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Diabetes and Hypertension: Correlation Between Glycosylated Hemoglobin (HbA1c) and Serum Nitric Oxide (NO),

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Abstract: Diabetes mellitus and hypertension frequently coexist. Formation of HbA1c and serum NO have recently documented as disease markers for diabetes and hypertension respectively. This study was aimed to find the correlation between HbA1c and NO anomalies in coexisted diabetes and hypertension. A total of 100 diabetic patients were selected and divided into diabetic normotensive and diabetic hypertensive patients. Equal number of age and sex matched normal individuals were selected as control. The demography of the patients was done at routine. Their fasting blood glucose, urea, creatinine and serum NO levels were measured by spectrophotometer. The HbA1c was measured by fast ion exchange resin separation method. Parameters were correlated by modern statistical methods. Fasting blood glucose and HbA1c levels were significantly high ($p<0.01$) where as serum NO level was significantly low ($p<0.01$) in diabetic normotensive and diabetic hypertensive patients as compared to control. A significant negative correlation ($p<0.01$) was found when serum nitric oxide was correlated with serum glucose and HbA1c levels in diabetic normotensive and diabetic hypertensive patients. These findings suggest that the constellation of disturbed glycemic control and formation of advanced glycosylation end products like HbA1c critically contribute to anomalies of NO metabolism or vice versa and may help to explain frequent clinical coexistence of diabetes and hypertension.

Key words: Diabetes, hypertension, glycosylated end products, nitric oxide.

INTRODUCTION

Hypertension is about twice as prevalent in patients with diabetes as in those without disease. In patients with type II diabetes, elevated blood pressure usually occurs in association with obesity and insulin resistance. Endothelial dysfunction and pressor effects of hyperinsulinemia and hyperglycemia may contribute directly to raised arterial pressures; however, hypertension is generally considered a renal phenomenon[1]. The role of hyperglycemia has recently been studied as the most important factor in the onset and progression of diabetic complications during Diabetes Control and Complications Trials and United Kingdom Prospective Diabetes Study (UKPDS) epidemiological studies (Stratton *et al.,* 2000) Among the irreversible changes that occur as a result of hyperglycemia is the formation glycosylated conjugates like HbA1c through a reaction between sugars and the free amino groups on proteins. These chemically heterogeneous compounds are known to have a wide range of chemical, cellular and tissue effects implicated in the development and progression of hypertension in diabetes (Forbes *et al.,* 2003).

The major complications of diabetes include abnormalities of the vessel wall structure and function, which results in both micro and macrovascular disease. Vascular disease is two to eight times more common in diabetic than non-diabetic individuals, and remains the leading cause of death in diabetic patients[4]. 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

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juxtamedullary nephrons (Zats *et al.,* 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 (Ohisiho and Carmines, 1995; Walder *et al.,* 1991). Endothelium-derived nitric oxide is an important determinant of renal natriuresis and of peripheral vascular tone. Although the available data are conflicting, a number of studies have demonstrated impaired vascular response to endothelial dependent vasodilators or reactive hyperemia in diabetic patients. Suggesting impaired production or enhanced degradation of nitric oxide or depressed smooth muscle responses to nitric oxide. The mechanisms underlying this abnormality in vascular function are not well established Elliot *et al.,* (2001). Changes in renal medulla resulting in activation of the renin-angiotensin system may also contribute to sodium retention and hypertension in such patients. Raised capillary pressure has been implicated in the formation of diabetic microangiopathy in type I diabetes, in which it is elevated in those with the earliest signs of diabetic renal disease but remains normal in those without complications[11]. Due to these establishments, the present study was designed to investigate, and correlate the glycemic abnormalities such as formation of glycosylated hemoglobin with the bioavailable NO, its significance and implication to treat diabetic hypertensive patients before hand.

MATERIAL AND METHODS

The patients with 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 and M.S. General Hospital, Karachi were selected. However, patients suffering from type 1 diabetes mellitus, gestational diabetes and any known mental illness, macro vascular disease prior to diagnosis of type 2 diabetes or the patients who refused to participate in the study were excluded. Equal number of age and sex matched individuals were selected as control with no known history of hyperglycemia and hypertension. The aim and procedures were explained to patients and/or attendant and informed consent was obtained. Their diabetes age was more than five years. The diagnosis of diabetes was made according to the World Health Organization's (WHO) criteria (World, 1985). The study protocol was approved by the regulations of institutional ethical committee for the use of human subjects in research. The patients were divided into following groups (50 each) as follows:

Group I:Non-diabetic, normotensive control subjects.

Group II: Diabetic, normotensive patients.

Group III: Diabetic, hypertensive patients.

A previously structured questionnaire was used to record the demographic features of all subjects. Height and weight were noted for BMI (BMI=weight in Kg/height in m^2). BMI from 20.0 to 29.9 was considered non-obese over weight and >30.0 was considered as obese. Blood pressure was measured with standard mercury sphygmomanometer while the patient was sitting after resting for 10 min. Hypertension was defined as the blood pressure³140/90 mm Hg (Bakris *et al.,* 2000).

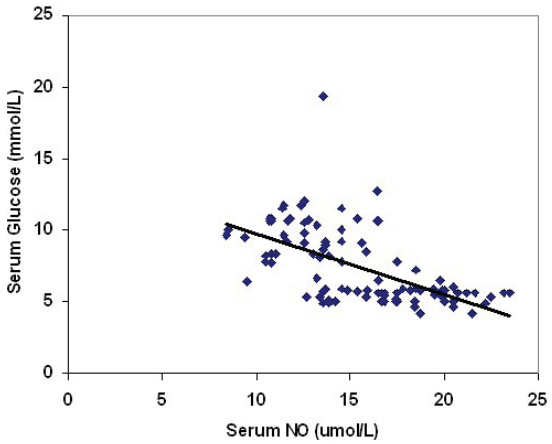
The blood samples of patients and control subjects were collected in lithium heparin coated tubes after the patients have been taken no drugs for the last 12 hours or more. An aliquot was taken separately in order to get serum. Blood samples were processed the same day for estimations, in accordance with the ethical guidance and regulation of institution and with generally accepted guidelines governing such work. Fasting blood glucose was measured by O-Toluidine method[14]. HbA1c was estimated by Fast Ion-Exchange Resin Separation Method (Human Gessellschaft fur Biochemica und Diagnostica mbH, Germany). Serum urea was measured by Thiosemicarbazide-Diacetyl monoxime method (Mather *et al.,* 1969). Serum creatinine estimated by modified Jaffe's method (Spierto *et al.,* 1979). Serum NO level was measured by measuring its metabolites (Nitrate+Nitrite) concentrations by spectrophotometric method (Smarason *et al.,* 1997).

Results are presented as mean±SD. Statistical significance and difference from control and test values were evaluated by Student's t-test. Correlation coefficient and regression analysis were used to describe the effects of NO on glucose, HbA1c, SBP, DBP and BMI. Scattergram was plotted by taking dependent variables on y-axis and independent variable (NO) on x-axis.

RESULTS AND DISCUSSION

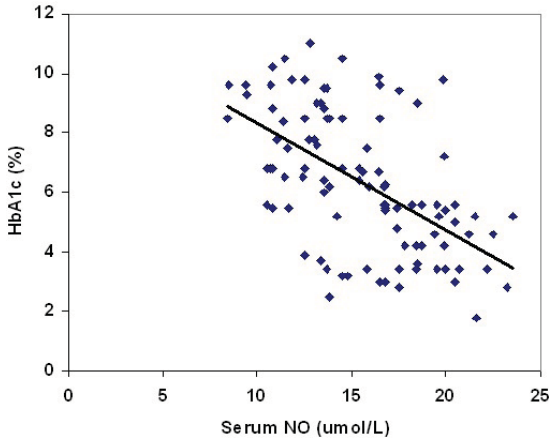
Table I shows the demographic and biochemical features in control, diabetic normotensive and diabetic hypertensive patients. The BMI values were found to be significantly high in diabetic normotensive and diabetic hypertensive patients as compared to control. Systolic and diastolic blood pressures were also found raised significantly in diabetic hypertensive patients as compared to control and diabetic normotensive patients.

Fasting glucose levels and HbA1c were significantly increased in diabetic normotensive patients and diabetic hypertensive patients as compared to control. Serum NO was observed significantly low in diabetic normotensive and diabetic hypertensive patients as compared to control. Serum urea and creatinine levels were significantly high in diabetic normotensive and diabetic hypertensive patients as compared to control. Strong negative correlation ($p<0.01$) was found between serum NO and serum glucose as well as HbA1c levels in diabetic normotensive and diabetic hypertensive patients. (Figures I and II).



r = 0.59, p<0.01

Fig. I: Correlation between serum nitric oxide and serum glucose in diabetic normotensive and diabetic hypertensive patients



r = 0.56, p<0.01

Fig. II: Correlation between serum nitric oxide and HbA1c in diabetic normotensive and diabetic hypertensive patients

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 diabetes and hypertension (Goiigorsky *et al.,* 2001).

This present study finds support in the observation that diabetes affects basal NO metabolism as a successive and significant negative correlation was observed in the level of endothelial NO at the onset of diabetic complications such as hyperglycemia and hypertension (Figure I). 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 (Komers *et al.,* 2000). 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) (Michel and Feron, 1997). The decrease production of NO during diabetic complications (Table I) 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 higher levels of HbA1c was observed in diabetic nephropathy patients during the present study (Table I). 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: 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 (Pflueger *et al.,* 1999). The same effects were also observed during hypertension in normoglycemic patients (Figure II) in present study due to variety of factors involved in impairment of NO metabolism.

Hemoglobin is one of many proteins that undergo non-enzymatic glycation, and glycosylated hemoglobin is a general term for hemoglobin non enzymatically glycosylated with glucose. Potential glycation sites of the hemoglobin-A molecule include the N-terminal amino acid valine of the four-polypeptide chains and all of the free amino groups of lysine residues. The predominant glycosylation site is the N-terminal valine residue of b-chain, which accounts for ~60% of bound glucose (Nuttall, 1998). HbA1c represents the most prevalent glycated species. Because erythrocytes are freely permeable to glucose, the rate of formation of HbA1c is directly proportional to the ambient glucose concentration in which the erythrocyte circulates and to the duration of exposure (Krishnamurti *et al.,* 2001). The distinctly different risks of microalbuminuria in patients with low HbA1c values and those with high values suggest that diabetes damages the kidney through several mechanisms. The mechanisms operating below the threshold HbA1c value of less than 10% (which corresponds to prevailing blood glucose concentration of less than 200 mg/dL or 11 mmol/L seem to be influenced by other components of the diabetes mellitus for example, abnormalities in plasma insulin concentrations (Abrass *et al,* 1994). One of the early alterations observed in diabetes mellitus that may lead to endothelial dysfunction is the decreased bioavailability of NO as observe during this study. In a series of previous studies it is demonstrated that supraphysiological concentrations of glucose are capable of scavenging NO (Bohlen and Lash, 1993). Specifically it is demonstrated that acute exposure to human endothelial cell to higher concentrations of glucose, at levels found in plasma of diabetic patients, results in significant blunting of NO responses. This effect of glucose may account for the acute hypertensive response to hyperglycemia (Bohlen and Lash, 1993). In addition, extrapolating from these in vitro data, each episode of hyperglycemia, as transient as it might be lead to the temporary decrease in the bioavailability of NO and the reversible impairment of NO-dependent functions of the endothelium in conjunction with abnormal angiotensin II.

Table I: Demographic and biochemical features of patients and control subjects

Parameters	Control (n=50)	Diabetic normotensive patients (n=50)	Diabetic hypertensive patients (n=50)
BMI (Kg/m^2)	21.35±2.56	27.18±2.59*	36.65±2.15*§
SBP (mm Hg)	124.24±8.48	124.96±5.94	143.4±12.67*§
DBP (mm Hg)	79.72±7.19	78.72±4.56	88.64±6.93*§
Fasting Glucose (mmol/L)	5.38±0.45	9.09±4.01*	9.46±2.56*§
HbA1c (%)	4.54±1.29	7.41±2.14*	8.15±1.65*§
Serum NO (µmol/L)	18.13±2.65	17.51±2.91*	13.01±2.49*§
Serum Urea (mmol/L)	10.78±2.46	9.62±2.02	11.24±2.50*§
Serum Creatinine (µmol/L)	111.82±45.97	116.52±32.59*	155.08±43.08*§

* $p<0.01$ as compared to control

§ $p<0.01$ as compared to diabetic normotensive patients

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