



Lipid Profile, Aip And Glycated Haemoglobin Level In Type-2 Diabetic Patients In Enugu, South-East Nigeria

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Abstract

Impaired lipid metabolism resulting from uncontrolled hyperglycaemia has been implicated in diabetes and glycated haemoglobin (HbA1c) used as predictor of uncontrolled hyperglycaemia. The present study is aimed at analyzing the HbA1c level, lipid profile and atherogenic index of plasma (AIP), in type-2 diabetics in Enugu. Ninety (90) type-2 diabetic patients (46 males and 44 females) drawn from the diabetic clinic and 40 apparently-healthy, non-diabetics (20 males and 20 females), all aged 45-70 years, were included in the study. Venous blood samples were collected from the subjects after 8 hours fasting and analyzed for HbA1c, fasting blood glucose (FBG), total cholesterol (TC), triacylglycerol (TG) and high-density lipoprotein (HDL-C), whereas low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C) and AIP were calculated using standard procedures. Data was analyzed with SPSS computer software version 11 using student's t-test at 95% confidence limit. The results showed significant increase ($P < 0.05$) in AIP and the lipid profile parameters, and highly significant increase ($P < 0.001$) in FBG and HbA1c in the diabetic patients. LDL-C was not significantly increased in the test subjects compared to controls. Blood pressure was significantly increased ($P < 0.05$) whereas body mass index (BMI) revealed central obesity in the patients. Significant increase in HbA1c, AIP and BMI was observed in the diabetic patients compared to the control. All the subjects had AIP in the medium CV risk group. The study suggests that the diabetic patients might also be predisposed to the risk of complications from dislipidaemia.

Key-Words: Hyperglycaemia, Dislipidaemia, LDL-C, Enugu

Introduction:

The global prevalence of diabetes is predicted to rise from 135 million in 1995 to 300 million by 2025 (Khaw et al, 2001). Type-2 diabetes mellitus (also known as non insulin-dependent diabetes mellitus), is a condition whereby the pancreas retains some ability to produce insulin but this is inadequate for the body's needs, making the body to become resistant to the effect of insulin (Khaw et al, 2001). Various blood glucose threshold concentrations have actually been proposed for the diagnosis of diabetes (Monnier et al., 2003), in relation to risk of complications of diabetes, particularly retinopathy (Alberti and Zimmet, 1998).

Glycated haemoglobin (HbA1c) concentration, an indicator of average blood glucose concentration over three months, has been suggested as a diagnostic or screening tool for diabetes (Marshall and Barth, 2000). It was initially postulated that the HbA1c reflects the status of metabolic control over three months. Thus, there is a likelihood of a rapid dissipation leading to a significant change in HbA1c within 4-6 weeks upon instituting rapid metabolic control (Chandalia, 2010). Similarly, stress and transient hypoglycemia is known to elevate HbA1c level (Chandalia and Krishnaswamy, 2002). Salivary enzymes have also been suggested as biochemical markers for assessment and monitoring of type-2 diabetic patients with periodontal complications since the presence of type-2 diabetes has been shown to lead to a significant increase in salivary enzymes activities of periodontal patients (Ikekpeazu et al., 2011). AIP has been used by some practitioners as a significant predictor of atherosclerosis and has also been used to predict the risk of atherosclerosis in hypertensive postmenopausal women in South-East Nigeria (Nwagha and Igweh, 2005). The combined use HbA1c and AIP together in the monitoring of type-2 diabetics have not been reported.

In the present study, we evaluated the fasting blood glucose (FBG), lipid profile, AIP and HbA1c of type-2 diabetic patients in Enugu, South-East Nigeria. We also tested the hypothesis that the diabetic patients tested might be at the risk of possible complications as a result of their blood lipid profile. The results

of this study will be useful in evaluating, monitoring and ultimately managing these patients to reduce the rate of development of complications of dislipidaemia which might predispose the patients to atherosclerosis as a result of diabetes.

Subjects and methods

Selection and Description of Participants

We studied type-2 diabetic patients attending the diabetic clinic of the University of Nigeria Teaching Hospital, (UNTH) Ituku-Ozalla, Enugu State, Nigeria. Study subjects comprised of a total of ninety (90) type-2 diabetic patients (46 male and 44 females volunteers) aged between 45 and 70 years and resident in Enugu, South-East Nigeria. The control group comprised of forty (40) age-matched, apparently-healthy non-diabetic volunteers (20 males and 20 females) also resident in Enugu. Adequate approval was granted by the institution's ethics committee before the commencement of the study. Fasting blood glucose (FGB), two hours postprandial (2HPP) glucose test and urinalysis were performed on the type-2 diabetic patients, who were already visiting the diabetic clinic and based on physical examination, history taking and clinical presentations, the subjects for the study were selected. All the diabetic test study participants were enrolled into the study during their visit to the clinic, after giving informed consent to participate in the study. The patients were on different antidiabetic therapy.

Blood Pressure and Anthropometric Measurement

Study subjects' blood pressure was measured with a random zero mercury sphygmomanometer (Hawksley & Sons, Ltd.; Lancing, United Kingdom). The measurement included, after a supine rest of 5 minutes, three measurements in the supine position, one in the standing position, and two in the sitting position at 5-minute intervals. The mean of all six measurements was used as the systolic and diastolic blood pressures. Hypertension was defined according to the WHO-International Society of Hypertension and Sixth Joint National Committee recommendations ($\geq 140/90$ mmHg) (WHO, 1999).

Information on age, sex and anthropometric measures were obtained from all patients and control subjects. Weights (in kg) were taken without shoes, to the nearest 0.5 kg. Heights (in metres) were taken to the nearest 0.5 cm with subjects standing erect without shoes or headgear. Body Mass Index (BMI) was derived by dividing the weight by the square of the height. BMI of ≥ 25 to 29.99 kg/m^2 was used to define overweight while a value $\geq 30 \text{ kg/m}^2$ was used to define obesity (WHO, 1999). Waist circumference was taken as the average of two measurements taken after subject inspiration and after expiration at the midpoint between the lowest rib and the iliac crest with subjects in the supine position.

Sample Collection and Processing

Fasting blood samples were taken from the patients by clean veni-puncture from the antecubital fossa using 5ml disposable syringe and needle under aseptic conditions. The samples were collected without undue pressure to either the arm or the plunger of the syringe to avoid stasis. Four (4) mls of blood were collected and distributed into sterile plain tube, Ethylenediaminetetracetic acid (EDTA) bottle and fluoride oxalate bottle respectively. The samples in the plain tubes were allowed to clot and then centrifuged at 3000rpm for 5 mins to obtain clear serum samples, which were used for total cholesterol (TC), triacylglycerol (TG) and High-density lipoprotein cholesterol (HDL-C) assays. The anticoagulated samples in the fluoride oxalate and the EDTA bottles were separated and used for fasting blood glucose estimation and glycated haemoglobin (HbA1c) test respectively. The study was conducted between the months of May-August, 2010. Blood samples were assayed at the Department of Chemical Pathology, University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Enugu. All analysis were performed immediately the samples were processed and when immediate analysis was not possible, the serum samples were stored frozen (-20°C) and lipid analysis done within 3 days.

Analytic Methods

Serum TC assay was done by enzymatic-spectrophotometric method of Allain et al, (1974); whereas HDL-C estimation was carried out using precipitation/ enzymatic-

spectrophotometric method Groove, (1979). Enzymatic-spectrophotometric method of Buccolo and David (1973) was used for TG estimation; while LDL-C and VLDL-C were calculated using Friedewald's formula (Friedewald et al, 1972). The kit reagents were manufactured by Biosystems S.A, Barcelona Spain. Fasting blood glucose was done by glucose oxidase method using Randox kit reagents whereas glycated hemoglobin (HbA1c) estimation was done with In2it (1) ^(R) A1c cartridges (Bio-Rad Laboratories Deeside, CH5, 2 NU, UK) which uses the well-established boronate affinity chromatography method to separate the glycated fraction from the non-glycated fraction. Atherogenic index of plasma (AIP) was calculated as $\log(\text{TG}/\text{HDL-C})$.

Statistical Analysis

The statistical analysis of the data obtained was carried out with the statistical Package for Social Sciences (SPSS) computer software version 11 using unpaired student's t-test at 95% confidence limit and 3 degrees of freedom, with P-values of (<0.05) being considered as significant. The results were expressed as mean plus/minus standard deviation (mean \pm SD).

Results

A total of 90 type 2 diabetic patients and 40 control subjects participated in the study. Average age range of the subjects (type 2 diabetic patients and control subject) at time of study was (range 45 - 70 years). There were 46 (51.1 %) males and 44 (48.9%) females in the diabetic group. The mean duration of the disease in the test subjects was $7.48\text{years} \pm 6.33$ months. Table 1 shows the mean \pm SD of HbA1c, FBG, lipid profile, body weight, waist circumference and blood pressure in the type-2 diabetics and control subjects. From the result, there were highly significant increase ($P<0.001$) in the HbA1c and FBG in the test subjects compared to the control. Significant increases ($P<0.05$) were also recorded in the lipid profile parameters, total cholesterol (TC), triacylglycerol (TG) and high-density lipoprotein (HDL-C), as well as body weight, blood pressure and waist circumference whereas low-density lipoprotein (LDL-C) showed non-significant increase in the test subjects compared to the controls. Although

type-2 diabetic patients had higher BMI than control subjects, the anthropometric difference between the two groups was more striking when the waist circumference was compared. Mean waist circumference were respectively 96.3 ± 11.3 and 88.2 ± 8.6 among diabetic and control subjects, ($p < 0.05$). Blood pressure of the test subjects ($155/95\text{mmHg}$) was significantly increased ($P < 0.05$) compared to the controls ($138/85\text{mmHg}$). Mean BMI among type-2 diabetic patients was $28.93 \pm 4.13 \text{ kg/m}^2$ compared to $22.93 \pm 2.02 \text{ kg/m}^2$ among control subjects ($p < 0.02$). Overweight occurred in 46 (51.1%) of diabetic subjects and 9 (22.5%) of control subjects; while obesity (BMI $> 30.0 \text{ kg/m}^2$) was recorded in only 5 (5.6%) type 2 diabetic patients and none of the control subjects (Table 2). The largest number of diabetic patients included in the study, 44 (48.9%) were on Sulphonylurea therapy, whereas the least number 7 (7.8%) were on Thiazolidenidiones. (Table 3).

Discussion

Diabetes mellitus increases the risk of atherosclerotic vascular disease, mostly in people who have other known risk factors such as dislipidaemia, hypertension and obesity. The present study assessed the levels of FBG, HbA1c, AIP, BMI, Blood Pressure, waist circumference and lipid profile in type-2 diabetics and the results showed significant increases in all the studied parameters except for the LDL-C in the test subjects compared with the controls, with HbA1c, and FBG being highly significant ($P < 0.001$). LDL-C was however not significantly increased ($P > 0.05$) in the test subjects in comparison with the controls. Universally, atherogenic index of plasma (AIP) calculated as $\log(\text{TG}/\text{HDL-C})$ has been used by some practitioners as a significant predictor of atherosclerosis (Dobiasova and Frohlich, 2001; Tan et al., 2004). The AIP of the diabetic patients in our present study was found to show a statistically significant increase ($P < 0.05$) in the diabetic patients compared to the controls. AIP ($\log(\text{TG}/\text{HDL-C})$), has been successfully used as an additional index when assessing cardiovascular (CV) risk factors (Dobiasova and Frohlich, 2001) and studies have suggested that AIP values of -0.3 to 0.1 are associated with low-

risk, whereas (0.1 to 0.24) and (above 0.24) are associated with medium-risk and high- risk of CV disease respectively (Dobiasova, 2006).

The present study showed that both the test and control subjects had their AIP in the range of the medium CV risk group, based on the suggested classification of Dobiasova, (2006). The subjects in the present study showed abnormality of lipid metabolism which maybe secondary to insulin deficiency as indicated by the poor glycaemic control of the diabetic patients, even with anti-diabetic medication.

Previous studies have reported the normal range of HbA1c in healthy persons to be about 4%–5.9% (Dobiasova, 2006). Highly significant values, observed in our study subjects ($9.62 \pm 1.23\%$), show that type-2 diabetics also stand the risk of having higher HbA1c levels as a result of persistently elevated blood sugar and might be at the risk dislipidaemia which might further predispose them to long-term vascular complications.

Persistent elevations in blood sugar (and therefore HbA1c) increase the risk for the long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, etc (Monnier et al., 2003; Gautier et al., 2010). Although there are no conclusive studies to demonstrate the superiority of BMI or waist circumference as an indicator of diabetes, at least there is an indication of waist circumference being an important indicator of progression to diabetes (Gautier et al., 2010).

Our data shows that central obesity (BMI = $25-29.9 \text{ kg/m}^2$) is common in the sample of type 2 diabetes patients in the present study (BMI = $28.93 \pm 4.13 \text{ kg/m}^2$) and is similar to the association between obesity and diabetes shown in other studies (Daousi et al., 2006). This study has shown that whereas obesity as defined by BMI might be rare, central obesity is quite common among Nigerian type-2 diabetic patients. This is crucial in the management of type-2 diabetic patients as central obesity is the form of obesity that is associated with cardiovascular morbidity and mortality (Stensvold et al., 1993; Daousi et al., 2006).

The World Health Organization (WHO) defined metabolic syndrome based on BMI ≥ 30 kg/m² while hypertension was defined according to the WHO–International Society of Hypertension and Sixth Joint National Committee recommendations as ($\geq 140/90$ mmHg) (WHO, 1999). The diabetic subjects in our study had mean blood pressure above the cut-off for hypertension, which might predispose them further to cardiovascular risk whereas the control subjects had mean blood pressure below than the cut-off point. This implies that cholesterol and blood pressure lowering programmes should be considered for these patients for adequate management of their condition. Also long-term glycaemic control should also be considered as this should help in correcting the lipid abnormalities secondary to insulin deficiency.

Although previous studies have shown that essential metals such as potassium, calcium and zinc are abundant in edible fruits such as banana, mango and water melon, which are so abundant in Nigeria (Maimuna et al., 2012), diabetic patients should cut down on such fruits intake because of their sugar content. Raised glycated haemoglobin (HbA1c) concentration, even in men without diabetes, is a marker of greater absolute risk, and preventive treatment with blood pressure or cholesterol lowering drugs should be considered in such patients. The test subjects appeared to be the affected group with blood pressure, waist circumference and BMI significantly increased, in comparison with the control. Although our control subjects did not have raised HbA1c, they had their AIP in the range for medium CV risk. This however could pose a health problem in future. The raise AIP could be as a result of the type of diet common in this part of the country (mostly saturated fat). A variety of intervention studies show that patients with type 2 diabetes who succeed in losing weight often enjoy modest improvements in glycaemic control and cardiovascular risk profiles, as long as the weight loss is maintained.

Conclusion

Our study showed that the type-2 diabetic subjects studied might be at risk of complications resulting from dislipidaemia due to their unfavourable lipid profile results and also that central obesity is more common in the studied subjects. High level of HbA1c was also observed in the test population, suggesting that these patients might also be at the risk of atherosclerosis as a result of lack of long-term glycaemic control despite the anti-diabetic medication. The control subjects also had their AIP in the range of medium CV risk and this calls for attention even with the normal HbA1c. Adequate enlightenment in addition to constant exercise is thus required, on the need to keep the blood lipids in check.

Table-1 Lipid profile, AIP, HbA1c, blood pressure, body weight and waist circumference in type-2 diabetic patients and control subjects (non-diabetics).

Parameters	Type-2 Diabetics N= 90	Non-Diabetics N= 40	P-Value
HbA1c (%)	9.62 ± 1.23	4.49 ± 0.19	P< 0.001*
FBS (mmol/L)	11.41 ± 1.53	4.10 ± 0.31	P< 0.001*
TC (mmol/L)	5.22 ± 1.93	4.54 ± 0.33	P<0.05*
TG (mmol/L)	2.27 ± 0.98	1.1 ± 0.23	P<0.05*
HDL-C (mmol/L)	1.89 ± 0.98	1.54 ± 0.18	P<0.05*
LDL-C (mmol/L)	2.69 ± 0.92	2.65 ± 0.35	P>0.05
VLDL-C (mmol/L)	0.70 ± 1.21	0.49 ± 0.10	P<0.05*
AIP	0.20 ± 0.17	0.14 ± 0.73	P<0.05*
Blood Pressure (mmHg)	155/95	138/ 85	P<0.05*
Waist Circumference (cm)	96.3 ± 11.3	88.2 ± 8.6	P<0.05*
Weight (kg)	102 ± 5.7	96 ± 3.2	P<0.05*
BMI (Kg/m ²)	28.93 ± 4.13	22.93 ± 2.02	P<0.05*

* : Statistically significant compared to controls.

Table-2 Body mass index in type-2 diabetic patients and control subjects.

Body Mass Index (Kg/m ²)	Percentage (%) of test subjects (n=90)	Percentage (%) of control subjects (n= 40)
<19.5	6.7%	15%
19.5-23.9	21.1%	7.5%
23-24.9	15.6%	24.4%
25-29.9	51.1%	22.5%
>30	5.6%	0%

Table-3: Antidiabetic Drug Therapy In The Study Test Subjects.

Antidiabetic drug	Percentage Utilization (n=90)
Insulin	17.8%
Metformin	22.2%
Sulfonylureas	48.9%
Alpha glucosidase inhibitors	3.33%
Thiazolidenidiones	7.8%

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