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Serum Homocysteine Levels and Blood Pressure in Type 2 Diabetic Patients in Zaria, Northern Nigeria.

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Abstracts

Hyperhomocysteinaemia and hypertension are established cardiovascular risk factors for cardiovascular diseases. However, knowledge on the contributions of these risk factors in diabetic patients is limited. The purpose of this study was to assess serum homocysteine concentrations and blood pressures in Type 2 diabetic patients in Zaria, Kaduna State, Northern Nigeria. In this cross-sectional study, serum homocysteine levels and blood pressures were assessed in 140 men and women with type 2 diabetes aged 20-60 years in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. Diabetes mellitus status was confirmed biochemically according to World Health Organization diagnostic criteria for classification of diabetes mellitus. Hypertension was confirmed according to World Health Organization diagnostic criteria. Serum homocysteine concentration was measured using ELISA method. Data for selected clinical/demographic variables were obtained from fasting blood samples using an interviewer-administered questionnaire. The mean values of serum homocysteine, Waist circumference (WC), Hip circumference (HC), WC: HC, Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) in diabetic patients were significantly higher (p=0.000) than those of controls, whereas the mean values of age, weight (WT), height (H) and Body Mass Index (BMI) in diabetic patients were similar to those of controls (p=0.972, p=0.271 and p=0.825 respectively).

We observed that that High levels of homocysteine and high blood pressures all contributes to the development of cardiovascular complications in people with type 2 diabetic mellitus.

Key words: Homocysteine, Blood Pressures, Type 2 Diabetes Mellitus, Cardiovascular Disease Zaria.

Introduction

Diabetes mellitus (DM) is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves (WHO, 2020). About 422 million people worldwide have DM, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to DM each year. International Diabetes Federation (IDF) predicts that the number of people living with DM will rise in Africa from 21.5 million in 2014 to 41.5 million by 2035 (IDF, 2015).

Homocysteine (HCY) is a sulfurous α-amino acid which acts as an intermediate molecule in methionine metabolism (Ganguly and Alam, 2015). When HCY is produced in the body it can be metabolized via either sulphuration pathway, demethylation pathway or remethylation pathway (Ghaedi *et al*, 2007). Only a small fraction (2%) of plasma total HCY circulates in thiol form. The remainder is a mixture of disulfide derivatives, including homocysteine, homocysteine-cysteine mixed disulfides and protein-bond disulfides (Ghaedi *et al*, 2007). Hyperhomocysteinemia has been identified as an independent risk factor for cardiovascular disease (CVD). Many studies have demonstrated that hyperhomocysteinaemia is linked to

endothelial damage (Cao *et al,* 2015), atherosclerosis (McCully, 2015) and venous thrombosis (Heijer *et al,*1998).

Hypertension, also known as high or raised blood pressure, is a condition in which the blood vessels have persistently raised pressure. Blood pressure is created by the force of blood pushing against the walls of blood vessels (arteries) as it is pumped by the heart. The higher the pressure, the harder the heart has to pump. Hypertension is clinically defined as systolic blood pressure readings of $\geq$ 140 mmHg and/or the diastolic blood pressure readings of $\geq$ 90 mmHg. Hypertension is the strongest or one of the strongest risk factors for almost all different cardiovascular diseases acquired during life, including coronary disease, left ventricular hypertrophy and valvular heart diseases, cardiac arrhythmias including atrial fibrillation, cerebral stroke and renal failure (Sverre, 2018).

The purpose of this study was to assess the serum HCY levels and blood pressures in Type 2 diabetic patients. We speculated that both hyperhomocysteinemia and hypertension may contribute to the adverse cardiovascular disease consequences in Type 2 diabetic patients.

Materials and Methods

The study was conducted in the Departments of Chemical Pathology and Medicine of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. A total of 240 subjects aged 20-60 years were recruited for the study. We sourced the subjects (140) 55 males and 85 females with mean age of 52±1.2 years from among diabetic patients attending Medical Out-patient Department (MOPD) Clinic and 100 apparently healthy subjects with mean age of 50±1.4 years. The apparently healthy subjects (controls) were recruited from the population of the study area.

Diabetes mellitus status and subject selection was confirmed biochemically according to World Health Organization diagnostic criteria for classification of diabetes (WHO, 1999). The approval of the study was obtained from the Ethical Committee of the College of Medical Sciences, Ahmadu Bello University, Zaria, in accordance with Helsinki declaration. Written informed consent was obtained from every participant.

Blood Pressure and Anthropometric Measurements

Systolic and diastolic blood pressure was recorded using an Andon BPM an automatic blood pressure monitor (Model: KD-595). Blood pressure was recorded as the average of the last two of three consecutive readings, obtained from the right arm of seated subjects at 1-min intervals after a 10-min rest period. Weight was measured to within 0.1 kg before breakfast and following a 12-h fast, with subjects wearing light clothing and no shoes, using (HANA) a mechanical bathroom scale (Model:BR). Height was measured to within 0.1 cm using a wall-mounted stadiometer. BMI was calculated as weight in kilograms divided by the square of height in meters (2). Waist Circumference and Hip Circumference were measured to within 0.1cm using a standard tape. Waist Circumference to Hip Circumference ratio was calculated as Waist Circumference in centimeters (cm) divided by Hip Circumference in centimeters.

Serum /plasma biochemistry

Hettich Universal 32 Centrifuge (Germany) was used to spin the blood specimens. Serum Homocysteine concentration was assayed using a commercially ELISA Kit (Nelsin Medical, Limited, China). Serum homocysteine concentrations were read on a micro-plate reader (RT-6000 Rayto China). Serum glucose concentrations were analyzed using an automatic analyzer (Serial no.6-8096; Selectra XL Series, Vital Scientific, Netherlands) and a commercial enzymatic kit (ELiTech Group Empowering IVD, Sees, France) by the glucose oxidase method. Serum cholesterol triacylglycerol concentrations and high-density lipoprotein cholesterol (HDL) were also analyzed with the Selectra XL Series, Vital Scientific, Netherlands; Serial no.6-8096; analyzer using a commercial enzymatic kit (ELiTech Group Empowering IVD, Sees, France). Low density lipoprotein cholesterol (LDL) concentrations were calculated using Friedewald equation (Friedewald *et al,* 1972). Atherogenic indices (ratio) were calculated as the concentration of total cholesterol divided by

the concentration of high-density lipoprotein cholesterol. Glycated Haemoglobin (HbA1c) was assayed using a commercially available Kit (Labcare Diagnostic, Gujarat, India) by the Ion Exchange Resin Method. Beckman Coulter DU-520 general purposes UV/VIS Spectrophotometer (Germany) was used to measure the concentrations of HbA1C,

Statistical analyses

We used SPSS software for Windows (version 21; SPSS, IL) to perform statistical analyses. Serum HCY, lipids, glucose, plasma and glycated haemoglobin concentrations obtained from diabetic patients were compared with those of apparently healthy individuals (controls) using two-tailed student's t-test. Similarly, the results of Age, WC, HC, WC: HC, WT, H, BMI, SBP and DBP obtained from diabetic patients were compared with those of apparently healthy individuals (controls) using two-tailed student's t-test. *A p-value of* < 0.05 was considered statistically significant.

Results

The mean values of Age, WC, HC, WC: HC, WT, H, BMI, SBP and DBP were compared between diabetic patients and controls as shown in Table 1. The corresponding values for the above variables were 52±1.2 vs 50±1.4 (p=0.857), 93±1.7 vs 79±1.3 (p=0.000), 98±2.2 vs 87±1.3 (p=0.000), 1.0±0.1 vs 0.1±0.1 (p=0.000), 70±2.3 vs 70±1.3 (p=0.972), 1.9±1.6 vs 1.61±0.1 (p=0.271), 26±1.3 vs 27±1.1, (p=0.825) 132±3.3 vs 91±2.2 (p=0.000) and 83±3.5 vs 49±3.2 (p=0.000) respectively. The results showed that systolic blood pressure (SBP) and diastolic blood pressure (DBP) as well as WC, HC, WC: HC in diabetic patients were higher than those of controls, whereas age, WT, H and BMI in diabetic patients were similar to those of controls.

The mean values of HCY, TC, TG, HDL, LDL, TC: HDL, FBG and HbA1c were compared between the two groups as shown in Table 2. The corresponding values for the above variables were 10.5±1.5 vs 5.8±1.1 (p=0.007), 4.1±0.1 vs 2.0±0.2 (p=0.000), 1.5±0.1 vs 1.3±0.1 (p=0.001), 0.7±0.1 vs 2.2±0.1 (p=0.000), 2.7±0.1 vs 2.5±0.2 (p=0.000), 7.9±0.7 vs 1.4±0.2 (p=0.000), 7.9±0.4 vs 4.5±0.1, (p=0.000) and 7.9±0.2 vs 5.5±0.5 (p=0.000) respectively. The results of homocysteine and other biochemical analytes were higher among diabetic patients than those of control group, whereas HDL concentration in diabetic patients was lower than those of control subjects.

Table 1: Anthropometric and Clinical Parameters (Mean±SEM) in Diabetic Patients and Controls

Subjects	N	Age (Years)	WC (cm)	HC (cm)	WC: HC	WT (Kg)	H (m)	BMI	SBP (mmHg)	DBP (mmHg)
Subjects	140	52±1.2	93±1.7	98±2.2	1.0±0.1	70±2.3	1.9±1.6	26±1.3	132±3.3	83±3.5
Controls	100	50±1.4	79±1.3	87±1.3	0.1±0.1	70±1.3	1.6±0.1	27±1.1	91±2.2	49±3.2
P-Value		0.857	0.000	0.000	0.000	0.972	0.2705	0.825	0.000	0.000

n=Number of subjects, WC=Waist circumference, Hip circumference, WC: HC=Waist circumference to Hip circumference ratio, WT=Weight, H=Height, BMI=Body mass index, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, SEM=Standard Error of Mean

Table 2: Biochemical Analytes (Mean±SEM) in Diabetic Patients and Controls

Subjects	N	HCY (µmol/L)	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	TC: HDL	FBG (mmol/L)	HbA1c (%)
Subjects	140	10.5±1.5	4.1±0.1	1.5±0.1	0.7±0.1	2.7±0.1	7.9±0.7	7.9±0.4	7.9±0.2
Controls	100	5.8±1.1	2.0±0.2	1.3±0.1	2.2±0.1	2.5±0.2	1.4±0.2	4.5±0.1	5.5±0.5
P-Value		0.007	0.000	0.001	0.000	0.000	0.000	0.000	0.000

n=number of subjects, tHCY= total homocysteine, TC= total cholesterol, TG= triglyceride, HDL= high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, TC: HDL=total cholesterol-high density lipoprotein cholesterol ratio, FBG=fasting blood glucose (FBG), HbA1c=glycated haemoglobin and SEM=Standard Error of Mean

Discussion

Hyperhomocysteinaemia and hypertension are established risk factors for cardiovascular diseases. The results obtained in the present study showed that serum HCY levels were significantly higher in diabetic patients than in controls. This indicates that the diabetic patients under this study had mild hyperhomocystienaemia. This is consistent with the reports of previous studies (Maria and Ester, 2012; Saurabh *et al.,*2016). However, Platt *et al.* (2017) found lower serum HCY levels in diabetic patients than in controls. The mechanisms by which HCY promotes cardiovascular disease are uncertain. Increased HCY level has shown a pre-dilation towards promotion of platelet adhesion to endothelial cells and has also been associated with higher levels of prothrombotic factors such as β-thromboglobulin, tissue plasminogen activator and factor VIIc (Zhang *et al.*, 2012). These lead to the augmentation of thrombus formation.

McAnulty *et al.* (2005) suggested that elevated HCY level has both atherogenic and thrombogenic effects, causes endothelial dysfunction by increasing oxidative stress and in part decreases the release of NO as well as impairing vasodilation.

To date, the mechanisms behind the Type 2 DM correlation with HCY levels have been difficult to identify. Daniel *et al.* (2017) reported that decreased insulin secretory responsiveness, caused by the destructive production of reactive oxygen species (ROS) as a result of elevated HCY levels leads to insulin resistance. It has also been suggested that in patients with insulin resistance, there is hepatic acceleration of glucocorticoid secretion that also leads to enhanced HCY catabolism and decreased plasma HCY levels (Daniel *et al.*, 2017).

Earlier study on Type 2 diabetic patients reported a strong association between elevated HCY levels and early CVD events (Daniel *et al.*, 2017; McAnulty et al 2005). Moderately raised serum HCY as obtained in this study are indications that Type 2 diabetic patients are at risk of early CVD events.

The present study showed that the mean values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were higher in subjects compared to controls. This present study indicated that the diabetic patients were hypertensive when the WHO classification was applied. This is in agreement with the work of Ghulam *et al.* (2019). According to Angelo *et al.* (2018), activation of phosphotydalinositol-3-kinase (PI 3-kinase) pathway has been associated with production of nitric oxide (NO), particularly due to an increased rate of endothelial nitric oxide synthase (eNOS) gene expression. Down regulation of PI 3-kinase in vascular cells were observed in insulin resistance state and this may suppress the vasodilative effect of insulin via NO production which in turn causes endothelial dysfunction and promotes the development of hypertension (Cusi *et al.,* 2000). This could be the reason for the presence of hypertension seen in the present study. Furthermore, PI 3-kinase also stimulates several

pro-atherogenic mechanisms which are mainly vascular smooth muscle cell (VSMC) proliferation and migration, leukocyte adhesion to endothelial cells and platelet aggregation (Angelo *et al.,* 2018). The presence of hypertension expedites the development of cardiovascular complications in diabetic patients like stroke, myocardial infarction, retinopathy, neuropathy and nephropathy (Ghulam *et al.,* 2019). The concomitant presence of hypertension in diabetic patient increases the mortality and morbidity of these patients (Epstein and Sowers, 1992).

Conclusion

This study showed that hyperhomocysteinaemia and hypertension both contribute to the development of cardiovascular complications in Type 2 diabetes mellitus.

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