Blood Lipids and Its Atherogenic Indices in Alloxan Induced Diabetic Male Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ODC and SCM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ODC and TU managed the analyses of the study. Authors ISIO, OEO and BNE managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Diabetes mellitus is a group of metabolic disorders which result to excessive accumulation of blood sugar over a prolonged period. Due to higher risk of diabetes mellitus to cardiovascular disease, it is crucial to identify and address these cardiovascular risks. This study assessed the effects of diabetes on levels of some blood lipids and its atherogenic indices in diabetic male rats.

Methods: This is an experimental study that involved 40 apparently healthy adult male albino rats (wistar strain) which were randomly assigned to five groups (A, B, C, D and E) of eight (8) animals each. Group A (Normal Control of No intervention for 72 hours), Group B (Diabetic rats of 72 hours post diabetes induction), Group C (metformin treated diabetic rats), Group D (Diabetic Control untreated) and Group E (Normal Control of 3 weeks post diabetes induction). Seven milliliters of

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fasting blood sample were collected from all the subjects. Serum levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were determined using standard methods. Atherogenic indices, non HDL cholesterol (Non HDL-c), cardio risk ratio (CRR), atherogenic index of plasma (AIP), atherogenic coefficient (AC) and atherosclerosis index (AI) were calculated. It was analyzed statistically using SPSS version 23.0.

Results: The mean values of HDL-c was significantly higher in the treated diabetic group when compared with untreated diabetic control (P<0.05) while TC, TG, LDL-c, VLDL-c, Non HDL-c, CRR, AIP, AC and AI were significantly lower in treated diabetics when compared to the untreated diabetic control (P<0.05). Also, blood mean levels of HDL-c were significantly lower in the diabetic groups (treated and untreated) when compared with non diabetic control (P<0.05) while TC, TG, LDL-c, VLDL-c, Non HDL-c, CRR, AIP, AC and AI were significantly higher in the diabetic groups (treated and untreated) when compared with non diabetic control (P<0.05).

Conclusion: The study suggests that atherogenic indices can serve as predictive pointer for future cardiovascular event especially, when LDLc value is normal. Also hyperglycemia could cause significant alterations of lipids, but metformin treatment has showed not only hypoglycemic effect, but also anti-hyperlipidemic properties.

1. INTRODUCTION

Diabetes mellitus (DM) also simply known as diabetes, is a group of metabolic disorder which result to excessive accumulation of blood sugar over a prolonged period [1]. Diabetes is a chronic condition that arises when the pancreas produce insufficient insulin, or when the body cannot effectively use the insulin produced. In other words, diabetes has been characterized as a chronic metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action or both [2,3]. Lipid abnormalities associated with diabetes are termed as dyslipidemia. Dyslipidemia is defined by presence of one or more than one abnormal serum lipid concentration [4]. Diabetes mellitus (DM) is a common comorbidity of hyperlipidemia, particularly, if glycemic control is poor, which in-turn is an important risk factor for atherosclerosis and coronary heart disease. Dyslipidemia is becoming increasingly prevalent all around the world as both insulin deficiency and insulin resistance affects enzymes and pathways of lipid metabolism. Type 2 diabetes is a major risk factor for cardiovascular disease (CVD). The risk of CVD mortality in patients with type 2 diabetes (T2DM) is 2-4 times that observed in individuals without diabetes [5]. It has been observed that about seventy- eight percent of type 2 diabetic patients die from cardiovascular disease, due to premature atherosclerosis which involves dyslipidemia [6]. Many factors contribute to cardiovascular disease risk in diabetes but lipid abnormalities are major contributors. Due to higher risk of diabetes mellitus to cardiovascular disease, it is crucial to identify and address these cardiovascular risks. The measurement of LDL cholesterol alone does not provide sufficient measure of atherogenic risk in hyper-triglyceridemic patients, and a second or several markers are warranted [7].

Early assessment and control of cardiovascular risk factors in patients with T2DM has a positive effect on reducing the risk of CVD and death in patients and improving the prognosis of patients. Work done by VinodMahato et al. [8] and Swaminathan et al. [9] showed a significant increased level of Cardiac risk ratio (CRR) in diabetes mellitus when compared with controls. This shows that cardiac risk ratio could also serves as a good predictor of cardiovascular risk. According to American Heart Association, a value of (>3.5) shows greater propensity towards cardiovascular diseases. Adu et al. [10] also reported a significant increase value of Atherogenic Coefficient (Ac) of diabetes when compared with the non- diabetes subjects which is in agreement with the work of Ikewuchi [11].

Atherogenic Coefficient (AC), calculated as (Non-HDL-c)/HDL-c) or (TC-HDL-c)/HDL-c) is a measure of cholesterol in LDL, VLDL, IDL fractions with respect to good cholesterol or HDL-c [12]. The atherogenic index of plasma (AIP) is a good predictor of the risk of atherosclerosis and coronary heart disease [13, 14]. The association of TGs and HDL-c in this simple ratio reflects the balance between risk

and protective lipoprotein forces [15]. A study has suggested that the visceral fat area in patients with T2DM is associated with AIP [16].

Non HDL-c is calculated as total cholesterol minus HDL-c. Non-HDL-c has been observed to have the strongest relationship with small dense LDL-c (sdLDL-c) levels when compared with other lipid measurement [17]. The predictive value of non-HDL-C for cardiovascular risk and mortality is better than that of LDL-c [18]. The strong association between non-HDL-c and sdLDL-c adds additional support for using the non-HDL-c level as a predictor of CVD mortality [17].

Therefore, one of the objectives of this study is to assess the cardiovascular risk of diabetes mellitus individuals using Non HDL cholesterol (TC – HDL-c), Cardio Risk Ratio (TC/HDL-c), Atherogenic Index of Plasma (Log TG/HDL-c), Atherogenic Coefficient (TC- HDL-c/ HDL-c) and Atherosclerosis Index (LDL-c/HDL-c).

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty (40) adult male albino rats (wistar strain) weighing between 100 – 176 g and between 8-10 weeks old, obtained from the animal breeding unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used as experimental animals. The rats were kept in cages for two weeks in the Animal House of the College of Health Sciences Nnamdi Azikiwe University Nnewi Campus to acclimatize and had free access to food and water. The animals were maintained under standard laboratory conditions (12 hours light /12 hours dark cycle, temperature of about 37±2ºC). All the rats were fed a commercial diet (Vital feed, Nigeria) during the experiment. The protocol was in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use.

2.2 Experimental Design

The Forty (40) adult male wistar rats were randomly assigned to five groups (A, B, C, D and E) of eight (8) animals each. Table 1 summarized the experimental design.

2.3 Induction of Diabetes

In group B, C and D, wistar rats were fasted for 12 – 15 hours, after which diabetes was induced by a single intraperitoneal injection of freshly dissolved Alloxan monohydrate (Sigma-Aldrich, USA) (130 mg/kg b.w) dissolved in 0.5 mL of 10 percent normal saline maintained at 37°C [19, 20]. Normal control rats (Group A and E) received a similar volume of normal saline alone. After 72 hours of alloxan injection, the animals were fasted overnight and their fasting blood glucose was measured. The rats having fasting blood glucose level greater than 200 mg/dL were selected for the study. Fasting blood glucose level of all the diabetes induced rats were greater than 200 mg/dL and were all selected for the study.

2.4 Blood Sample Collection and Serum Preparation

The animals were fasted overnight and sacrificed using chloroform as an anesthetic agent. Blood samples of Groups A and B rats were collected 72 hours post intraperitoneal injection, and that of groups C, D and E rats were collected 3 weeks after confirmation of diabetes. Fasting Blood sample (7 mL) was collected from all the animal through cardiac puncture after 12 hours fasting into a well- labeled, sterile plain bottle. The blood samples were centrifuged at 4000 rpm for 5 minutes after allowing the blood to clot and retract from walls of the sample container at

Table 1. Showing different groups and treatment 72 hours post diabetic induction

Groups	Treatment
Group A (Normal Control of 72 hours)	Received 0.5 mL of 10 percent normal saline only + No intervention for 72 hours
Group B (Diabetic rats)	Induced diabetes + sacrificed 72 hours of post diabetes induction
Group C (Diabetic rats treated)	Induced diabetes + 500 mg/kg, p.o metformin drug treatment for 3 weeks after confirmation of diabetes
Group D (Diabetic Control)	Induced diabetes + without metformin drug treatment for 3 weeks after confirmation of diabetes
Group E (Normal Control of 3 weeks)	Received 0.5 mL of 10 percent normal saline only + NO intervention for three 3 weeks of study

room temperature. The sera were separated from the whole blood into a new container and then stored in a refrigerator at −20°C. The analysis was carried out within one week of sample collection. Haemolysed blood samples were not used to avoid error in the result. The blood sample was used for the analysis of total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) using colorimetric method while low density lipoprotein cholesterol (LDL-c), and very low density lipoprotein cholesterol (VLDL-c), non HDL cholesterol (non HDL-c), cardio risk ratio (CRR), atherogenic index of plasma (AIP), atherogenic coefficient (AC), and atherosclerosis index (AI) were calculated as follows:

In mmol/L LDL = (Total cholesterol – (Triglycerides/2.2) – HDL cholesterol)   [21]

In mmol/dL VLDL = (Triglycerides/2.2)   [21]

Atherogenic Index of Plasma (AIP) = Log TG/HDL-c.   Normal Ranges: ≤ 0.1 [22]

Cardio Risk Ratio (CRR) = TC/HDL-c.
Normal Ranges: ≤ 3.5 [23]

Atherogenic Coefficient (AC) = TC- HDL-c/ HDL-c.   Normal Ranges: ≤ 3.0 [24]

Atherosclerosis Index (AI) = LDL-c/HDL-c.
Normal Ranges: ≤ 3.3 [23]

Non HDL cholesterol = TC – HDL-c.
Normal Ranges: ≤ 3.3 [25]

Triglycerides were determined by glycerol phosphate oxidase method [26]. Cholesterol was determined using cholesterol oxidase method [27]. High density lipoproteins cholesterol was determined by precipitation with phosphotungstic acid in the presence of magnesium ions [28].

2.5 Method of Data Analysis

The Statistical Package for Social Sciences (SPSS) [29] version 23 was used for statistical analysis and the variables were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used to compare the mean difference among groups, and Post Hoc multiple comparism was used to assess inter group variability. The level of significance was considered at $P < 0.05$.

3. RESULTS

In Table 2, there was a significant high mean levels of total cholesterol and low density lipoprotein cholesterol in diabetic rats (Group B) and (Groups C and D) when compared with control Groups A and E respectively ($P < 0.05$). Also, their mean blood levels were significantly higher in Group D when compared with Groups B and C ($P < 0.05$). Furthermore, there was a significant higher mean level of total cholesterol and low density lipoprotein cholesterol in Group C when compared with the Group B ($P < 0.05$).

There was no significant difference in the blood mean levels of triglycerides observed when diabetic Group B was compared with control Group A ($P > 0.05$), but a significant increase was observed when diabetic Groups C and D were compared with control Group E ($P < 0.05$). Also, a significant increase in mean level of triglyceride was observed in Group D when compared with Group B and C ($P < 0.05$). No significant difference was seen when diabetic rats Group C is compared with Group B ($P > 0.05$).

Blood mean level of high density lipoprotein cholesterol was significantly lower in the diabetic rats (Group B) and (Groups C and D) when compared with non diabetic control Groups A and E respectively ($P < 0.05$). However in Group D, the mean level was also significantly lower when compared with Groups B and C ($P < 0.05$). No significant difference exists in the mean level of high density lipoprotein cholesterol in Group B when compared with Group C ($P > 0.05$).

Blood mean level of very low density lipoprotein cholesterol was significantly higher in diabetic rats (Groups B and D) when compared with control Group E ($P < 0.05$). Very low density lipoprotein cholesterol mean value in Group D was significantly higher when compared with Groups (B and C) ($P < 0.05$). However, there was no significant difference in the blood mean level in Group B when compared with Groups A and C ($P > 0.05$).

The blood mean level of non HDL cholesterol was significantly higher in the diabetic rats (Group B) and (Groups C and D) when compared with non diabetic control Groups A and E respectively ($P < 0.05$). However, the mean level in Group D was significantly higher when compared with Groups B and C respectively, while in Group B, it was significantly

Table 2. Fasting blood lipids and glucose in diabetic and apparently healthy non diabetic male rats (group A-E)

Parameter (mmol/L)	Group A n=8	Group B n=8	Group C n=8	Group D n=8	Group E n=8	F-value	P-value
TC	3.26 ±0.17	3.69 ±0.26[acd]	4.39 ±0.29[bde]	4.96 ±0.37[bce]	3.35 ±0.12	63.254	< 0.001[*]
TG	1.54 ±0.07	1.76 ±0.19[d]	1.85 ±0.22[de]	2.72 ±0.31[bce]	1.55 ±0.04	49.947	< 0.001[*]
HDLc	1.52 ±0.07	1.30 ±0.13[ad]	1.32 ±0.06[de]	0.98 ±0.08[bce]	1.54 ±0.06	58.103	< 0.001[*]
LDLc	1.04 ±0.21	1.59 ±0.26[acd]	2.24 ±0.31[bde]	2.74 ±0.44[bce]	1.10 ±0.15	51.736	< 0.001[*]
VLDLc	0.70 ±0.03	0.79 ±0.09[d]	0.84 ±0.10[de]	1.24 ±0.14[bce]	0.70 ±0.02	50.308	< 0.001[*]
FBS	4.83 ±0.32	17.58 ±4.36[ac]	9.27 ±1.77[bde]	19.09 ±2.83[ce]	4.86 ±0.33	61.847	< 0.001[*]

Key: [a]- Significant when compared with Group A (Normal Control of 72 hours), [b]- Significant when compared with Group B (Diabetic Rats),[c]- Significant when compared with Group C (Diabetic Rats), [d]- Significant when compared with Group D (Diabetic Control), [e]- Significant when compared with Group E (Normal Control of 3 weeks), Group A (Normal Control of 72 hours) - Received 0.5mL of 10 percent normal saline only, Group B (Diabetic Rats) - Induced diabetes + sacrificed 72hours of post diabetes induction Group C (Diabetic Rats) - Induced diabetes + 500 mg/kg, p.o metformin drug treatment for 3 weeks after confirmation of diabetes, Group D (Diabetic Control) - Induced diabetes + without metformin drug treatment for 3 weeks after confirmation of diabetes, Group E (Normal Control of 3 weeks) - Received 0.5mL of 10 percent normal saline only, TC- Total Cholesterol, TG- Triglycerides, HDLc- High Density Lipoprotein Cholesterol, LDLc- Low Density Lipoprotein Cholesterol, VLDLc- Very Low Density Lipoprotein Cholesterol and FBS- Fasting Blood Sugar

Table 3. Lipids atherogenic indices in diabetic and apparently healthy non diabetic male rats (group A-E)

Parameter	Group A n=8	Group B n=8	Group C n=8	Group D n=8	Group E n=8	F-value	P-value
Non-HDL	1.74 ±0.19	2.39 ±0.21[acd]	3.08 ±0.29[bde]	3.98 ±0.43[bce]	1.81 ±0.14	96.648	< 0.001[*]
CRR	2.15 ±0.16	2.84 ±0.23[ad]	3.33 ±0.25[de]	5.09 ±0.68[bce]	2.18 ±0.12	94.539	< 0.001[*]
AIP	0.01 ±0.00	0.13 ±0.06[ad]	0.14 ±0.05[de]	0.44 ±0.07[bce]	0.01 ±0.00	116.229	< 0.001[*]
AC	1.15 ±0.16	1.84 ±0.23[ad]	2.33 ±0.25[de]	4.09 ±0.68[bce]	1.18 ±0.12	94.480	< 0.001[*]
AI	0.69 ±0.16	1.22 ±0.23[ad]	1.69 ±0.26[de]	2.81 ±0.58[bce]	0.71 ±0.10	63.294	< 0.001[*]

Key: [a]- Significant when compared with Group A (Normal Control of 72 hours), [b]- Significant when compared with Group B (Diabetic Rats), [c]- Significant when compared with Group C (Diabetic Rats), [d]- Significant when compared with Group D (Diabetic Control), [e]- Significant when compared with Group E (Normal Control of 3 weeks), Group A (Normal Control of 72 hours) - Received 0.5ml of 10 percent normal saline only, Group B (Diabetic Rats) - Induced diabetes + sacrificed 72hours of post diabetes induction Group C (Diabetic Rats) - Induced diabetes + 500 mg/kg, p.o metformin drug treatment for 3 weeks after confirmation of diabetes, Group D (Diabetic Control) - Induced diabetes + without metformin drug treatment for 3 weeks after confirmation of diabetes, Group E (Normal Control of 3 weeks) - Received 0.5ml of 10 percent normal saline only, Non HDL- Non HDL cholesterol, CRR- Cardio Risk Ratio, AIP- Atherogenic Index of Plasma, AC- Atherogenic Coefficient and AI- Atherosclerosis Index

lower when compared with Group C (P < 0.05). Blood mean levels of cardio risk ratio, atherogenic index of plasma, atherogenic coefficient, and atherosclerosis index were all significantly higher in diabetic rats (Group B) and (Groups C and D) when compared with Control Groups A and E respectively (P < 0.05). Furthermore in Group D, mean levels of cardio risk ratio, atherogenic index of plasma, atherogenic coefficient and atherosclerosis index were also significantly higher when compared with Groups B and C (P < 0.05). There was no significant difference in their mean levels when Group B is compared with Group C (P > 0.05) as showed in Table 3.

4. DISCUSSION

Diabetes is commonly associated with abnormalities in plasma lipids and lipoproteins levels. In particular, it usually presents with concomitant elevations in plasma lipids. Abnormalities in the lipid profile are one of the most common complications in diabetes and are associated with an increased risk of coronary heart disease; therefore, an ideal treatment for diabetes should have a favorable effect on the lipid profile in addition to offering good glycemic control [30]. The high concentration of triglycerides, total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and lower concentration of high density lipoprotein cholesterol observed in diabetic rats compared to normal rats group, agreed with report of Pierre et al. [31]. Also, significantly lower serum concentration of triglycerides, total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein concentration and higher level of high density lipoprotein cholesterol observed in diabetic treated rats compared to untreated diabetic rats group was in agreement with the work of Vishnu et al. [32]. This finding suggests that an elevated glucose level will occur upon induction of diabetes, and this would give rise to corresponding increase in plasma lipids concentration. Hyperlipidaemia is a factor of cardiovascular diseases associated with diabetes mellitus. It is often characterized by elevated cholesterol, triglycerides, phospholipids and other lipoproteins [33]. Several mechanisms may account for the atherogenic lipid abnormalities in diabetic patients. Dysfunctional adipose tissue is less sensitive to insulin and has reduced hormone-sensitive lipase activity compared with normal adipose tissue. As a result, there is an increased breakdown of intracellular triglycerides and increased release of free fatty acids into the circulation, leading to fatty infiltration in the liver, muscles and pancreatic β-cells leading to predisposition to type 2 diabetes. Increased hepatic free fatty acids contribute to increased hepatic triglycerides synthesis in turn resulting in elevated concentrations of very low density lipoproteins particles. Various lipases contribute to remodeling of very low density lipoproteins to small, dense low density lipoproteins particles. In addition, cholesterol ester transfer protein exchanges triglycerides from very low density lipoproteins to cholesterol found in high density lipoproteins and low density lipoproteins, leading to cholesterol-rich atherogenic very low density lipoproteins particles. High density lipoproteins particles that undergo these modifications are cleared more readily by the kidney, resulting in lower high density lipoproteins cholesterol levels [34].

Several epidemiological studies have found that in type 2 diabetic patients, metformin improves vascular function and reduces cardiovascular events and mortality by mechanisms that are not entirely attributed to its anti- hyperglycemic effects [35]. This study finds significant higher plasma level of non HDL cholesterol, cardio risk ratio, atherogenic index of plasma, atherogenic coefficient, and atherosclerosis index in diabetic animals compared to control group. Also, a significant lower level was observed in non HDL cholesterol, cardio risk ratio, atherogenic index of plasma, atherogenic coefficient and atherosclerosis index in metformin treated diabetic animal compared with untreated animal. Findings of this study is in agreement with the work of Rajesh et al. [36] which finds atherogenic index of plasma higher in diabetic animals compared to normal control, which upon treatment with metformin lowers the atherogenic index. The results showed that metformin have a beneficial effect in type 2 diabetes, including improved lipid profiles, and enhanced endothelial function. This would eventually improve atherogenic risk in diabetic subjects. Several studies have suggested that metformin may improve some of the features of the metabolic syndrome as it not only improves insulin sensitivity in the liver and muscle, as its primary anti-hyperglycemic mechanism of action, but also induces additional beneficial effects on several metabolic abnormalities associated with the metabolic syndrome [37,38,39]. A study also reported that metformin induced improvement of metabolic disorders that is associated with the energy state of the body, reduction of

macrovascular morbidity and mortality, anti-atherogenic, anti-inflammatory and antioxidant effects [40].

5. CONCLUSION

In conclusion, atherogenic indices can serve as predictive pointer for future cardiovascular event especially, when LDLc value is normal. Also hyperglycemia could cause significant alterations of lipids, but metformin treatment has showed not only hypoglycemic effect, but also anti-hyperlipidemic properties.

ETHICAL APPROVAL

Ethical approval was sought and obtained from the Research Ethics Committee of the Federal Medical Centre (FMC) Umuahia with reference FMC/QEH/G.596/VOL.10/282. The protocol was in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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