Reduced nitrate level in individuals with hypertension and diabetes

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ABSTRACT

Background: Nitric oxide (NO) turnover is vital for proper endothelial function to maintain a healthy vascular system. Various risk factors responsible for hypertension and diabetes may disrupt this homeostasis, leading to decreased bioavailability and/or bioactivity of NO, which potentiates endothelial dysfunction. Plasma NO is a useful indicator of NO homeostasis and vascular endothelial function. Since endothelial function plays a key role in the development and progression of diseases like diabetes and hypertension, we sought to investigate the NO profile in patients having diabetes and hypertension and determine the relationship of NO turnover with the disease. **Materials and Methods:** For this purpose, three groups were studied for the NO production. The first group consisted of 74 hypertensive patients, the second group consisted of 72 diabetic patients and the third group consisted of 60 healthy controls. Nitrate synthase activity was evaluated by measuring nitrate level using an automated sample injector connected to an automated NO detector – Ion liquid chromatograph. **Results:** The plasma concentration of NO was found to be significantly lower in both essential hypertensive patients and diabetic patients without complications as compared to the healthy controls ($P < 0.05$). **Conclusion:** This data confirms that different factors like hyperglycemia and blood pressure are seen to have immense influence on NO production.

Key words: Diabetes, hypertension, nitric oxide

INTRODUCTION

Endothelium, which is an inert single-cell lining covering the internal surface of blood vessels, plays a crucial role in vascular homeostasis by regulating vascular tone and structure.[1] Nitric oxide (NO) is the most pivotal molecule secreted by endothelium and thus is a major mediator of endothelial function. The production of NO is catalyzed by family of enzymes called as nitric oxide synthases (NOS), which convert the amino acid L-arginine to L-citrulline and NO.[2,3] Three isoforms of NOS, specific to different organ systems, exist. Apart from playing an important role in vasodilation, NO is also critically involved in the regulation of other protective properties of the healthy endothelium by playing an important role in a wide range of physiological processes like platelet and leukocyte aggregation,[4] leukocyte adhesion,[5] cell proliferation and vasoconstriction.[6,7] Evidence suggests that NO plays a major role in regulating blood pressure and glucose levels, and thus impaired NO bioactivity forms an important component of hypertension and diabetes. The physiological importance of NO in the regulation of blood pressure is evidenced by the fact that pharmacological inhibition of NO synthases leads to severe hypertension, vascular injury, and glomerulosclerosis in experimental animals.[8] Moreover, endothelial NOS (eNOS) knockout mice exhibit hypertension,[9] thus providing further

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support for the importance of NO in the regulation of blood pressure. Likewise, in diabetic subjects also, it has been demonstrated that the vasodilating response to stimuli is diminished and that this anomaly is related to glycemic control.[10] *In vivo* studies have demonstrated that hyperglycemic spikes induce an endothelial dysfunction in both diabetic and normal subjects.[11-13] Moreover, a significant association between eNOS gene polymorphisms and type 2 diabetes suggests a genetic link between NO production and diabetes.[14] Endothelial dysfunction is associated with disruption of vascular homeostasis leading to proinflammatory and prothrombotic phenotype of the endothelium; thus, it may play a pivotal role in the development and progression of secondary complications both in diabetes and hypertension.

While it is apparent that NO turnover has a definitive influence on the etiology of many common disorders, much remains to be done to substantiate NO targeted therapies for the treatment of such disorders. Likewise, a systematic detail of ascertaining the relationship of NO dynamics with such disorders needs to be elucidated before NO-targeted therapeutic proposition is considered. As NO rapidly changes into stable oxidized metabolites, such as nitrite and nitrate, in all parts of the body, the amount of the stable form in plasma should reflect vascular activities and circulatory changes in the body. Therefore, pathophysiological changes such as atherosclerosis, endothelial dysfunction, pro-inflammation and inflammation seen in diabetic and hypertensive patients may be understood by measuring NO metabolites in the peripheral blood. Our study is an attempt to measure NO metabolite (NOx: nitrate) in the serum of normotensive controls, diabetic subjects and hypertensive subjects and analyze it in relation to the effects of disease.

MATERIALS AND METHODS

Study design

The study was conducted on 74 hypertensive patients (40 men, 34 women; mean age of 55±10 years), 72 diabetic patients without complications (37 men, 35 women; mean age of 55±10 years) and 60 healthy volunteers (33 men, 27 women) from a similar ethnic background without any health problems (aged 45–65 years) who served as healthy controls.

Both hypertension and type 2 diabetes were diagnosed according to the criteria of the World Health Organization. Normal blood pressure was defined as systolic blood pressure (SBP) <140 mm Hg and diastolic blood pressure (DBP) <90 mm Hg. Hypertension was defined as either SBP ≥160 mm Hg or DBP of ≥95 mm Hg, or both, with a well-documented history of long-term high blood pressure. Patients were excluded if they had any history of certain vascular complications (i.e., cardiac, cerebral, or peripheral vascular diseases), congestive heart failure, renal dysfunction (serum creatinine concentration > 1.5 mg/dl), malignancy, or hematological diseases, and if they had taken any antihypertensive/hyperlipidemic medications such as angiotensin converting enzyme inhibitors (ACEI)/statins that might influence NO levels. Participants were instructed to refrain from eating for 18 hours, drinking beverages containing alcohol or caffeine, or smoking for at least 24 hours before blood sampling.

To exclude the aging effect possible, only those aged less than 65 years were examined. The samples to be assayed were taken from those who agreed with the experimental use of the research, and a signed informed consent was obtained from all the patients who participated in the study.

Analytical methods

About 2 ml of whole blood was drawn from each subject into heparinized tubes, which were promptly chilled in an ice bath. Plasma was isolated by centrifugation (15 min at 13,000 rpm) and then stored at −80°C till further analysis. For deproteinization, equal amount of acetonitrile was added to the plasma followed by centrifugation at 13,000 rpm for 30 min. The supernatant was collected and pellet discarded. The samples obtained were kept at −80°C until the time of NO metabolite analysis. Nitrate level in the plasma samples was measured using an automated sample injector connected to an automated NO detector – Ion liquid chromatograph (Dionex, Model ICS-2500).

Statistical methods

The entire data was statistically analyzed using SPSS program. Data were expressed as the mean±SEM and were compared by analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS

The clinical characteristics of hypertensive, diabetic and normal control subjects are summarized in Table 1. With the exception of systemic blood pressure (measured at the time of the study) in case of hypertensive subjects, no significant difference in these characteristics was observed between the patient and control groups. The average plasma nitrate level in essential hypertensive patients was 39.7±13.27 μmol/L (range 30.855–46.63 μmol/L), which showed significant

Table 1: Clinical characteristics of the study groups

Characteristic	Control	Hypertension	Diabetes
No. of patients (men/women)	60 (33/27)	74 (40/34)	72 (37/35)
Age, years	55±10	55±10	55±10
Systolic blood pressure, mm Hg	128±4	159±5	128±4
Diastolic blood pressure, mm Hg	71±3	101±4	71±3
Heart rate, beats per minute	69±2	68±3	68±3
Body mass index, kg/m^2	25±2.5	26±2	25±3.6
Waist to hip ratio	0.9±0.05	0.9±0.04	0.96±0.049
Serum cholesterol, mmol/L	4.5±0.9	4.7±0.55	4.6±1.2
Serum HDL, mmol/L	1.1±0.5	1.2±0.4	1.1±0.32
Serum LDL, mmol/L	2.0±0.5	2.1±1.1	2.3±1.25
Serum VLDL, mmol/L	0.8±0.05	0.9±0.05	1.17±0.4
Serum triglycerides, mmol/L	1.6±0.4	1.7±0.5	2.66±0.94
Serum creatinine, µmol/L	61±17	62±18	59.6±20.4
Blood urea nitrogen, mmol/L	8.2±2	8±2.3	8.3±2.5
Serum uric acid mmol/L	0.4±0.12	0.38±0.12	0.34±0.13

Table 2: Mean nitrate level of patients with essential hypertension, diabetes and control subjects

	Control subjects (n = 60)	Essential hypertensive subjects (n = 74)	Diabetic subjects (n = 72)
Nitrate level	100.48±32.46 µmol/L	39.7±13.27 µmol/L	24.95±15.79 µmol/L

difference compared to the normotensive healthy group (100.48±32.46 µmol/L). Patients having diabetes without complications had a plasma nitrate level of 24.95±15.79 µmol/L (range 16.808–30.748 µmol/L), which is also significantly less compared to the control group [Table 2].

DISCUSSION

The inverse correlation between the plasma nitrate concentration and hypertensive group makes it imperative that blood pressure plays an important role in the downregulation of NO production in these subjects. The observations concerning the end product of NO metabolites are non-univocal in hypertension; many reports in fact show their decrease, while others demonstrate their increase. However, our results are in agreement with data showing the inverse correlation between the two. A study conducted on hypertensive patients has shown decreased level of NOx and guanosine 3',5'-cyclic monophosphate (cGMP) compared to normotensive subjects, and antihypertensive agents such as calcium channel blockers or ACEI were effective in recovering those levels.[15] It has been seen that SBP and DBP inversely correlated with plasma and urinary nitrate owing to the decline of antioxidative activity (i.e., lipid peroxidation enhanced by the lack of antioxidant activities) which was associated with decreased NO production and the severity of hypertension.[16] Li *et al*, reported a positive association between NOx and BP in normotensive African Americans who carry the "a" allele of eNOS4 polymorphism.[17]

Hypertension can produce structural damage to aortic endothelial cells in animals, and pressure overload is associated with a direct toxic effect on human endothelium; impairment of the release of NO from vascular endothelial cells may thus contribute to the reduced plasma nitrogen oxide concentrations in patients with essential hypertension. Decreased synthesis of NO might also result from abnormal handling of intracellular calcium and a consequent reduction in the activity of NOS.[18] Increased production of superoxide anions in oxidative stress which rapidly deactivate NO is a characteristic feature of experimental models of hypertension.[19,20] It is also seen that plasma indexes of lipid peroxidation are increased in patients with hypertension.[21] Studies have shown that hypertension impairs endothelium-dependent dilation of rat coronary arteries as a result of superoxide anion mediated degradation of NO.[19] Mice with disruption of the gene for eNOS have elevated BP levels compared with control animals, suggesting a genetic component to the link between impaired NO bioactivity and hypertension.[22]

Our results also showed inverse correlation between NO level and diabetic state and this fact clearly underlines that hyperglycemia is a major determinant factor in serum NOx levels. It is widely recognized that hyperglycemia induces impairment of the endothelial function via increased oxidative stress[23] which is a characteristic feature of diabetic individuals. The hyperglycemic state stimulates the production of advanced glycosylated end products,[24] enhances the polyol pathway[25] and activates protein kinase C leading to oxidative stress.[26,27] A reduced content of glutathione, an important antioxidant in erythrocytes,

has been demonstrated in diabetic patients.[28,29] Also, reduced radical-trapping antioxidant parameter (TRAP) and increased lipid peroxidation levels support the *in vivo* presence of increased oxidative stress in diabetes.[30-32] Reduced NO availability may not only be of relevance to the development of atherosclerotic complications in diabetes, but also interfere with insulin-mediated postprandial glucose disposal and possibly contribute to the development of insulin resistance.

Various studies have reported a significant decrease of plasma nitric oxides in patients with type 2 diabetes mellitus without any complications.[33,34] Decreased NO bioavailability to smooth muscle cells in no complicated group has also been demonstrated in different studies.[35] Our results coincide with these reports, and we presumed that the cascade of NO bioactivity and availability on smooth muscle cells was impaired in the early affected stage of diabetes mellitus and followed the decrease of endothelial NO production.

Reduced NO availability in diabetes mellitus and hypertension underlines its relevance to the development of secondary complications in these clinical conditions. Alteration of NO metabolism and increased oxidant stress, previously demonstrated in diabetic patients, have been demonstrated to be involved in the pathogenesis of macrovascular events, which are increased in hypertensive as well as diabetic patients.[36-39]

To the horizon of our knowledge, our work represents the first study of this kind done in Kashmir, India. Moreover, the distinct features of Kashmir including unique geographic locale, traditions, culture and genetically pure and ethnic population prompted us to take this particular study and compare NO levels in diseased and normal states. Our study is a preliminary attempt to investigate the correlation of NO turnover with diabetes and hypertension. However, further studies need to be done to gain information for better understanding of the key events relevant to the designing and establishing the role of NO assays in diabetes and hypertension. In conclusion, our findings indicate that NO concentrations were significantly lower in men and women with essential hypertension and type II diabetes, supporting the hypothesis that altered NO pathway is a central defect leading to diabetes and hypertension. The inverse correlation observed in this cohort of population is in agreement with the observations of some of our global peers, while other studies have noted elevated levels in both diabetes and hypertension. Our study clearly shows that NO turnover has a definitive influence on the etiology of these disorders; however, the non-univocal findings point to a need for robust multicentric studies in order to substantiate NO targeted therapies for these disorders.

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