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Research Article

EFFECTS OF AGEING ON LEVELS OF NT-PROBNP [AMINO TERMINAL-PRO BRAIN NATRIURETIC PEPTIDES] IN ELDERLY DIABETES MELLITUS TYPE-II FEMALES

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ABSTRACT
To investigate the serum NT-proBNP, as a biomarker of cardiovascular diseases-Left ventricular hypertrophy, in females with diabetes type-II and its association with conventional cardiovascular risk factors i.e., Total Cholesterol, Triglycerides, HDL-Cholesterol, LDL-Cholesterol, and Blood Glucose. NT-proBNP were measured by fluoroturbidometry method. 40 diabetes type-II females and 20 without diabetes age matched females selected. The correlations between NT-proBNP and other variables of cardiovascular risk factors were assessed. The levels of NT-proBNP concentration in diabetes type-II female patients were significantly elevated. The MDA concentrations of 40 subjects were high as compared to controls, P<0.001. NT-proBNP levels were significantly correlated (P<0.05) with conventional cardiovascular risk factors (age, Glucose, Total Cholesterol, Triglycerides, HDL-Cholesterol, LDL-Cholesterol, Urine Albumin, and blood pressure). Serum levels of NT-proBNP is currently one of the most commonly proposed as a choice of cardiac biomarker. Serum levels of NT-proBNP increased significantly in diabetes type-II female patients. It may be a good marker for Left Ventricular Dystrophy and an oxidative stress event that is implicated in various pathological conditions.
Keywords: NT-proBNP, Lipid Peroxidation, Malondialdehyde, Cardiovascular Disease, Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus-Type II is recognized as one of the leading causes of morbidity and mortality. Diabetes mellitus-Type I increases the free radical's activity[1]. Diabetes mellitus-type II is associated with - increased activity of free radical-induced[2] lipid peroxidation, in tissues accumulation of lipid peroxidation products,[3] a substantially higher prevalence of atherosclerositic and cardiovascular mortality.[4] The mechanisms of free radicals formation in diabetes mellitus-type-II includes increased non-enzymatic and auto-oxidative glycosylation, metabolic stress resulting from changes in energy metabolism, and the levels of inflammatory mediators.[4]

The concentrations of plasma lipid peroxides, and malondialdehyde, are significantly high during congestive heart failure[5]. There was a significant negative correlation between malondialdehyde and left ventricular ejection fraction (LVEF)[6]. The pericardial levels of 8-iso-prostaglandin F2a (8-iso-PGF2a), is a specific non-enzymatic peroxidation product of arachidonic acid, increases with increase in functional severity of heart failure[7], and is also associated with ventricular dilatation[8]. Oxidative stress markers other than lipid peroxides, levels of 8-hydroxy-2-deoxyguano sine (8-OHdG), a marker of oxidative DNA damage, are elevated in serum and urine of patients with heart failure, and urinary 8-OHdG reflects the clinical severity of congestive heart failure (CHF) on the basis of symptomatic status and cardiac dysfunction[9]. Studies indicated that oxidative stress is involved in severity of heart failure. Levels of oxidative stress are also elevated in the myocardium of patients with heart failure. Reactive Oxygen Species cause damage to lipid cell membranes in the process of lipid peroxidation. In this process, several aldehydes, including 4-hydroxy-2-nonenal (HNE), are generated and the amount of HNE is increased in the human failing myocardium[10]. The presence of 8-OHdG has also been detected in nuclei of cardiac myocytes in patients with dilated cardiomyopathy[10]. The levels of oxidative stress are elevated in both body fluid including serum, urine and pericardial effusion and myocardium of patients with heart failure. Different oxidative stress levels stimulate cellular proliferation, trigger apoptosis or produce necrosis in various cells[6]. Reactive Oxygen Species (ROS) can exert a graded effect on the cardiac myocyte phenotype[11]. Lower levels of oxidative stress stimulate hypertrophy and higher levels of oxidative stress induce apoptosis1[12]. Higher rates of reactive oxygen species production contribute to the transition from compensatory left-ventricular hypertrophy to heart failure[13].

Lipid peroxidation is an autocatalytic free radical-mediated destructive process whereby poly-unsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides.[14-15]. By-products of lipid peroxidation i.e., conjugated dienes and malondialdehydes (MDA) are increased in the circulation of diabetic patients. MDA, a three carbon dialdehyde, is generated as a relatively stable end product from the oxidative degradation of poly-unsaturated fatty acids (PUFA)[16]. This free radical-driven lipid peroxidation has been causatively implicated in the aging process[17], atherosclerosis[18], and cancer[19]. Serum MDA has been used as a biomarker of lipid peroxidation and has served as an indicator of free radical damage. Similarly, NT-proBNP is used to assess the left ventricular dystrophy of heart muscles during the breathing not properly symptoms.

The NT-proBNP usually increases in the elderly subjects suffering cardiac ailments with or without diabetes. The hypothesis of the current study is that elevated levels of malondialdehyde as thiobarbituric acid reactive substances (TBARS)[20] are associated with increased risk of cardiovascular complications in patients with Type-II diabetic females. The aim of this study was to investigate the serum NT-proBNP levels, as a biomarker of cardiac ailments, and associated immunological disorders with lipid peroxidation in diabetes type-II patients, and other conventional cardiovascular risk factors in elderly females.

MATERIALS AND METHODS

Forty diabetic females with a median age of 62.9 years were selected from the Escorts Heart Institute and Research Centre New Delhi. And 20 non-diabetic healthy females [considered

as control] with a median age of 62.5 years were selected. All subjects gave written informed consent. Venous blood samples were taken without stasis after a 12-hour fast and a 30 minute rest in a supine position. Plasma glucose (Glu), serum total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), BUN, Creatinine (CT), and uric acid (UA) were measured immediately after blood collection. Plasma Glucose, serum TC, TG, HDL-C, UA, and the other biochemical variables were measured by standard enzymatic methods using a BM Hitachi 902 analyzer (all reagents were from Roche Diagnostics); low-density lipoprotein cholesterol (LDL-C) was estimated[21]. Blood pressure was measured with the patients in the supine position after 5 minutes resting period. Blood pressure was the mean value of at least two measurements of patients and the control subjects on the same day. For the determination of MDA, serum was stored at -80^{0}C without the addition of exogenous antioxidants before MDA analysis. After thawing the samples, measurements of MDA in term of TBARS were performed[22] in a double-beam spectrophotometer (Shimadzu, Japan). The within-run and between-run precisions of these assays analyzed in two pooled-control sera. The replicate analyses were of 6.62% and 6.81% for within-run precision assay, and 7.62% and 7.19% for between-run assay with the MDA concentration averaging (SD) 2.283 (0.153), 4.42 (0.267) and 2.285 (0.169), 4.472 (0.354) µmol/L. The percentage recovery for this MDA determination in serum sample and standard were 97.6% and 98.6% respectively. All analyzes were undertaken using $p < 0.05$ (two-tailed) as the significant statistic.

RESULTS

Clinical characteristics of the healthy controls and diabetes type-II patients are shown in Table 1. The median level for serum MDA concentrations in subjects and controls were 2.84 µmol/L and 1.71µmol/L respectively (2.5^{th}/97.5^{th} percentile = 0.734-2.848 µmol/L, and 2.31-3.52µmol/L, respectively). In this study, the age in both groups were not significantly different ($P = 0.06$). Diabetes type-II patients had elevated MDA (≥2.86 µ mol/l), The serum MDA levels, a product of lipid peroxidation as the oxidative stress marker from diabetes type-II patients were significantly higher than the MDA levels from healthy controls (P<0.001). The conventional cardiovascular risk factors (Glucose, TC, TG, LDL-C, Urine Albumin, and systolic blood pressure) were also significantly higher (P<0.05) in diabetes type-II patients than healthy controls. HDL-C levels were also significantly lower in diabetes type-II patients (P<0.001). The serum MDA levels were significantly correlated (P< 0.05) with the conventional cardiovascular risk factors (age, Glucose, TC, TG, LDL-C, HDL-C, Urine Albumin, and blood pressure) using the Spearman rank correlation test (Table-2).

DISCUSSION

Increasing evidence in experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes type-II. Free radicals are formed disproportionately in DM by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation which may play an important role in the development of complications in T2D patients. The levels of MDA (as TBARS) were significantly elevated in diabetes type-II patients.[16-17]. In the present study, the serum MDA levels were also significantly elevated in diabetes type-II patients. The free radical activity is increased in the type 2 diabetic mellitus[1,2] which leads to a higher incidence of atherosclerotic and cardiovascular disease.[4]The absolute level of atherogenic lipoproteins in the circulation is a major factor in the risk of cardiovascular diseases, and this oxidative modification of blood lipids further increased this risk[23]. Oxidized LDL particles are more readily internalized by macrophages and thereby enhance foam cell formation which, in the vascular wall, favors smooth muscle cell proliferation, increases platelet adhesion and the anticoagulant activity of the endothelium[24,40]. These consequences of oxidative stress, aggravates the development of complications in diabetes mellitus patients. In our study, the diabetes type-II patients had increased lipid peroxidation and decreased levels of reduced glutathione, glutathione reductase, glutathione peroxidase, glutathione and G6PDH[25]. During ageing phenomenon there is an increased lipoperoxide level, which is correlated to cardiovascular complications[25] i.e., atherosclerosis, Alzheimer's disease[26], and cancerous conditions. Lipid peroxidation is an autocatalytic free-radical-mediated destructive process whereby Poly Unsaturated Fatty Acids (PUFA) undergoes degradation to form lipid hydroperoxides. These decompose to form a wide variety of products i.e., ketone, alkenals, low-molecular mass hydrocarbons, alkanals, free fatty acids, hydroxyl aldehydes, and MDA. The methods most widely used is based on the reaction of MDA with TBA, in which one molecule of MDA reacts with two molecules of TBA to form a stable pink to red color that absorbs maximally at 532nm[27,35]. The median level for serum MDA concentrations in subjects and controls were 2.84 µmol/L and 1.71µmol/L respectively (2.5^{th}/97.5^{th} percentile = 0.734-2.848 µmol/L, and 2.31-3.52µmol/L, respectively). Diabetes type-II patients had elevated MDA (≥2.68 µ mol/l). These results were close to previous researchers as determined by gas chromatography-mass spectrophotometry, 1.30 ± 0.07µmol/L[28] and 1.67 ± 0.16 µmol/L[29]. Gliesner et al[30] showed no statistically significant differences were found for any of the oxidative stress markers (PCG) assessed between patients with diabetic patients and controls. In addition, weight, height, and routine metabolic tests, including creatininemia and cholesterol levels, were similar between the groups. The lack of significant differences between healthy controls and patients with diabetics suggested that treatment is able to counteract the increase in free radical production. Ahmed et al[31] observed profound increases in proteolytic products of glycated and oxidized proteins in diabetic patients, concurrent with much lower increases in protein glycation and oxidation adduct residues. Nitric oxide (NO) is an important vascular target for reactive oxygen species (ROS). Superoxide neutralizes Nitric Oxide, and the peroxynitrite formed is a source of hydroxyl radicals that can cause endothelial damage[33-39]. A significant negative correlation coefficient between blood glucose and vitamin C has been observed. Patients with diabetes or the metabolic syndrome have low levels of the antioxidant vitamin C[34], and also control the diabetes[35]. In conclusion, increased oxidative stress caused an increased lipid peroxidation, measured as serum malondialdehyde (TBARS) concentration. The Females suffering with diabetes type-II had significantly high NT-proBNP concentration than the healthy controls (P<0.001). A significant correlation was found with the traditional cardiovascular risk factors (age, Glucose, TC, TG, LDL-C, HDL-C, UA, and blood pressure). This is a serious cause of concern for the elderly females having diabetes. Appropriate measures should be taken for the prevention of cardiovascular disorders.

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Table 1: Characteristics in Elderly Females type 2 diabetic Indian patients
[Values as medians and interquartile (Q1-Q3)]

Parameters	Non-Diabetic Controls [n=20]	Diabetic Females[n=40]
BMI	23.83	26.5
(Q1-Q3)	19.75-26.21	23.26-28.14
Weight (Kg)	59.5	56.4
(Q1-Q3)	52.4-67.9	51.9- 65.9
Systolic blood pressure (mmHg)	123.26	132.84
(Q1-Q3)	115.14-134.8	121.5-142.7
Diastolic blood pressure (mmHg)	74.8	78.4
(Q1-Q3)	70.1-79.5	72.8-88.1
Fasting blood glucose (mg/dL)	85.7	119.5
(Q1-Q3)	78.5-96.4	97.5-151.6
Blood Urea Nitrogen (mg/ dL)	10.8	13.9
(Q1-Q3)	9.1-13.4	11.6-16.5
Creatinine (mg/dL)	0.79	0.94
(Q1-Q3)	0.69-0.79	0.77-1.04
Uric acid (mg/dL)	4.7	6.3
(Q1-Q3)	4.2-6.5	5.1-6.9
Total cholesterol(mg/dL)	169.8	208.6
(Q1-Q3)	161.5-191.7	188.3-231.8
Triglycerides (mg/dL)	131.4	178.4
(Q1-Q3)	124.0-180.4	128.4-295.2
HDL	43.1	56.8
(Q1-Q3)	37.1-48.6	49.5-66.7
LDL cholesterol (mg/dL)	91.8	113.4
(Q1-Q3)	84.5-94.8	91.7-128.9
MDA (µ mol/L)	1.71	2.84
(Q1-Q3)	1.354-2.1	1.95-3.59
NT-proBNP(pg/ml)	8.5	3698
(Q1-Q3)	0.4-14.8	2608.5-4987.2

TABLE 2.CORRELATIONS BETWEEN SERUM NT-PROBNP WITH CARDIOVASCULAR RISK FACTORS

	NT-proBNP (Amino Terminal–proBrain Natriuretic Peptide)	
Parameters	**r**	**(p-Value)**
Total cholesterol	0.447	0.005
Triglycerides	0.241	0.035
HDL-Cholesterol	0.284	0.021
LDL-Cholesterol	0.318	0.016
MDA	0.410	0.012
Plasma Glucose	0.484	0.005
Serum Uric acid	0.366	0.001
BUN	0.01	0.030
Systolic blood pressure	0.210	0.034
Diastolic blood pressure	0.188	0.017

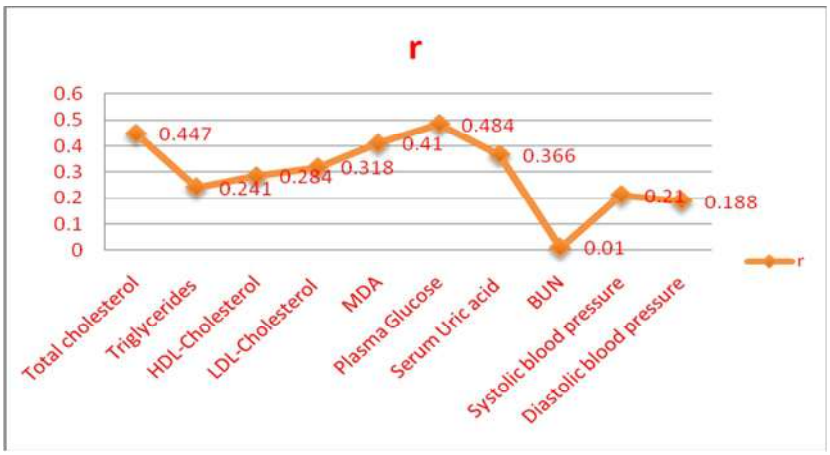

FIGURE: CORRELATIONS BETWEEN NT-PROBNP WITH CARDIOVASCULAR RISK FACTORS

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