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Relationship between nitric oxide and sialic acid concentrations in south Indian type 2 diabetic patients

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ABSTRACT

Nitric oxide and sialic acid variables are indicators of the acute phase response. This study was planned to investigate the relationship between the level of serum nitric oxide and sialic acid in type 2 diabetes with and without nephropathy. Fasting venous blood samples were taken from 90 subjects of which 30 were of type 2 diabetic patients with nephropathy (group I), 30 type 2 diabetes without any micro vascular complications (group II) and 30 healthy individuals without diabetes (group III). The serum samples were analyzed for serum nitric oxide and sialic acid levels. The higher levels ($p<0.01$) of serum sialic acid were observed in group I and II as compared to controls. Significantly low levels of serum nitric oxide ($p<0.01$) were observed in group I and II patients as compared to controls. Results indicated that variations in the serum nitric oxide and sialic acid are the major biochemical indicators for micro and macro vascular complications of diabetes nephropathy. The present study revealed a progressive increase in serum sialic acid with decreasing nitric oxide concentration in diabetic nephropathy patients. Furthermore, several variable risk factors may associate serum sialic acid and nitric oxide levels of the diabetic patients.

Key words: Diabetic nephropathy, sialic acid, nitric oxide

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by elevation of blood glucose concentration and is associated with increased prevalence of microvascular complications. The two major classes of diabetes are: type 1 diabetes (T1DM) and type 2 diabe-tes (T2DM). T1DM is the classical form of diabetes, treatment this form of diabetes accounts for 5 to 10% of all diabetics and these subjects cannot survive without insulin (1) · T2DM is a group of genetically determined diseases which may be controlled by diet, hypoglycemic agents and/or exogenous insulin (2) . Type 2 diabetes stands for about 85% to 95% of the people with diabetes in developed countries and an even higher percentage in developing countries (3).

In India, the epidemiological data shows an increasing trend in the diabetic population, rising from 19 million in 1995 to 57 million in 2025. Although Type 2 diabetes is widespread and tests to identify it are widely accessible, however the disease remains under diagnosed. Roughly 25% of the people with a new diabetes diagnosis already have microvascular diseases. One of the additional devastating aspects of type 2 diabetes is the numerous complications such as diabetic retinopathy, kidney nephropathy and peripheral neuropathy.

Diabetic nephropathy has been the leading cause of deaths due to end stage renal disease in diabetes that affects more than 40% of diabetic patients (4). Although several factors are involved in the genesis of diabetic nephropathy, endothelial vascular dysfunction antedates the development of nephropathy and appears to contribute to the diabetes associated renal injury (5).

The endothelial dysfunction associated with diabetes has been attributed to a lack of bio-available nitric oxide (NO) (6). NO-dependent vasodilation has been shown to be an important factor in the maintenance and regulation of vascular tone in the renal microcirculation (7). Inhibition or genetic deletion of endothelial nitric oxide synthase (eNOS, NOS III) induces opening of the inter-endothelial junctions and increases vascular permeability in microvascular beds, suggesting that nitric oxide (NO) production may play an important role in regulating the endothelial barrier function (8,9). Functionally significant polymorphisms in eNOS have been recognized in human diabetic nephropathy (10). Evidence that glomerular arteriolar resistances are regulated by basal NO levels is supported by observations of vasoconstriction in afferent and efferent arterioles of both superficial cortical (11) and juxtamedullary nephrons (12) following NO synthesis inhibition.

The serum sialic acid (N-acetyl neuraminic acid) concentration is a marker of the acute phase response, since many of the acute phase proteins (e.g. al-acid glycoprotein, fibrinogen and haptoglobin) are glycoproteins with sialic acid as the terminal sugar of the oligosaccharide chain (13). Circulating serum sialic acid, an inflammatory marker has been shown to be a strong predictor of microvascular mortality (14). Several general population studies and those carried out in diabetic patients with complications have pointed that serum sialic acid as a marker of inflammation (15,16). Sialic acid is basically released from terminal oligosaccharide chain of some glycoproteins and glycolipids of the acute phase of the disease (17).

Approximately 25% of the people with a new diabetes diagnosis already have microvascular disease, suggesting that they already have had the disease for 4–7 years by the time of diagnosis (18, 19). Hence, the present study was undertaken to investigate the function of the biochemical parameters such as serum nitric oxide and sialic acid in the prediction of the microvascular complications in Type 2 diabetes of south Indian population to recognize the development of nephropathy in diabetes as rapid biomarkers.

MATERIALS AND METHODS

2.1. Selection criteria

This study was conducted in Tamilnadu which is geographically southern part of India. Sixty Type 2 diabetic patients who visited the out-patient and in-patient departments of hospitals in various parts of Tamilnadu we recruited in the study and 30 healthy individuals who came for general checkup were considered as the controls. The Type 2 diabetic patients were divided into 2 groups based on the presence and absence of the diabetic complications (Group- I: 30 Type 2 diabetes mellitus patients with nephropathy; Group-II: 30 Type 2 diabetes mellitus patients without microvascular complications). All the patients, as well as the controls, were completely informed about the rationale, the procedures and the hazards of the study. Once taking voluntary informed consent, all the subjects were integrated in the study. The work was carried out in agreement with the ethical standards laid down the 1964 Declaration of Helsinki.

2.2. Sample Collection

Fasting venous blood (5 ml) was collected from all the above-mentioned groups. The samples were centrifuged, separated and stored at 4°C until analysis.

2.3. Serum Sialic Acid

Serum sialic acid was estimated by Ehrlich method (20). Samples of 200 µL were mixed with 400 µL of 02 N H_2SO_4 and incubated in a water bath at 80^{o}C for 1 hour. Then, 1 mL of 10% trichloroacetic acid (TCA) was added and mixed, after which the solution was centrifuged at 3,000 rpm for 5 minutes. The supernatant (500 µL) was collected for analysis. It was diluted with 2 mL of distilled water and a further 500 µL of Ehrlich reagents (2.0 g of p–dimethylaminobenzaldehyde (DMAB) in 50 mL of 95% ethanol and 50 mL of concentrated hydrochloric acid) were added. The solution was boiled in the water bath for 30 minutes. The reaction was stopped by cooling the sample tubes in an ice bath. Absorbance of the color was measured by a spectophotometer (Shimdzu) at 565 nm. The pure sialic acid (20-100 µg/tube) was used as the standard and 0.2 N H2 SO4 - 10% TCA was used as a blank.

2.4. Serum Nitric oxide Estimation

Serum nitric oxide was estimated by the method of Smarason et al., 1997 (21). The level of NO was estimated as nitrite, a NO metabolite, in control and case group samples, because NO is a highly reactive free radical gas that is a ready oxidizer and remains stored in tissues as Nitrates or Nitrites. Thus NO concentration can be estimated by measuring concentrations of Nitrate or Nitrite by Nitrate reductase or metallic catalyst, followed by the calorimetric Griess reaction to measure NO_2 levels. Sample solutions were taken in test tubes and treated with Griess reagent (1% Sulphanilamide, 0.1% Napthylethylenediamine dihydrochloride and 2.5% hydrochloric acid). The calorimetric reaction was allowed to proceed for 10 minutes at room temperature, and optical density was measured at 550nm using a spectrophotometer. The concentrations of Nitrite were calculated from a standard curve established with serial dilutions of sodium nitrite.

2.5. Statistical Analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 12 for Windows. Results are expressed as mean SEM. Statistical significance and difference from control and test values were evaluated by Student's t-test.

RESULTS

The clinical characterization of the study subjects are shown in Table 1. Mean age of Group I, II and III were 41±8, 45.5±6 and 43.5±7 years respectively. The study groups were well matched for age and sex with their respective control groups.

Table 2 shows the comparison of the biochemical markers between the controls and the cases with diabetes (with and without nephropathy). There was a significant increase in sialic acid (2.26±0.25) in cases with diabetes (Group II and III) as compared to that of controls (1.69±0.28) at the p value less than 0.01. But there was significant decrease in nitric oxide concentration in cases with diabetes (15.56±2.488) as compared to that of controls (18.35±2.458) at the p value less than 0.001.

Table 3 shows the relationship between sialic acid and nitric oxide concentrations in diabetic subjects with and without nephropathy complication (Group I and Group II respectively). The table depicts significant increase in sialic acid concentration in Group I (2.35± 0.25) as compared with Group II (2.16±0.46) at the p value less than 0.001. The table shows the significant decrease of Nitric oxide concentration in Group I (13.65±2.473) compared with Group II (16.95±2.467)

DISCUSSION

Diabetes is associated with altered endothelial vascular and inflammatory, acute phase responses. Vascular endothelium is a subject for deregulation, dysfunction, insufficiency and failure in diabetic nephropathy (22). The present study finds support in the observation that diabetes affects basal NO metabolism as a successive and significant decrease was observed in the level of serum NO at the onset of diabetic complication, nephropathy (Table 2). The NO is a paracrine mediator acting as a potent vasodilator in various vascular beds. In the kidney, NO controls both afferent and efferent vascular tone, the ultrafiltration coefficient and medullary blood flow (23). NO is synthesized as a by product of conversion of its physiological precursor L-arginine to Lcitrulline. This reaction is catalyzed by a family of enzymes known as NO synthases (NOS) (24). The decreased production of NO during diabetic complications supposed to be the consequence of reduced production of NO by NOS and inactivation of NO by reactive oxygen species produced either by glycosylated proteins. The present study demonstrates the decreasing trends of serum Nitric oxide in diabetic patients with the progression of diabetic nephropathy.

Inflammation plays a major role in the pathogenesis of type 2 diabetes mellitus and its complications (25). Hence inflammatory markers or acute phase markers have gained the importance as indicators and predictors of diabetic process. Serum Sialic acid is one of the acute phase response markers that are found to be associated with diabetes mellitus (26). The vascular permeability is regulated by sialic acid moieties through shedding of vascular endothelial sialic acid into the circulation. It is well established that vascular endothelium carries a high level of sialic acid (27), and the vascular damage leads to its release into the circulation. Previously researchers found the increased concentration of sialic acid in type 2 diabetes. Similarly we observed a significant rise in serum sialic acid levels in diabetic subjects compared to controls. Likewise, diabetic patients with nephropathy have high concentration of

sialic acid compared to diabetic patients without complications. Therefore, sialic acid may act as an indicator for early diabetic complication process.

Table 1. Clinical characterization of study subjects

Parameters	Group I Diabetes with Nephropathy (n=30)	Group II Diabetes without Nephropathy (n=30)	Group III Controls (n=30)
Sex (M/F)	15/15	15/15	15/15
Age (Years)	45.5±6	43.5±7	41±8
Weight (Kg)	55.6±8.4	58.5±6.8	63.5±6.3
Height (Cms)	153.6±8.5	162.3±14.6	157±24.9
Duration of DM (Years)	14.5±2.4	11.6±3.8	--
Family History (%)	78	74	52

Table 2. Comparison of Serum Sialic acid and Nitric oxide parameters in cases and controls

Biochemical Parameters	Cases (Group I and Group II) n=60	Controls (Group III) n=30	p value
Sialic acid (mmol/L)	2.26± 0.25*	1.69± 0.28*	<0.01
Nitric oxide (µmol/L)	15.56±2.488**	18.35±2.458**	< 0.001

*Mean± S.D, * p value <0.01, ** p value < 0.001, n= number of individuals.*

Table 3. Comparison of Serum Sialic acid, Nitric oxide in Group I1 and Group II

Biochemical Parameters	Group I with Nephropathy (n=30)	Group II without Nephropathy (n=30)	p value
Sialic acid (mmol/L)	2.35± 0.25*	2.16±0.46*	<0.01
Nitric oxide (µmol/L)	13.65±2.473**	16.95±2.467**	< 0.001

*Mean± S.D, * p value <0.01, ** p value < 0.001, n= number of individuals.*

CONCLUSION

In conclusion, the present study suggests that increased serum sialic acid levels and decreased nitric oxide levels are strongly associated with the development of diabetic nephropathy in South Indian diabetic patients. These findings strengthen the hypothesis that an increase in circulating serum sialic acid and nitric oxide is an early manifestation of diabetic renal disease. Further research would be of help to clarify the role of sialic acid and nitric oxide in the development of diabetic renal disease.

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