.244
SR.CREATININE	0.111		0.360
	0.559		0.050
SERUM SIALICACID	0.219	0.360	
	0.244	0.050	

In table 4 shows there is significant positive correlation between serum sialicacid and Creatinine excretion ($p<0.0001$ and r 0.71); serum sialic acid and serum creatinine levels (p 0.05 and r 0.36).

Discussion:

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.

Sialic acid acts as a cofactor of many cell surface receptors and positivelyassociated with most of the serum acute phase reactants.Sialic acid can be used as a marker of acute phase response as many proteinsof immune response are actually glycoproteins which have sialic acid as the terminalsugar on their oligosaccharide chain.

Serum sialic acid concentration is elevated in pathological states when there isdamage to tissue, tissue proliferation and inflammation. Sialic acid regulatesvascularpermeability. The vascular endothelium carries a high concentration of sialic acidhence extensive microvascular damage associated with NIDDM result in its sheddinginto the circulation. This leads to an increase in vascular permeability and increased serum sialic acid concentration.

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In the present study the mean serum sialic acid values were 1.798±0.240 in controls and 2.247±0.243 in normoalbuminurics of NIDDM. In the study of crook and associates found that elevated plasma sialicacid concentrations were associated with the presence of microvascular complications in large group of type1 diabetes subjects. Plasma sialic acid concentration was associated with several known risk factors for the development of diabetic micro and macro vascular disease, i.e., diabetes duration, glycemic control(HbA1c), hyperlipedimia, waist hip ratio, hypertension and smoking and low level of physical exercise.

Research studies have shown that the concentration of sialicacid is serum is elevated in pathological states when there is tissue damage, tissue proliferation and inflammation. Studies have also indicated that vascular permeability is regulated by sialic acid moieties. The vascular endothelium carries a high concentrations of sialicacid and hence extensive microvascular damage associated with non insulin dependent diabetes mellitus (NIDDM), could account for its shedding into the circulation leading to an increase in vascular permeability and overall increased serum sialic acid concentrations.

Tissue injury caused by diabetic vascular complications stimulates local cytokine secretion from cells involved in the complications such as endothelium and macrophages, which are known to be major sources of cytokine production and this induces an acute phase response. The diabetic process stimulates cytokine production from cells throughout the body, and these cytokines play a direct role in the causation of vascular complications.

In the study of Chen and associates, serum sialic acid levels found to be elevated in NIDDM patients. The mechanisms for the increased concentration of serum sialic acid in NIDDM patients with and without diabetic nephropathy are unknown, but elevated synthesis, reduced catabolism, or both must be present. Sialic acid usually occurs as a terminal component of glycoproteins and glycolipids. Consequently, we can rule our reduction in glomerular filtration as a cause of increased sialic acid concentration as also demonstrated in the present study. In humans, a large quantity of sialic acid is found in so-called acute phase reactants, i.e., orosomucoid, α1-antirypsin, haptoglobin, and fibrinogen.

In the study of Prajna, Ashok kumar and Srinidhi increased in sialic acid levels, in type 2 diabetics without any complications and type 2 diabetics with nephropathy, when compared to controls and they were statistically significant. Tissue injury caused by diabetic vascular complications stimulates localcytokine secretions from cells involved in the complications such as macrophages andendothelium. This induces an acute phase response which involves the release ofacute phase glycoproteins with sialic acid from the liver into the general circulation again leading to increased serum sialic acid concentrations.

Another possible explanation for the increased serum sialicacid is that there may be a difference in the ratio between the two forms of erythrocyte sialidases which are important in maintaining the viability of the erythrocyte and its survival in the circulating blood.

Melidonis A, Tournis S in their study showed that sialic acid levels werehigher in type 2 DM patients compared to control group. Among diabetic patients,those with signs of nephropathy had higher levels of sialic acid than those without nephropathy.

In another study, done by Shahid SM and Mahaboob T, it was shown thatserum sialic acid levels were significantly increased in both diabetic and diabeticnephropathy patients as compared to controls. This elevation was significantly higherin diabetic nephropathy patients compared to diabetic without nephropathy.

In a study done by Krishnamurthy U, Halyal SS, serum sialic acid levels foundto be elevated in NIDDM patients with microalbuminuric when compared to controlsand demonstrated that there is a progressive rise in serum sialic acid levels withincrease in urinary albumin excretion in NIDDM patients.

M. Usman K., Mansoor A also demonstrated increased serum sialic acidconcentration in NIDDM patients as compared to controls.

In the study of Martin A.Crook, Peter Trutt, John C.Pickup concluded that serum sialic acid levels were significantly elevated in a relatively small group of NIDDM patients and were correlated with hypertension and retinopathy. A larger study of circulating sialic acid concentration as a risk factor for the development or marker of diabetic angiopathy is therefore justified.

Summary and conclusion:

Type2 Diabetes mellitus is the predominant form of diabetes worldwide, and the most common endocrine disorder characterized by metabolic abnormalities and long term complications such as retinopathy, nephropathy and neuropathy. Diabetic nephropathy remains a major cause of morbidity and mortality for thepersons either T1DM, or T2DM. Diabetic nephropathy occurs in about 25-30% of diabetic patients.

The present study was undertaken to study the serum sialic acid levels and to assess whether there is a relationship between sialic acid parameter with FBS and serum creatinine in diabetic patients towards the development of diabetic nephropathy who attended outpatient department at Osmania general hospital were taken for case study. 30 age and sex matched healthy persons were taken ascontrols. A statistically significant difference was observed in values of FBS, serum creatinine, serum sialic acid and controls. In our study positive correlation was observed between serumsialic acid and urinary microalbumin in cases. It was also observed that serum sialicacid concentrations were strongly associated with several risk factors like glycemic status, renal dysfunction (creatinine) and urine albumin eaxcretion for thedevelopment of micro and macrovascular complications. These markers wereclinically correlated with increasing concentration of sialic acid. It is concluded that increase in circulating serum sialic acid is an earlymanifestation of diabetic renal disease (micro vascular complications) and serum sialicacid levels in NIDDM is helpful in assessing the progress of disease and identifying the risk category for complications, such as diabetic nephropathy which are main causes for mortality and morbidity among diabetes mellitus patients. Further studies would be helpful to clarify the role of sialic acid in the pathogenesis of diabetic renal disease.

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