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Original Research Article

Correlation between endothelial dysfunction, inflammatory status, oxidative stress and total (nitrite/ nitrate) in subjects with diabetes mellitus type 2

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ABSTRACT

Background: Diabetes Mellitus is a systemic metabolic disorder associated with Endothelial dysfunction and increased systemic inflammatory state with oxidative stress leading to increased Cardiovascular risk. This study planned to correlate the level of Endothelial dysfunction with oxidative stress and inflammatory status.

Methods: Study was conducted in 60 Diabetes Mellitus subjects of both genders with duration of more than two years. Endothelial dysfunction assessed as Augmentation Pressure and Augmentation Index generated from Radial artery waveforms by tonometer using Spygmocor PWA system. Plasma Total Nitrite/ Nitrate, High sensitive C - Reactive Protein, Malondialdehyde and Glutathione were measured.

Results: Out of total 60 Diabetes Mellitus subjects 16 subjects were with Coronary Artery Disease. There was no significant difference in High sensitive C - Reactive Protein, Glutathione, Malondialdehyde and Total Nitrite/ Nitrate between Diabetes Mellitus with Coronary Artery Disease and without Coronary Artery Disease, however significant difference (p=0.02) was observed Augmentation Pressure between Diabetic alone (12.8±5.19 mm of Mercury) and diabetics with Coronary Artery Disease (16.13±33.47 mm of Mercury) and Augmentation Index (p=0.04) between Diabetic alone (29.8±5.68 mm of Mercury) and diabetics with Coronary Artery Disease (40.01±5.74). As endothelial function is age dependent the subjects were divided into three age groups (20-40 years, 40-60 years and more than 60 years). High sensitive C - Reactive Protein, Glutathione, Malondialdehyde, Total Nitrite/ Nitrate and Augmentation Index did not differ in the three age groups while Augmentation Pressure (p=0.0096) showed significant difference between age group 20-40 years (10.59±3.24) and age group more than 60 years (15.83±3.92).

Conclusions: There is significant endothelial dysfunction observed in Diabetes Mellitus subjects and Diabetes Mellitus with coronary artery disease showed greater endothelial dyfunction. Thereby concluding that Diabetes Mellitus subjects were at higher risk for development of coronary artery disease and as endothelial dysfunction is an early event, it may have some prognostic value.

Keywords: Augmentation pressure, Augmentation index, Diabetes mellitus, Endothelial dysfunction, Oxidative stress

INTRODUCTION

Globally, an estimated 422 million adults are living with Diabetes Mellitus (DM), according to the latest 2016 data from the World Health Organization.[1] Diabetes prevalence is increasing rapidly and the number is projected to almost double by 2030.[2] India, the second most populous country

of the world, has been severely affected by the global diabetes epidemic.[3]

Diabetes Mellitus is a metabolic disorder characterized by increased mortality rates and importantly implicated in the atherogenetic process. The manifestation of endothelial dysfunction is not only associated with cardiovascular disease but may precede its development, as shown in a study of offspring of hypertensive patients.[4]

The Endothelial dysfunction is characterized by reduction of the bioavailability of vasodilators, particularly nitric oxide (NO), and/or an increase in endothelium-derived contracting factors.[5] The resulting imbalance leads to an impairment of endothelium-dependent vasodilatation, which is the functional characteristic of endothelial dysfunction. Endothelial cells (ECs) synthesize and release different molecules that orchestrate metabolic, vascular, and cellular responses. Among them, nitric oxide (NO) is a key regulatory molecule of paramount importance for endothelial function and vascular tone relaxation.[6,7] Reduced endothelial cell nitric oxide synthase (eNOS) expression and/or NO bioavailability are associated with decreased EC survival and with endothelial dysfunction.[8] The regulation of NO metabolism is particularly important in diabetes mellitus, since the activation of eNOS has been demonstrated to be under the insulin control.[9,10]

Diabetes is also associated with a systemic inflammatory state that may impair endothelial function and contribute to atherosclerosis.[11] A diabetic patient there is an increase of circulating levels of inflammatory markers.[12-14] Moreover, higher levels of inflammatory markers are a predictor of increased cardiovascular risk in diabetic patients and on the other hand, augmented levels of circulating inflammatory markers also relate to the incidence of diabetes mellitus.[15,16] Oxidative stress, caused by the overproduction of Reactive Oxygen Species (ROS) plays an important role in the activation of pathogenic pathways involved in diabetic complications. Increased oxidative stress in the vasculature is a major contributor of endothelial dysfunction in DM via the superoxide production and impairment of NO bioavailability in the vascular wall. The concept of mild chronic vascular inflammation as part of the pathophysiology of cardiovascular disease, most importantly hypertension and atherosclerosis, has been well accepted. Indeed, there are links between vascular inflammation, endothelial dysfunction and oxidative stress.[17]

The prevalence of diabetes mellitus is rising worldwide and has reached epidemic dimensions. Diabetes mellitus places patients at high cardiovascular risk. Diabetes and its associated complications have become a public health problem of considerable magnitude. Cardiovascular disease causes most of the excess morbidity and mortality in diabetes mellitus. Adults with diabetes are at a 2- to 4-fold increased risk of cardiovascular events relative to those without diabetes.[18] Cardiovascular disease accounts for up to 80% of premature excess mortality in Diabetes mellitus.[19] Both systemic and coronary endothelial dysfunction have been demonstrated to be independent predictors of cardiovascular events.[20,21]

So, this study was conducted to correlate endothelial Dysfunction with inflammatory markers, oxidative stress and Nitric Oxide levels in subjects diagnosed with Type 2 Diabetes Mellitus.

METHODS

The study was conducted with NIMS Institutional Ethics Committee approved protocol and in compliance with GCP, Schedule Y (Drugs and Cosmetic Act, 1940 and any amendments therein) and ethical guidelines for biomedical research on human participants (Indian Council of Medical Research, 2006) at department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad. Written Informed Consent was taken before subjects were enrolled into the study.

Both gender aged between 18 to 65 years with a documented type II Diabetes Mellitus of ≥ two years duration were included into the study.

All procedures were performed in a sound proof temperature controlled room. Brachial blood pressure was recorded in duplicate in the nondominant arm using a validated Mercury sphygmomanometer. Radial arterial waveforms (20 cardiac cycles) were recorded with a high-fidelity tonometer and validated transfer function was then used to generate a corresponding central aortic pressure waveform. An averaged composite radial waveform was calculated from which specially designed software (Sphygmo Cor TM; PWV Inc., Australia, version 8) derived an aortic BP waveform, in real time, using a validated transfer function algorithm. Augmentation Pressure (AP) is quantified as the amount of pressure added to the systolic pressure peak based on the reflected wave and Augmentation index AIx) calculated, as the difference between the second systolic peak and inflection point, expressed in percentage. An average of nearest two measurements was taken.

Plasma glutathione estimation was determined by using Ellman's method, where thiol group of glutathione reacts with 5, 5'-dithio-bis (2-nitrobenzoic acid) to give golden yellow colour complex measured at 412nm.[22] Serum Malondialdehyde (MDA) was measured by using trichloroacetic acid and thiobarbituric acid and absorbance of the supernatant was read at 540nm against blank.[23] Nitric Oxide (NO) was measured in form of total (Nitrite/ Nitrate) in human serum by employing colorimetric detection with Griess reagents.[24,25] Human high-sensitivity CRP C-Reactive Protein was measured by using commercially available ELISA kits manufactured by Calbiotech Inc, USA as per the manufacturers' recommendation.

The study was done in a 60 type 2 Diabetes Mellitus subjects. All changes represent absolute differences, rather than percentage. All values represent mean±SD. p value of less than 0.05 was statistically significant.

RESULTS

Baseline clinical characteristics

A total of 60 type II diabetes mellitus subjects participated in this study conducted at department of clinical pharmacology and therapeutics, Nizam's Institute of Medical Sciences, Hyderabad, India. The baseline clinical characteristics of participants are summarized in Table 1. Out of there 60 subjects 14 were with Coronary Artery Disease (CAD). As Endothelial Function is age dependent the study population was sub grouped in to 3 groups based on age (20-40 years, 40-60 years and >60 years).

Table 1: Demographic characters of the study population.

Character	Total Group n = 60	Type II DM n= 44	CAD n = 16
Age (years)	48.3±8.85	47.4±8.91	50.7±8.21
Male:Female ratio	46:14	35:11	11:3
Coronary artery disease(number)	16	--	--
Smoker(number)	08	7	5
Alcoholic(number)	10	3	3

Table 2: Inflammatory marker (high sensitive C - Reactive Protein), oxidative stress (malondialdehyde and glutathione), total (nitrite/ nitrate) and endothelial function (augmentation pressure, augmentation index in three different groups.

Parameter	Total group (n= 60)	Diabetes mellitus (n= 44)	Coronary artery disease (n= 16)
hs CRP[@] (mg/L)	8.61±1.94	5.73±2.02	5.28±1.73
Glutathione[@] (µmol/L)	235.46±80.89	227.35±74.04	257.76±96.41
Malondialdehyde[@] (nanomol/ml)	5.92±1.55	5.89±1.53	6.0±1.65
Total (Nitrite/ Nitrate)[@] (µmol/L)	23.05±2.51	23.25±5.59	22.49±5.47
Augmentation Pressure[*] (mm of Mercury)	13.75±4.98	12.89±5.19	16.13±3.47
Augmentation Index[#] (Ratio)	32.53±7.26	29.8±5.68	40.01±5.74

@ p >0.05 between all the three groups. *p=0.02 between diabetes mellitus and coronary heart disease. # p=0.04 between total group and coronary artery disease and p=0.0001 between diabetes mellitus and coronary artery disease

Table 3: Inflammatory marker (high sensitive C - Reactive Protein), oxidative stress (malondialdehyde and glutathione), total (nitrite/ nitrate) and endothelial function (augmentation pressure, augmentation index in three age groups.

Parameter	Age group 20-40 years (n = 13)	Age group 40-60 years (n = 41)	Age group >60 years (n = 06)
hs CRP@ (mg/L)	5.92±1.64	5.65±2.01	4.89±2.31
Glutathione@ (µmol/L)	225.12±62.13	235.36±88.67	247.88±69.22
Malondialdehyde@ (nanomol/ml)	5.83±1.72	5.77±1.48	6.74±1.36
Total (Nitrite/ Nitrate)@ (µmol/L)	25.01±4.05	22.94±5.23	20.51±8.89
Augmentation Pressure* (mm of Mercury)	10.59±3.34	14.41±5.22	15.83±3.92
Augmentation Index@ (ratio)	29.67±7.97	32.90±7.04	34.83±7.28

@ p >0.05 between all the three groups. * p=0.0096 age group 20-40 years and age group > 60 years

Levels of inflammatory marker (high sensitive C - Reactive Protein), oxidative stress (malondialdehyde and glutathione), total (nitrite/ nitrate) and endothelial function (augmentation pressure, augmentation index in the three groups of the study. Inflammatory status (high sensitive C reactive protein (mg/L) in total group 8.61±1.94; diabetes mellitus 5.73±2.02 and coronary artery disease 5.28±1.73), oxidative stress measured by glutathione (µmol/L) in total group 235.46±80.89; diabetes mellitus 227.35±74.04 and coronary artery disease 257.76±96.41) and malondialdehyde (nanomol/ml) in total group 5.92±1.55; diabetes mellitus 5.89±1.53 and coronary artery disease 6.0±1.65) and nitric oxide as total (nitrite/ nitrate) (µmol/L) in total group 23.05±2.51; diabetes mellitus 23.25±5.59 and coronary artery disease 22.49±5.47) showed no statistical difference between total group, Diabetes Mellitus Group and Coronary Artery Group.

Augmentation Pressure (mm of mercury) did not show any statistical difference (p>0.05) when compared between total group (13.75±4.98), diabetes mellitus group (12.89±5.19) and coronary artery group (16.13±3.47). But augmentation pressure showed statistical difference (p=0.02) when compared between diabetes mellitus group and coronary artery group. Augmentation index (ratio) did not show any statistical difference when compared between total group (32.53±7.26) and diabetes mellitus group (12.89±5.19). But Augmentation Index is statistical difference (p=0.04) between Total group and coronary heart disease and p=0.0001 between diabetes mellitus and coronary heart disease.

Levels of Inflammatory Marker (High sensitive C - Reactive Protein), oxidative stress (malondialdehyde and glutathione), total (nitrite/ nitrate) and endothelial function (augmentation pressure, augmentation index when the total study population is divided into different age groups. In all the three age groups High sensitive C - Reactive Protein (20-40 years- 5.92±1.64, Age Group 40-60- 5.65±2.01 years and >60 years- 4.89±2.31), Oxidative stress in form of Glutathione (20-40 years- 225.12±62.13, age group 40-60-235.36±88.67 Years and >60 years- 247.88±69.22), and malondialdehyde (20-40 years- 5.83±1.72, age group 40-60-5.77±1.48 years and >60 Years- 6.74±1.36), and nitric oxide as total (nitrite/ nitrate) (20-40 years- 25.01±4.05, age group 40-60-22.94±5.23 years and >60 years- 20.51±8.89), did not show any statistical difference (p>0.05). The same pattern was seen with augmentation pressure (20-40 years-10.59±3.34, age group 40-60- 14.41±5.22 years and >60 years- 15.83±3.92). While comparing augmentation index there was statistical difference between age group 20-40 (29.67±7.97) years and age group (>60 years 34.83±7.28).

DISCUSSION

In 1990, endothelial dysfunction was first described in human hypertension in the forearm vasculature. Impairment of vasodilatation has also been described in type 1 and type 2 diabetes, coronary artery disease, congestive heart failure, and chronic renal failure. The pathophysiology of endothelial dysfunction is complex and involves multiple mechanisms.

The results from this study confirm that diabetes mellitus subjects with CAD are having a significant Endothelial dysfunction when compared to subjects with diabetes mellitus alone. Subjects with diabetes mellitus showed lesser levels of glutathione, total nitrite/nitrate, elevated levels of MDA and hS CRP levels in our study. In spite of no statistical difference between the Diabetes Mellitus subjects with CAD and without CAD in above markers, Augmentation Pressure and Augmentation Index measures of Endothelial dysfunction have varied significantly between these groups. Findings from our study are concurring with results from Paola Gargiulo et al, who found that Diabetic patients with and without CAD show significantly impaired peripheral vascular function compared to non-diabetic patients without CAD.[26] ED in diabetic patients without CAD is comparable to patients with CAD without DM. In another study by Gissette Reyes-Soffer et al, showed patients with CAD, the concomitant presence of T2DM is independently associated with greater ED.[27]

In diabetes, various mechanisms can trigger endothelial dysfunction. Conditions like insulin resistance, as seen in type 2 diabetes where insulin signalling is altered, hyperglycemia leads to advanced glycation end products (AGE), which extinguish Nitric Oxide (NO) and impair endothelial function.[28] AGE induce ROS and promote vascular inflammation, with enhanced expression of interleukin-6, VCAM-1, and MCP-1.[29] This forms a vicious circle in conditions like diabetic nephropathy, as renal failure delays the clearance of AGE, further promoting vascular and renal injury.[30] Acute hyperglycemia by itself can reduce NO and attenuate endothelium-dependent vasodilation in humans in vivo.[31]

Diabetes Mellitus regulation of NO metabolism is particularly important, since the activation of eNOS has been demonstrated to be under the insulin control.[32,33] In pathological conditions, like diabetes mellitus, NOX activity and superoxide production are increased.[34] Oxidative stress plays an important role in the development of insulin resistance and both micro- and macro-vascular diabetic complications.[35] Diabetes Mellitus is associated with an impairment of cellular autophagy.[36] Proinflammatory status of the vessel wall is directly linked to Oxidative excess. NO bioavailability is reduced by Inflammation and C-reactive protein (CRP) has been implicated to decrease eNOS activity.

Acute inflammatory states have been implicated to impair endothelium-dependent vasodilatation through inflammatory mediators like tumour necrosis factor-α (TNF-α) and decrease eNOS expression in ECs.[37] Diabetes is considered to be a systemic inflammatory state that can impair endothelial function and contribute to atherosclerosis.[38] In diabetic patients there is an increase in circulating levels of inflammatory markers like C reactive protein, TNF- Alfa, and intercellular adhesion molecule-1.[39,40] This increase in inflammatory markers is considered as a double edged sword as on one hand this is predictor of increased cardiovascular risk in diabetic patients and on the other hand, they also relate to the incidence of diabetes mellitus.[41,42]

In Diabetes Mellitus Endothelial dysfunction contributes to cardiovascular morbidity.[43] Others mechanisms contributing to development of diabetes-induced endothelial dysfunction include prostanoid vasoconstrictors production and increased oxidative degradation of NO.[44] Atherogenesis and increases vascular resistance are a result of deficient NO production along with other mechanisms of NO deficiency, increased oxidative degradation, and NO deficiency caused by asymmetric dimethylarginine (ADMA) finally

contributing to Cardiovascular morbidity and mortality in Diabetes Mellitus.[45,46]

Diabetes Mellitus individuals are at increased risk for development of cardiovascular disease (CVD). There is profound association between like hood for development of atherosclerotic plaque formation and Diabetes Mellitus although the exact mechanisms of association between these two are not completely defined. Reduced NO and increased oxidative stress may activate matrix metalloproteinases (MMP), mainly MMP-2 and MMP-9, which weaken the fibrous cap. Thrombogenicity and its severity are contributed by reduced NO and inhibition of platelet aggregation.[31] In type 1 or type 2 diabetes CVD is the primary cause of death and it accounts for highest component of health care expenditures in people with diabetes.[47,48] In prediction of stroke and cerebrovascular disease in CAD, Diabetes Mellitus is considered to be a strong independent risk factor.[49]

Reduced NO bioavailability, increased oxidant excess, and expression of adhesion molecules contributes to Endothelial Dysfunction both for its initiation and progression of atherosclerotic plaque formation and finally to triggering of cardiovascular events. Because endothelial dysfunction is an early event, it may be of prognostic value. As the measures of endothelial dysfunction become clinically applicable, this may translate into improved methods of risk assessment that help in predicting, preventing and treating cardiovascular disease.

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