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Serum Nitric Oxide and Sialic Acid: Possible Biochemical Markers for Progression of Diabetic Nephropathy

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***Abstract*—**This study was designed to investigate the role of serum nitric oxide and sialic acid in the development of diabetic nephropathy as disease marker. Total 210 diabetic patients (age and sex matched) were selected followed by informed consent and divided into four groups (70 each) as I: control; II: diabetic; III: diabetic hypertensive; IV: diabetic nephropathy. The blood samples of all subjects were collected and analyzed for serum nitric oxide, sialic acid, fasting blood glucose, serum urea, creatinine, HbA1c and GFR. The BMI, systolic and diastolic blood pressures, blood glucose, HbA1c and serum sialic acid levels were high ($p<0.01$) in group II as compared to control subjects. The higher levels ($p<0.01$) of BMI, systolic and diastolic blood pressures, blood glucose, HbA1c, serum urea, creatinine and sialic acid were observed in group III and IV as compared to controls. Significantly low levels of GFR and serum nitric oxide ($p<0.01$) were observed in group III and IV as compared to controls. Results indicated that serum nitric oxide and sialic acid are the major biochemical indicators for micro and macrovascular complications of diabetes such as hypertension and nephropathy. These should be taken into account during screening procedures regarding identifications of the diabetic patients to get them rid of progressive renal impairment to ESRD.

***Keywords*—**Diabetic nephropathy, hypertension, nitric oxide, sialic acid.

I. Introduction

DIABETIC nephropathy has been the leading cause of deaths due to end stage renal disease (ESRD) in diabetes that affects more than 40% of diabetic patients [1]. Although several factors are involved in the genesis of diabetic nephropathy, glomerular hyperfiltration with increased intraglomerular pressure antedates the development of nephropathy and appears to contribute to the diabetes-associated renal injury [2]. The endothelial dysfunction associated with diabetes has been attributed to a lack of bioavailable nitric oxide (NO) [3]. NO-dependent vasodilation has been shown to be an important factor in the maintenance and regulation of vascular tone in the renal microcirculation [4]. 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 [5] and juxtamedullary nephrons [6] following NO synthesis inhibition. Both cortical and medullary renal blood flows have been shown to decrease with systemic inhibition of NO in diabetic & non-diabetic rats [7,8].

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 [9]. Circulating serum sialic acid, an inflammatory marker has been shown to be a strong predictor of cardiovascular mortality [10]. Several general population studies and those carried out in diabetic patients with complications have pointed to serum sialic acid as a marker of inflammation in cardiovascular disease [11,12]. Sialic acid is basically released from terminal oligosaccharide chain of some glycoproteins and glycolipids of the acute phase [13].

The current study aimed to point towards the exploitation of serum nitric oxide and sialic acid levels in terms of endothelial dysfunction and vascular damage to identify the progression of nephropathy in diabetes as easy-to-detect and quick biomarkers..

II. Subjects and Methods

A total of 210 patients with type 1 and type 2 diabetes mellitus of either sex admitted in diabetic wards or visiting out patient departments of Civil Hospital Karachi, Jinnah Postgraduate Medical Center Karachi or M.S. General Hospital, Karachi were selected and divided into four groups (70 each). The aim and procedures were explained to patients and/or attendant and informed consent was obtained. The mean age of patients was 54.84±9.75 (mean±SEM) years. Their diabetic age was more than five years. The diagnosis of diabetes was made according to the World Health Organization's (WHO) criteria [14]. The patients suffering from gestational diabetes, any known mental illness, macrovascular disease, endocrinological disorders prior to diagnosis of diabetes mellitus, or patients who refused to participate in the study were excluded. The study protocol was approved by the regulations of institutional ethical committee for the use of human subjects in research. The groups of patients and control subjects were as follows:

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Group I: Non-diabetic, normotensive control subjects.
Group II: Diabetic, normotensive patients.
Group III: Diabetic, hypertensive patients.
Group IV: Diabetic, hypertensive patients with nephropathy.

A structured questionnaire was used to record the demographic characteristics of all subjects. Height and weight were noted for the calculation of Body Mass Index [(BMI=weight in kilograms/height in meters)^2]. Blood pressure was measured with the help of standard mercury sphygmomanometer while the patient was sitting after resting for 5-10 minutes. Hypertension was defined as blood pressure 150/100 mm Hg [15].

The blood samples of patients and control subjects were collected after the patients have been taken no drugs for the last 12 hours or more. An aliquot was taken separately in order to get serum. Blood samples were processed same day for estimations, in accordance with the ethical guidance and regulation of institution and with generally accepted guidelines governing such work. The serum nitric oxide metabolites (nitrate+nitrite) were measured by previously described spectrophotometric method [16]. The serum sialic acid was measured by Ehrlich's method [17]. The fasting blood glucose, serum urea and creatinine were measured by routine spectrophotometric methods. The HbA1c was measured by fast ion exchange resin separation method (Human Gessellschaft fur Biochemica und Diagnostica mbH, Germany). The glomerular filtration rates (GFR) were estimated by modern and well-established equation method [18].

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.

III. Results

The tables I-III summarize the results in terms of mean±SEM. In diabetic patients BMI, systolic and diastolic blood pressures, blood glucose, HbA1c and serum sialic acid levels were found to be significantly high ($p<0.01$) as compared to control subjects (Table I). The BMI, systolic and diastolic blood pressures, blood glucose, HbA1c, serum urea, creatinine and sialic acid levels were observed to be significantly high ($p<0.01$) in diabetic hypertensive patients (Group III) (Table II) as well as in diabetic nephropathy patients (Group IV) (Table III) as compared to control subjects. On the other hand, significantly low levels of GFR and serum nitric oxide ($p<0.01$) were observed in diabetic hypertensive patients (Group III) and in diabetic nephropathy patients (Group IV) as compared to normal control subjects.

TABLE I
SERUM NITRIC OXIDE, SIALIC ACID AND OTHER PARAMETERS IN PATIENTS OF DIABETIC PATIENTS

Parameters	Controls (Group I)	Patients (Group II)
BMI (Kg/m^2)	22.53±2.46	26.21±3.56*
Systolic BP (mm Hg)	125.77±7.88	130.22±4.59*
Diastolic BP (mm Hg)	77.48±3.98	83.78±6.84*
Blood Glucose (mmol/L)	5.35±1.39	8.89±3.50*
HbA1c (%)	4.59±1.29	7.42±2.14*
Serum Urea (mmol/L)	9.86±2.65	9.65±2.02
Serum Creatinine (mmol/L)	110.76±18.86	112.82±14.63
GFR (mL/min)	80.12±6.28	79.93±9.34
Serum Nitric Oxide (µmol/L)	18.13±2.65	17.50 ± 2.90
Serum Sialic acid (mmol/L)	1.69±0.27	1.98 ± 0.25*

n=70
Values are mean±SEM
*$p<0.01$ as compared to control subjects

TABLE II
SERUM NITRIC OXIDE, SIALIC ACID AND OTHER PARAMETERS IN DIABETIC HYPERTENSIVE PATIENTS

Parameters	Controls (Group I)	Patients (Group III)
BMI (Kg/m^2)	22.53±2.46	35.41±3.15*
Systolic BP (mm Hg)	125.77±7.88	140.85±11.45*
Diastolic BP (mm Hg)	77.48±3.98	89.63±6.90*
Blood Glucose (mmol/L)	5.35±1.39	9.58±2.35*
HbA1c (%)	4.59±1.29	8.88±2.43*
Serum Urea (mmol/L)	9.86±2.65	11.25±2.85*
Serum Creatinine (mmol/L)	110.76±18.86	162.88±45.83*
GFR (mL/min)	80.12±6.28	44.36±17.84*
Serum Nitric Oxide (µmol/L)	18.13±2.65	13.01 ± 2.49*
Serum Sialic acid (mmol/L)	1.69±0.27	2.1 ± 0.37*

n=70
Values are mean±SEM
*$p<0.01$ as compared to control subjects

TABLE III
SERUM NITRIC OXIDE, SIALIC ACID AND OTHER PARAMETERS IN DIABETIC HYPERTENSIVE PATIENTS WITH NEPHROPATHY

Parameters	Controls (Group I)	Patients (Group IV)
BMI (Kg/m^2)	22.53±2.46	42.38±5.69*
Systolic BP (mm Hg)	125.77±7.88	156.85±11.29*
Diastolic BP (mm Hg)	77.48±3.98	108.95±9.93*
Blood Glucose (mmol/L)	5.35±1.39	13.78±3.65*
HbA1c (%)	4.59±1.29	11.69±2.84*
Serum Urea (mmol/L)	9.86±2.65	21.76±5.55*
Serum Creatinine (mmol/L)	110.76±18.86	198.46±38.53*
GFR (mL/min)	80.12±6.28	29.53±7.89*
Serum Nitric Oxide (µmol/L)	18.13±2.65	10.99 ± 2.09*
Serum Sialic acid (mmol/L)	1.69±0.27	2.2 ± 0.38*

n=70
Values are mean±SEM
*p<0.01 as compared to control subjects

IV. DISCUSSION

The last 20 years have brought about a lucid realization that the vascular endothelium is not a mere barrier between intravascular and interstitial compartments. In fact, the vascular endothelium has received the status of an organ, albeit a widely spread one, which is responsible for the regulation, hemodynamic, angiogenic vascular remodeling and metabolic, synthetic, inflammatory, antithrombogenic, and prothrombogenic processes. As any other organ, the vascular endothelium is a subject for dysregulation, dysfunction, insufficiency and failure in diabetic nephropathy [19]. Diabetes is associated with altered endothelial vascular and inflammatory, acute phase responses.

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 endothelial NO at the onset of diabetic complications such as hypertension (Table 2) and nephropathy (Table 3). 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 [20]. NO is synthesized as a by product of conversion of its physiological precursor L-arginine to L-citrulline. This reaction is catalyzed by a family of enzymes known as NO synthases (NOS) [21]. The decrease 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 or directly from vascular endothelium as high level of HbA1c was observed in patients of diabetes, hypertension and nephropathy during the present study. However, this only incompletely explains reduced relaxant responses of microvessels to agonists such as bradykinin in the presence of HbA1c [22]. Several mechanisms could account for a reduced responsiveness of the diabetic renal vasculature to NO-dependent vasodilation: 1) inactivation of NO and/or 2) a reduced sensitivity of the vascular smooth muscles cells (VSMC) to NO, 3) diminished autoregulatory adjustment in renal vasculature resistance, 4) baroreflex-mediated alterations in renal sympathetic nerve activity, and 5) increased production of NO antagonists such as endothelin 1, and quenching of NO by AGEs during micro and macrovascular complications [4]. The same effects were also observed during hypertension in normoglycemic patients (Table I) in present study due to variety of factors involved in impairment of NO metabolism.

The present study demonstrates the increasing trends of serum sialic acid in diabetic patients with the progression of complications such as nephropathy (Tables I-III). The results are completely in accordance with the recent studies in same area. A relationship between serum sialic acid and microvascular complication has been observed before in small scale studies for type 1 and type 2 diabetes [11,23].

Serum sialic acid is a marker of acute phase response [24,25]. Acute phase glycoproteins with sialic acid as a component of the oligosaccharide side chain being produced by liver, stimulated by proinflammatory cytokines. Therefore the two most likely explanations for the present findings are either or both of the following:

1) 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 the major sources of cytokine productions [26] and this induces an acute phase response.

2) The diabetic process stimulates cytokine production from cells throughout the body, and these cytokines play a direct role in the causation of vascular complication. The latter is supported by evidence that proinflammatory cytokines cause endothelial dysfunction by increasing capillary permeability, inducing prothrombotic properties and promoting leukocyte recruitment by synthesis of adhesion molecules and chemoattractants [27].

The realization that microalbuminuria is a non specific marker of inflammation in the general population further suggests that cytokinemia from a variety of causes leads to microvascular abnormalities. The need for early predictors of diabetic vascular complications such as nephropathy has recently been reviewed [28]. Some patients with microalbuminuria have quite advanced renal structure changes and microalbuminuria may here be a marker of microvascular damage that has already been occurred [29]. If circulating sialic acid increases before microangiopathy develops, it may be an early signal of processes such as hypercytokinemia that cause or drastically increase the risk of renal failure.

In conclusion, it is evident from the present study that serum nitric oxide and sialic acid are the major biochemical indicators for micro and macrovascular complications of

diabetes mellitus such as hypertension and nephropathy. These should be taken into account during screening procedures regarding identifications of the diabetic patients to get them rid of progressive renal impairment to end stage renal failure.

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