Relationship between serum nitric oxide and sialic acid in coexisted diabetes, hypertension and nephropathy

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Abstract: This study was designed to study the relationship between serum nitric oxide and sialic acid in patients of diabetic nephropathy. Total 210 diabetic patients including 115 males and 95 females, suffering from diabetes and nephropathy (DN) were selected followed by informed consent and approval from institutional ethical committee. Equal number of age and sex matched normal healthy subjects were selected without any known history of hyperglycemia, hypertension and renal insufficiency as controls. Fasting blood samples from patients and controls were collected and analyzed for serum nitric oxide, sialic acid, fasting blood glucose (FBG), serum urea, creatinine, HbA1c and golmerular filtration rate (GFR). The raised levels ($p<0.05$) of systolic and diastolic blood pressures, BMI, FBG, HbA1c, serum urea, creatinine and sialic acid were noted in DN patients as compared to controls. Significantly lower levels of GFR and serum nitric oxide ($p<0.05$) were observed in DN patients as compared to controls. Strong negative correlation was found between serum sialic acid and nitric oxide levels in patients diabetic nephropathy ($p<0.05$). The relationship between the levels of serum nitric oxide and sialic acid may be considered as a strong biochemical indicator for micro and macro vascular complications of diabetes such as hypertension and nephropathy. These parameters 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: Diabetes, nephropathy, nitric oxide, sialic acid.

INTRODUCTION

Diabetes mellitus is associated with a four to eightfold increased risk of macro and micro vascular diseases, compared with the general population (Soedamah-Muthu *et al.,* 2008). 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 (Shahid and Mahboob, 2008). 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 (Omer *et al.*, 1999). The endothelial dysfunction associated with diabetes has been attributed to a lack of bioavailable nitric oxide (NO) (James *et al.,* 2004). NO-dependent vasodilation has been shown to be an important factor in the maintenance and regulation of vascular tone in the renal microcirculation (Pflueger *et al.,* 1999). 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 (Lockhart *et al.,* 1994) and juxtamedullary nephrons (Zats and Nucci, 1991) 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 (Ohishio and Carmines, 1995; Walder *et al.,* 1991). NO can interact with proteins containing heme or thiols to decrease respiration and potentially trigger apoptosis or cell necrosis or both by inhibiting cytochrome oxidase, thus activate the inflammatory process (Sivitz and Yorek, 2010).

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 (Crook *et al.,* 2001). Circulating serum sialic acid, an inflammatory marker has been shown to be a strong predictor of cardiovascular mortality (Sriharan *et al.,* 2002). 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 (Gavella *et al.,* 2003; Crook *et al.,* 2002). Sialic acid is basically released from terminal oligosaccharide chain of some glycoproteins and glycolipids of the acute phase (Spunda *et al.,* 1996). The current study aimed to point towards the exploitation of serum nitric oxide and sialic acid levels to identify the development of nephropathy in diabetes as easy-to-detect and quick biomarkers.

SUBJECTS AND METHODS

A total of 210 patients of diabetes mellitus suffering from nephropathy (persistent albuminuria) of either sex

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admitted in diabetic wards or visiting out patient departments of Civil Hospital Karachi, Jinnah Postgraduate Medical Center Karachi and Darul Sehat Hospital, Karachi were selected. The patients included 115 males and 95 females. 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 (WHO, 1994). Equal number of age and sex matched normal healthy subjects were selected without any known history of hyperglycemia, hypertension and renal insufficiency as controls. 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.

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 (Bakris *et al.,* 2000).

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 at the 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 (Smarason *et al.,* 1997). The serum sialic acid was measured by Ehrlich's method (Crook *et al.,* 1993). The fasting blood glucose (FBG), 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 (Levey *et al.,* 2000).

Results are presented as mean±SEM. Statistical significance and difference from control and test values evaluated by Student's *t*-test. Correlation coefficients were used to describe the effects of two variables by Pearson's Correlation test. Statistical significance was assumed at the $p<0.05$ level. All statistical analyses were done by using statistical package for social sciences (SPSS) version 17.0 for Windows (Chicago, IL. USA).

RESULTS

The table 1 summarizes the comparison of means for clinical characteristics of controls and patients in terms of mean±SEM. The levels of BMI, systolic and diastolic BP, FBG, HbA1c, serum urea, creatinine and serum sialic acid were found to be significantly high ($p<0.05$) in DN patients as compared to controls, whereas GFR and serum nitric oxide levels were significantly low ($P<0.05$) in DN patients as compared to controls. A strongly significant and negative trend was found in the correlation between serum sialic acid and nitric oxide in DN patients during development of nephropathy in diabetes (fig. 1) that shows the impact of coexistence of impairments in serum sialic acid and nitric oxide levels in patients.

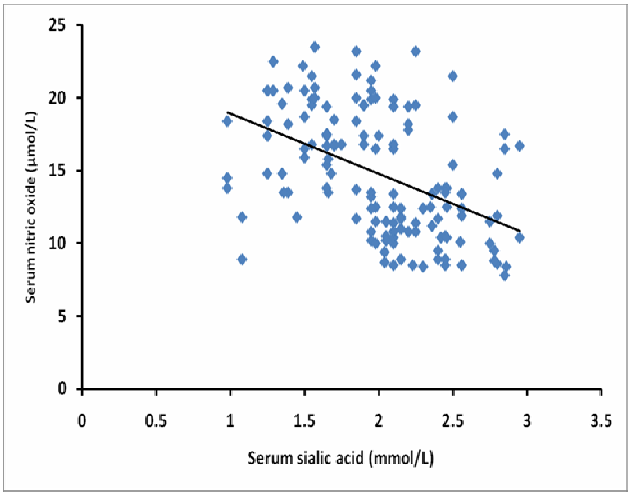

Fig. 1: Correlation between serum sialic acid and nitric oxide in diabetic nephropathy patients ($r=-0.558$, $p<0.05$)

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 (Goligorsky *et al.,* 2001).

In the present study, a significant decrease was observed in the level of endothelial nitric oxide level in coexistent diabetes and nephropathy, which indicates that diabetes affects basal nitric oxide metabolism (table 1). The nitric oxide is a potent vasodilator, acting as a paracrine

Table 1: Comparison of clinical characteristics of patients and controls

Parameters	Controls	Patients
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=210 Values are mean±SEM, *p<0.05 as compared to control subjects

mediator in various vascular entities. Nitric oxide controls afferent and efferent vascular tone in kidneys as well as the ultrafiltration coefficient and medullary blood flow (Komers *et al.,* 2000). Nitric oxide is synthesized as a by product of conversion of its physiological precursor L-arginine to L-citrulline followed by an enzymatic reaction, catalyzed by a group of enzymes known as nitric oxide synthases (NOS) (Michel and Feron, 1997). The low production of nitric oxide during diabetic complications supposed to be the consequence of reduced production NOS and inactivation of nitric oxide by reactive oxygen species (ROS) produced either by glycosylated proteins or directly from vascular endothelium as high level of HbA1c was observed in patients of diabetes 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 (Vallejo *et al.,* 2000). Several mechanisms could account for a reduced responsiveness of the diabetic renal vasculature to NO-dependent vasodilation could be explained by a couple of mechanisms. For instance, the inactivation of NO and/or a lower sensitivity of the vascular smooth muscles cells (VSMC) to nitric oxide diminished autoregulatory adjustment in renal vasculature resistance. The baroreflex-mediated alterations in renal sympathetic nerve activity, and increased production of nitric oxide antagonists such as endothelin 1, and quenching of nitric oxide by glycosylation products during micro and macrovascular complications can also be significant players in this game (Pflueger *et al.,* 1999).

This study also finds strong and statistically significant correlation between serum nitric oxide and sialic acid diabetic in diabetic nephropathy patients (fig 1). This shows a clear clinical relationship between serum sialic acid and nitric oxide regarding microvascular complication that has been observed before in small scale studies for type 1 and type 2 diabetes (Crook *et al.,* 2000; Linderberg *et al.,* 1991).

Serum sialic acid has been established as a predictor for acute phase response (Pickup *et al.,* 1997). Acute phase glycoprotein with sialic acid as a component of the oligosaccharide side chain being produced by liver is stimulated by proinflammatory cytokines. The vascular anomalies in diabetes causes tissue injury, thus 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 and this induces an acute phase response (Boumann and Gauldie, 1994).

The glycemic process stimulates cytokine production from cells throughout the body which in turn plays a key role in developing the vascular complications, such as hypertension and nephropathy. 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 (Mantovani and Bussolino, 1997).

The hypothesis that the cytokinemia leads to microvascular abnormalities provides realization that microalbuminuria is a non specific marker of inflammation in the general population. This reviews the need for early predictors of diabetic vascular complications such as nephropathy (Caramori *et al.,*2000). 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 (Yokoyama *et al.,* 1996). 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.

Therefore, it can be concluded that the serum nitric oxide and sialic acid are clinically correlated and biochemical predictors for micro and macro vascular complications of diabetes mellitus such as nephropathy. Their levels of correlation should be taken into account during screening

procedures regarding identifications of the diabetic patients to get them rid of development of renal impairment to end stage renal failure.

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