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Correlative analysis of total serum sialic acid with glycemic markers in Type II diabetes mellitus

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Abstract

Background: The present study aimed to evaluate the potential role of sialic acid in type II diabetes mellitus. Diabetes mellitus is characterized by chronically elevated blood sugar levels which are progressively associated with the development of complications. Sialic acid which is N-acetyl neuraminic acid (NANA) as an inflammatory marker can be clinically correlated with glycemic load.

Aim and objectives: The present study was carried out to evaluate the correlation between sialic acid and glycemic load in type 2 diabetes mellitus. Serum total sialic acid was analyzed to find out the correlation between FBS, 2 hours post-prandial blood sugar, and HbA1C.

Material and Method: The present study was a hospital-based cross-sectional study. It included the participation of 441 subjects and 402 healthy controls. Blood sugar analysis was done by estimating FBS, PP_2BS, and HbA1C which was correlated with serum total sialic acid.

Results: A total of 441 diabetes type 2 subjects of which 243 males and 198 females were enrolled. Control includes a total of 402 out of which 256 Males and 146 females. There was a significant correlation and mean difference found between sialic acid and glycemic markers ($P<0.001$) in type 2 diabetes mellitus subjects.

Conclusion: A significantly higher level of sialic acid (NANA) was found in type II diabetics. There was a positive correlation between glycemic markers and serum NANA.

Keywords: Total sialic acid, N-acetyl neuraminic acid (NANA), Glycated hemoglobin (HbA1C), Diabetes mellitus.

Introduction

The progressive rise of diabetes mellitus is a global challenge.[1] Diabetes mellitus is the most common endocrine disorder, the prevalence of which is rising alarmingly in India [2]. Prevalence of type II Diabetes mellitus in India was found to be 40.6 million in 2006, expected to increase to 79.4 million by 2030.**[3]** Elevated chronic hyperglycemia which is a key feature of diabetes mellitus ultimately affects a wide metabolic spectrum. Insulin resistance is the primary cause which results because of either diminished insulin action or secretion[4]. Chronic hyperglycemia progressively leads to macro and microvascular complications.[5]

N-acetyl neuraminic acid is also known as sialic acid, present on cell membranes as a component of glycoprotein. The level of NANA can be influenced by various factors, and elevated levels have been observed in conditions associated with inflammation and endothelial dysfunction, which are processes linked to vascular damage. Increased level of sialic acid indicates possible damage to cell membranes, especially cells of vascular tissue [6]. As a cell surface receptor, NANA is positively associated with most of the serum acute phase reactants.[7] Increased levels of NANA have been correlated with several pathologic conditions such as inflammation and malignancy.[8] In various studies it was observed that elevated levels of sialic acids have been found with the development of diabetes mellitus and related complications.[9]

On doing literature review it was found positive association of sialic acid in type 2 diabetes mellitus. Hence the study was undertaken to find out the correlation of serum sialic acid with blood glucose levels in diabetics and non-diabetics. The present project involves the analysis and correlation of serum total sialic acid with FPG (Fasting plasma glucose), PP_2BS, and HbA1C levels. Results were compared between two groups respectively diabetic subjects and controls.

Study design:

The present study is a hospital-based cross-sectional study. Subjects attending medicine OPD (Outpatient department) and NCD (Non-communicable disease) clinics for 1 year for regular checkups for blood sugar were selected. The WHO guideline for diabetes mellitus was used for the selection of study participants.[2] Prior informed consent was taken before commencing the study. The sample size was calculated according to prevalence of the type 2 diabetes mellitus in the region. This study included 441 subjects with type 2 diabetes of which 243 males and 198 females. Control includes a total of 402 out of which 256 males and 146 females.

Ethical consideration:

Before the commencement of the study permission from the ethical committee of the institute was obtained. The ethical committee approval reference number is "IEC/RESEARCH/3/2019".

Materials and Methods

Consent was taken from all the participants before the project. The selection of the participants is based on the following criteria.

Inclusion Criteria: Patients of the age group of 18-60 years having diabetes mellitus type 2.

Exclusion Criteria:

1. Patients with a history of pre-existing chronic kidney disease, and nephrotic syndrome as these conditions promote vascular discharge of sialic acid from cell membrane to circulation.

2. Patients with metabolic conditions like diabetic ketoacidosis, cerebrovascular accidents, preeclampsia, primary hypertensives, smoking, alcohol intake, and history of cardiac diseases are excluded.
3. Patients with autoimmune diseases were excluded.

Sample collection:

In a plain tube, blood was collected for serum sialic acid estimation. A whole blood sample for HbA1C was collected in an EDTA tube. After overnight fasting of 10-12 hours, a venous blood sample for fasting blood sugar was collected in a fluoride vacuette. A sample for PP_2BS was collected in a fluoride vacuette post 2 hours of meal. Serum was obtained by centrifugation at 3000 rpm for 10 minutes in plain tubes. FBS and PP_2BS were estimated by using a kit based on the glucose oxidase peroxidase method on the Vitros 5600 autoanalyzer system. Analysis of serum total sialic acid was done by acid ninhydrin method as given by Yao et al. [11] HbA1C was measured in whole blood by using HPLC techniques on NANO H110 OF LABSYSTEMS.

Parameters assay:

1. Blood glucose: FBS and PP_2BS:

A plasma glucose level of fasting and post-prandial blood sugar (post-meal of 2 hours) was estimated by glucose oxidase peroxidase method using a kit of Vitros dry test system (DTS). The analysis was carried out on Vitros 5600 DTS auto analyzer. Quality control and calibration are done before sample analysis regularly to ensure quality performance. QC was evaluated as CV% and it was 1.2% for intra-assay and 1.5% for inter-assay analysis.

2. Glycated Hemoglobin (HbA1c):

HbA1C was measured in whole blood by using HPLC (High-performance liquid chromatography) techniques on NANO H110 of LABSYSTEMS. Prior analysis of HbA1C instrument standardization and calibration was done.

3. Serum total sialic acid (NANA):

The method used for the serum total sialic acid was as proposed by Yao *et al.* It involves a reaction between sialic acid and ninhydrin in an acidic medium and subsequent color development which is read at 470nm using a spectrophotometer. The method consists following steps.

Preparation of Acid ninhydrin method:

Freshly prepared acid ninhydrin reagent was used in the procedure. 250 mg of ninhydrin was dissolved in 6 ml glacial acetic acid and 4 ml concentrated HCL, by thorough vortexing for 30 min.

Procedure:

Serum samples which were collected as FBS and PP_2BS were diluted respectively with saline by volume of 0.1 ml of serum which was mixed with 0.9 ml of saline. Subsequently, 4 ml of ethanol was added and precipitate is obtained by centrifugation. Later on, with obtained precipitate 1.0 ml of distilled water and 1.0 ml glacial acetic acid was added. In the next step

of protocol addition of 1.0 ml of acid ninhydrin done. The reaction mixture was thoroughly vortexed and then kept in boiling water for 10 minutes. After that the reaction mixture was cooldown under tap water. Absorbance of the reaction mixture was measured at 470 nm using spectrophotometer. Standard graph was obtained by using different concentration of standard of NANA (N-acetyl neuraminic acid). Based on the standard curve the concentration of serum total sialic acid was find out.

Statistical analysis:

An Independent sample t-test was done to see the mean difference between the two groups of measurement scale data. A P-value of less than 0.05 is considered significant at a 95% confidence interval. The Pearson correlation test was applied to find out the correlation between glycemic markers and serum sialic acid. The Graph Pad Prism and MS Excel were used in the analysis.

Results

In the present study, 441 diabetic subjects and 402 control groups were included to evaluate the correlation between glycemic status and sialic acid. The sample was analyzed with respective methods and correlated by appropriate statistical measures. Microsoft Excel and Graph Pad Prison software were used for statistical findings. Mean ± SD was used for the expression of data. The results are shown in Table 1.

Fasting blood glucose:

The analytical correlation of FBS in diabetic subjects and controls is shown in Table 1. The mean and SD of the two groups show significant differences. The obtained results were statistically analyzed and an unpaired student t-test was applied. P value was found to be <0.0001 which shows highly significant results.

Post Prandial blood sugar:

The PP_2BS analysis in diabetic subjects and controls is shown in Table 1. The mean and SD were statistically different. A highly significant difference was observed by applying an unpaired student t-test, P Value was <0.0001.

Serum sialic acid (NANA):

The mean values of serum sialic of the subjects and controls are shown in Table 1. The statistical method unpaired student t-test was applied to find out the correlation. The p-value was found to be <0.0001. A highly significant difference was found in the case group when compared to the control group.

Glycated Hemoglobin (HbA1C):

The statistical correlation of HbA1C between diabetic subjects and controls is shown in Table 1. The mean and SD were statistically different. A highly significant difference was noted by applying the student t-test, which was 28.60, and the Value was found p<0.0001.

The Correlation of fasting blood sugar, post-prandial blood sugar, and serum sialic acid in the diabetic subject group is shown in Table 2. There was a statistical difference found between FBS, PPBS, HbA1c, and sialic acid in diabetic subjects.

Table 1: Comparison of study biochemical parameters between Subjects and controls

Biochemical parameters	Case	Control	T Value	*P* value
FBS (mg/dl)	167 ± 58.14	84±6.4	26.5	<0.0001
PPBS (mg/dl)	240±65	120±9.3	27.4	<0.0001
HbA1C (%)	8.2±1.5	5.2±0.3	28.6	<0.0001
Sialic acid (mg/dl)	105±21	51±6	34.8	<0.0001

Table 2: Correlations of glycemic markers with sialic acids in diabetic subjects.

Categories	t value	*P* value	r value
FBS vs Sialic acid	24.8	<0.0001	0.542
PPBS vs Sialic acid	41	<0.0001	0.563
HbA1C vs Sialic acid	28.3	<0.0001	0.666

Discussion

Key findings:

Among metabolic disorders, diabetes mellitus represents the world's biggest health challenge. Statistical analysis of sialic acid shows a positive correlation with the level of blood glucose. We observed a distinct difference in serum total sialic acid between diabetes type 2 subjects (105±21) and controls (51±6) where P Value <0.0001. The Pearson correlation test r value between FBS and sialic acid was found 0.542 which shows a positive correlation. The r value between PP_2BS and Sialic acid was found 0.563 with a p value<0.0001 which also suggests a positive correlation. There was also a positive correlation between HbA1C and serum sialic acid with r value 0.668.

Sialic acid (NANA) can have an accompaniment role in laboratory investigation related to diabetes mellitus. NANA is considered one of the acute phase reactants.[12] Glomerular basement membrane having a negative charge which is maintained by the sialic acid. [13] It is important for the membrane permeability. Increased vascular permeability with subsequent release of endothelial sialic acid in the circulation leads to increased serum sialic acid levels.[14,15] As a part of the cell membrane sialic acid has an important role in vascular permeability. There is an increased level of sialic acid present in vascular endothelium. Chronic elevated blood sugar may lead to inflammatory conditions and cause possible vascular damage. This in turn causes the release of sialic acid from the cell membrane into the circulation which causes elevated levels of sialic acid.[16]

Limitations

The study conducted here is a cross-sectional study. Further study is required to support the findings related to establishing a sialic acid as a marker in the laboratory investigation of type 2 diabetes mellitus.

Conclusion

From the present study, it was observed that increased levels of sialic acid positively correlated with fasting blood sugar, 2 hours post-prandial blood sugar, and HbA1C. Therefore, sialic acid can be used as a companion marker in type 2 diabetes mellitus.

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Conflict of interest

There are no conflicts of interest.

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