# SERUM HOMOCYSTEINE LEVELS AND LIPID PROFILE IN TYPE 2 DIABETIC PATIENTS IN ZARIA, NORTHERN NIGERIA

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## ABSTRACT

**Background:** Diabetes mellitus (DM) is one of the most frequently occurring non-communicable disorders characterized by hyperglycemia. Diabetic individuals are highly prone to cardiovascular diseases (CVD) and hence it is necessary to search for advanced markers to assess the CVD risk.

**AIM:** The aim of this study was to evaluate serum homocysteine concentrations and lipids in Type 2 diabetic patients in Zaria, Kaduna State, Northern Nigeria.

**Materials and Methods:** The study was a cross-sectional type. A consecutive sampling method was employed to select the subjects who satisfy the study inclusion criteria. Informed consent for inclusion into the study was obtained from the subjects. Anthropometric measures, blood pressures were recorded for each of the subjects. This was followed the collection of blood specimens. Diabetes mellitus status was confirmed biochemically according to World Health Organization diagnostic criteria for classification of diabetes mellitus. We assessed 140 men and women with type 2 diabetes in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. Serum total homocysteine concentration was measured using ELISA method. Serum lipids concentrations were measured using a commercial enzymatic kit method.

**Results:** The mean values of homocysteine levels and lipids (TC, TG, HDL, LDL, TC: HDL), were higher among diabetic patients than those of control group (p=0.000). Similarly the mean values of other biochemical analytes (FBG and HbA1c) were also higher among diabetic patients when compared to control group (p=0.000).

**Conclusion:** High levels of homocysteine and dyslipidaemia are implicated in the development of cardiovascular complications in people with type 2 diabetes mellitus.

**Key words:** Type 2 Diabetes mellitus, Homocysteine, Dyslipidaemia, Cardiovascular

## 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 (World Health Organization, 2020).

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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 (International Diabetes Federation, 2015).

Homocysteine (HCY) is a thiol amino acid produced from methionine metabolism that does not participate in the synthesis of protein (Ghaedi*et al.,* 2007). When HCY is produced in the body it can be metabolized via either sulfuration 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 homocystiene, homocysteine-cysteine mixed disulfides and protein-bond disulfides (Ghaedi *et al.,* 2007).

DM is a major risk factor for cardiovascular disease (CVD) (Saurabh *et al.,* 2016). Framingham Heart Study and the Multiple Risk Factor Intervention Trial clearly showed the independent contribution of DM to cardiovascular risk, and the further incremental risk when other conventional risk factors such as lipids, smoking and hypertension are present (Syed *et al.,* 2014).

Homocysteine has generated considerable interest in recent years as both an emerging risk factor for cardiovascular disease and a sensitive biomarker of folate deficiency (even within the normal range of homocysteine concentrations) (Laima*et al.,* 2008).

Several studies have revealed that plasma HCY levels in patients with diabetes mellitus are increased as compared with those without diabetes (Laima*et al.,* 2008). Observational study has shown that an increase in plasma (HCY) along with systemic inflammation, coagulation factors, oxidative stress, ventricular hypertrophy and various dyslipidaemia subtypes, may work synergistically with DM in promoting CVD (Ganguly *et al.,*2015).

The relationship between plasma HCY and excess of CVD in diabetic patients remains to be clarified. Several studies have reported the existence of an interaction between plasma HCY levels and Type 2 DM and its vascular complications (Ganguly *et al.,* 2015; Lakshman *et al.,* 2012; Smulders *et al.,* 1999; Hoogeveen *et al.,* 1998).

The results obtained from various studies have demonstrated that elevated HCY levels predict the risk of death or coronary events in patients with Type 2 DM. However, conflicting results regarding the HCY level in patients with DM have been reported. Some studies found that plasma HCY levels were increased, unchanged or decreased in patients with type 2 DM. In spite of many research works on HCY in diabetic subjects in some parts of the world; there are no data available in developing countries including Nigeria. The present study is aim at evaluation HCY levels in diabetic patients so as to determine the possible link between HCY concentration and CVD in diabetic Nigerians.

## MATERIALS AND METHODS

### Study Location

The study was conducted in the Departments of Chemical Pathology and Medicine of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. Zaria is located in Kaduna state, northern part of Nigeria and shares a common border with Bauchi, Kano, Katsina, Niger and Plateau states. Kaduna state is the third most populous state in Nigeria (Nigeria- Population Census – 2006). The provisional population for the year 2006 census revealed that the state has a population of 6,066.562 million. The state has an average rainfall of 1.272.5 mm, with humidity of 56.64 %, daily wind speed of 176.12 km/h and average maximal temperature of $30^0$C.

The city is blessed with Formal and Informal schools as well as Islamic scholars and therefore, people from all parts of the country and neighboring countries such as Niger Republic, Benin Republic, Chad Republic and Cameroon come to the town to search for both western and Islamic knowledge. Majority of the population are Hausa and Fulani.

**Sample Size Determination**

The sample size for the study was determined from a standard formula for the calculation of minimum sample size (Oyejide. l992; Singha. 1996). Sample size n is given by the formula:

$n = (Z_1—a)^2(P)\ (1\text{-}P)/d^2$

Where n= minimum sample size, $Z_1$-a= value of standard normal deviate which at 95% confidence level has been found to be 1.96. P= the best estimate of the population prevalence obtained from literature review, d=difference between the true population rate and sample that can be tolerated, that is the absolute precision (in percentage point) on either side of the population. The current prevalence of DM in Nigeria is 5.1% (IDF, 2015).

$n= (1.96)^2\ (0.051)\ (1\text{-}0.051)/(0.04)^2$

n= 116

The calculated sample size for the study was 116. However, 10% was added to take care of attrition. Therefore, 140 diabetic patients were recruited for the study.

**Study Subjects**

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 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.

**Selection and Description of Participants**

A consecutive sampling method was employed to select the subjects who satisfy the study inclusion criteria. Informed consent for inclusion into the study was obtained from the subjects. The nature of the study was explained to the subjects using an appropriate language. A full medical history was obtained from the subjects by the physicians followed by clinical examination and collection of blood specimen. Diabetes mellitus status was confirmed biochemically according to World Health Organization diagnostic criteria for classification of diabetes (World Health Organization, 1999).

**Ethical Approval**

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.

**Clinical and Anthropometric Measures**

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 scales (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. 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.

**Biochemical Analysis**

Hettich Universal 32 Centrifuge (Germany) was used to spin the blood specimens. Serum Homocysteine concentration was assayed using a commercially ELISA Kit (Nelsin Medical Co., Limited, China). Serum homocysteine concentrations were read on a micro-plate reader (RT-6000 Rayto China).

Serum glucose concentrations, cholesterol, triacylglycerol, and high density lipoproteine cholesterol (HDL) were 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). Artherogenic indices (ratio) were calculated as the concentration of total cholesterol divided by the concentration of high density lipoprotein cholesterol. Glycated haemoglobin (HbA1c) in EDTA treated bottle 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, and glycated haemoglobin concentrations obtained from diabetic patients were compared with those of apparently healthy individuals (controls) using two-tailed student's t-test. $P< 0.05$ was considered statistically significant.

## RESULTS

Characteristics of the participants are shown in Table 1. The patients were made up of 55 males (39%) aged 30-60 (mean age 52 years) and 85 females (61%) aged 20-60 (mean age 50 years). Hausa/Fulani being the predominant ethnic group has the highest number of 96 (69%) followed by the combined minor ethnic groups with 34 (24%), Yoruba 8 (5%) and Igbo (1%).

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 2. 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 have shown that WC, HC, WC:HC, SBP and DBP 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 serum homocysteine and lipids (TC, TG, HDL, LDL, TC:HDL), as well as FBG and HbA1c were compared between the two groups as shown in Table 3. 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 the assessed 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: Characteristics of the Study Population**

| Subjects | n | Percentage (%) |
|---|---|---|
| Patients | 140 | 100 |
| **Sex** | | |
| Males | 55 | 39 |
| Female | 85 | 61 |
| **Ethnicity** | | |
| Hausa-Fulani | 96 | 69 |
| Yoruba | 8 | 5 |
| Igbo | 2 | 1 |
| Others | 34 | 24 |

n=number of patients

**Table 2: Anthropometric and Clinical Parameters (Mean±SEM) in Diabetic Patients and Controls**

| Variables | Patients (n=140) | Controls (n=100) | P-value |
|---|---|---|---|
| Age (Years) | 52±1.250±1.4 | | 0.857 |
| WC (cm) | 93±1.779±1.3 | | 0.000 |
| HC(cm) | 98±2.287±1.3 | | 0.000 |
| WC:HC | 1.0±0.10.1±0.1 | | 0.000 |
| WT(Kg) | 70±2.370±1.3 | | 0.972 |
| H (m) | 1.9±1.61.6±0.1 | | 0.270 |
| BMI | 26±1.327±1.1 | | 0.825 |
| SBP(mmHg) | 132±3.391±2.2 | | 0.000 |
| DBP(mmHg) | 83±3.549±3.2 | | 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 3: Biochemical Analytes (Mean±SEM) in Diabetic Patients and Controls**

| Variables | Patients (n=140) | Controls (n=100) | P-value |
|---|---|---|---|
| HCY | 10.5±1.55.8±1.1 | | 0.007 |
| TC | 4.1±0.12.0±0.2 | | 0.000 |
| TG | 1.5±0.11.3±0.1 | | 0.001 |
| HDL | 0.7±0.12.2±0.1 | | 0.000 |
| LDL | 2.7±0.12.5±0.2 | | 0.000 |
| TC: HDL | 7.9±0.71.4±0.2 | | 0.000 |
| FBG | 7.9±0.44.5±0.1 | | 0.000 |
| HbA1c | 7.9±0.25.5±0.5 | | 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) and HbA1c=glycated heamoglobin SEM=Standard Error of Mean

## DISCUSSION

There has been reported increased morbidity and high mortality due to CVD in diabetic patients with high (HCY) compared to diabetic patients without these abnormalities (Majumder *et al.,* 2017).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 Maria and Ester.(2012) and Saurabh *et al.*(2016).However, Platt *et al.*(2017)found that serum HCY levels were lower in diabetic patients than in controls. This may be attributed to methodology of assay of HCY, lack of consensus definition of hyperhomocysteinaemia, sample size, and environment. The mechanisms by which HCY promotes cardiovascular disease are uncertain.

Increased HCY level has shown a predialation 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 nitric oxide as well as impairing vasodialation.

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 (Mc Anulty *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 findings of the present study showed that the mean values of total cholesterol were significantly higher in diabetic patients compared to controls. In various *in vitro* studies, HCY was proved to play a role in increasing the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase) which in turn increases cholesterol synthesis (Simonen *et al.,* 2002). An increased cholesterol level promotes atherosclerosis and hence it is a risk factor for CAD than in non-CAD subjects (Simonen *et al.,* 2002). It was found that increase serum HCY levels positively correlated with severity of CAD (Simonen *et al.,* 2002). This might be the reason of increased serum total cholesterol seen in the present study.

The results obtained in the present study showed that the mean values of serum triglyceride were significantly higher in diabetic patients than those of controls. This indicates that there was dyslipidaemia in diabetic patients under the study. The hypertriglyceridaemia seen in the present study could be as a result of mobilization of free fatty acids to the liver due to the influence of insulin which can lead to increase synthesis of TG and in turn gives rise to pancreatic lipotoxicity in Type 2 diabetic patients. Increased oxidative stress has also been reported (Oluwafemi, 2019) in Type 2 diabetic patients and this could cause matrix alteration, thereby making diabetic patients more susceptible to the effect of increased plasma TG.

The findings of the present study showed that the mean values of serum high density lipoprotiene cholesterol (HDL) were significantly lower in diabetic patients compared with those of controls. This also indicates the presence of dyslipidaemia in this study. It was reported that HCY and lipid metabolism were interrelated at least in part via methyl group donors (Obeid and Herrmann, 2009). Moreover, hyperhomocysteinaemia in mice was associated with a decreased activity of hepatic thiolase and serum lecithin-cholesteolacylransferase (LCAT), which are two important enzymes involved in HDL metabolism (Kang *et al.,* 2012). This may be the reason of decrease serum levels of HDL seen in the present study.

The findings of the present study showed that the mean values of serum low density lipoprotiene cholesterol (LDL) were significantly higher in diabetic patients compared to those of controls. This contradicts the report of Bishwajit *et al.* (2018). According to Bishwajit *et al.* (2018), the levels of LDL cholesterol were lower in diabetic patients than those of controls. This may be attributed to methodology of assay of LDL, sample size, and environment.

In addition, clinical trials of cholesterol lowering have shown that lowering of LDL cholesterol in diabetic persons does reduce the incidence of CVD (Salim *et a.*, 2020).

## CONCLUSIONS

It can be concluded from the findings of this study that: mild hyperhomocysteinaemia (Novel risk factor for CVD) hypercholesterolaemia and dyslipidaemia are implicated in the development cardiovascular complications among diabetic patients in Zaria, Northern Nigeria.

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