Serum Lipids and Total Antioxidant Concentration in Alloxanized Diabetic Rats.

Obi-Ezeani C. N\textsuperscript{1}, Ogundede E. B\textsuperscript{2}, Nwagbara I. J\textsuperscript{3}, Ogundede A. O\textsuperscript{2}, Chukwu C. C\textsuperscript{4}

\textsuperscript{1}Department of Chemical Pathology, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka Anambra State, Nigeria.
\textsuperscript{2}Virology department, National Veterinary Research Institute P.M.B 01 Vom, Plateau State, Nigeria.
\textsuperscript{3}Enugu State Ministry of Health, Enugu State.
\textsuperscript{4}Chemical Pathology department, Annunciation Specialist Hospital Emene, Enugu State.

Abstract: This study was designed to determine the serum levels of lipids and TAC as biomarkers of cardiovascular complications and oxidative stress respectively in diabetic wistar rats. 20 albino wistar rats weighing between 150g and 200g were used in this study, they were divided into two equal groups. Group I was the control while Group II was the diabetic group. The animals were handled according to the institutional and international guidelines for the care and use of laboratory animals. Diabetes was induced in group II by a single intraperitoneal injection of alloxan monohydrate (150mg/kg body weight) while group I received normal saline. After four weeks, blood samples were collected after an overnight fast for biochemical assays. The mean Fasting Plasma Glucose FPG, Glycated Hemoglobin HbA1c, total cholesterol TC, low density lipoprotein LDL, very low density lipoprotein VLDL and triglyceride TG concentrations were significantly (p<0.05) elevated while high density lipoprotein HDL and total antioxidant concentrations TAC were significantly (p<0.05) reduced in the diabetic group when compared to the control group. From our study therefore, untreated diabetes resulted in alterations in the serum lipids as well as total antioxidant concentrations.

Keywords: Alloxan, Oxidative Stress, Reactive Oxygen Species, Lipid Profile, Total Antioxidant Concentration.

I. Introduction

Diabetes mellitus which is often referred to as diabetes is a chronic metabolic disorder characterized by high blood glucose concentration as a result of defects in insulin secretion or action. It is the primary cause of disability and hospitalization as well as results in significant financial burden. [1]. The World Health Organization (WHO) has predicted that about 171 million number of worldwide diabetic cases will reach 366 million or more by the year 2030 [2]. Diabetes is the main risk factor for atherosclerotic, thrombotic and cardiovascular disease. Hyperglycaemia probably contributes to diabetic complications by interfering with vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins [3]. Hyperlipidemia is one of the metabolic complications of both clinical and experimental diabetes [4], and up to 97% of diabetic patients are dyslipidemic which is highly correlated with atherosclerosis [5]. Alloxan is another diabetogenic agent used for induction of diabetes in various animal models which causes necrosis of the islets on administration and several features observed in human diabetes [6].

Both clinical and experimental studies have reported the pivotal role of oxidative stress in development and progression of diabetic complications resulting in increased production of free radicals or reactive oxygen species ROS which in turn leads to a decline in the antioxidant defense mechanism. Uncontrolled or poorly controlled diabetes causes increased production of ROS as a result of glucose oxidation, lipid peroxidation and non-enzymatic glycation of proteins, all of which contribute to the development of complications. Living organisms have developed complex antioxidant systems which include enzymes and non-enzymes to counteract reactive species and to reduce their damage [7][8]; however, the total antioxidant concentration TAC serves as a general marker of the antioxidant status. The present study therefore was designed to determine the serum levels of lipids and TAC as biomarkers of cardiovascular complications and oxidative stress respectively in alloxan induced diabetic rats.

II. Materials and Methods

Experimental Animals

20 albino wistar rats of both sexes weighing between 150g and 200g were used for this study. They were obtained and housed at room temperature in the animal house of College of Medicine, University of
Nigeria, Enugu Campus. The animals were maintained on a standard poultry diet (Vital Feeds, Jos) and water ad libitum, and were allowed acclimatization for a period of two weeks. The experimental animals were handled according to the institutional and international guidelines for the care and use of laboratory animals.

**Experimental Design**

The animals were randomly assigned into two groups I and II consisting of ten rats each. Group I was the control (non-diabetic) group while Group II was the diabetic group.

**Induction of Diabetes**

Experimental diabetes was induced in group II animals by a single intraperitoneal dose of alloxan monohydrate (Sigma Aldrich) at 150mg/kg body weight dissolved in freshly prepared normal saline while those in group I were given normal saline intraperitoneally after an overnight fast. On the 4th week, 10 rats from each group were fasted overnight, and blood samples collected for the estimation of biochemical parameters.

**Biochemical Assays**

After 4 weeks of study, blood samples were collected from the tail vein into fluoride oxalate, EDTA and plain tubes. Fasting Plasma Glucose FPG, total cholesterol TC, high density lipoprotein HDL and triglyceride TG were measured colorimetrically using commercial kits (Randox) while low density lipoprotein LDL and very low density lipoprotein VLDL were calculated using Friedwald’s equation. Glycated Hemoglobin HbA1c was measured in whole blood using micro-column chromatography. TAC was analysed using thiobarbituric acid reactive substance TBARS.

**Statistical Analysis**

This was done using SPSS version 17.0. All values were reported as mean ± standard deviation. Student’s t-test was used to compare the means, and statistical significance set at p < 0.05.

**III. Results**

**Biochemical Findings**

Table 1 shows the mean concentrations of FPG and glycated hemoglobin of alloxan-induced diabetic rats which were found to be significantly (p<0.05) elevated as compared to the control rats.

The mean serum concentrations of TC, LDL, VLDL and TG were significantly increased (p<0.05) in the diabetic group compared to the control group while the mean serum TAC and HDL concentration of the diabetic group decreased significantly (p<0.05) when compared to the control as shown in table 2.

| Table 1: FPG and HbA1c of Control and Diabetic Rats. |
|-----------------|-----------------|-----------------|
| **Group** | **FPG (mmol/l)** | **HbA1c (%)** |
| I | 5.0 ± 0.4 | 3.8 ± 0.3 |
| II | 13.7 ± 1.1 | 9.7 ± 1.4 |
| **p-value** | 0.001 | 0.001 |

| Table 2: Serum Lipid Profile and TAC of Control and Diabetic Rats |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Grp** | **TC (mmol/l)** | **HDL (mmol/l)** | **LDL (mmol/l)** | **VLDL (mmol/l)** | **TG (mmol/l)** | **TAC (mmol/l)** |
| I | 2.4 ± 0.2 | 1.3 ± 0.1 | 0.7 ± 0.2 | 0.5 ± 0.1 | 1.0 ± 0.1 | 1.4 ± 0.4 |
| II | 3.9 ± 0.2 | 0.6 ± 0.1 | 2.3 ± 0.3 | 1.1 ± 0.1 | 2.3 ± 0.1 | 0.7 ± 0.2 |
| **p-value** | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

**IV. Discussion**

In this study, administration of alloxan resulted in a significant elevation of blood glucose concentration when compared to the control rats. This result agrees with the earlier reports of [9][10][11]. The susceptibility of the pancreas to the action of alloxan induced free radical damage leads to massive reduction of insulin release resulting to insulin deficiency as well as increased blood glucose and a host of other metabolic changes.

In the present study, HbA1c was used as an indication of glycaemic control. The significant increase in HbA1c concentration in the diabetic rats when compared to the control may be attributed to persistent hyperglycaemia. Persistent hyperglycaemia or uncontrolled diabetes leads to an increased glycosylation of a
number of proteins as the excess glucose in blood reacts with these proteins. This result is in accordance with those of [12] and [13].

Serum lipid profile was used to assess development of cardiovascular complications in the present study which recorded elevated total cholesterol, LDL, VLDL and triglyceride levels whereas HDL levels decreased in alloxan-induced diabetic rats which is associated with cardiovascular diseases as seen in diabetes. The abnormal high levels of serum lipids in diabetes may be due to an increase in the activity of hormone sensitive lipase in insulin deficiency resulting in enhanced lipolysis and release of free fatty acids from adipose tissue [14][15] and peripheral depots resulting from underutilization of glucose. Some of the excess fatty acid produced is then metabolized to acetyl co A which is used in the synthesis of cholesterol in the liver, thus increasing cholesterol levels in diabetes. On the other hand, glucagon, catecholamines and other hormones may also enhance lipolysis. Therefore, the uninhibited action of lipolytic hormones on the fat depots may be responsible for this increase.

The high levels of LDLCould be due to diminished levels of LDL receptors resulting in increased circulation of LDL particles [16]. The decreased serum HDL levels in the present study may enhance CVD risk since HDL function is to remove cholesterol atheromas within arteries and transport them back to the liver for excretion and re-utilization.

The results support the works of [17][18][19] and [20] who reported marked increase in cholesterol, triglycerides, LDL, VLDL and decreased HDL in diabetic rats when compared to non-diabetic rats.

However, Jos [21] reported no correlation between diabetes mellitus and lipids in the study conducted with diabetes patients. Betteridge [22] reported significant alterations in serum lipids and lipoproteins profile associated with increased risk of coronary heart disease in diabetes. Moreover, oxidative stress may occur from glucose and lipid metabolic abnormalities which are associated with development of atherosclerosis and cardiovascular complications in diabetic patients [23].

In this study, diabetic rats exhibited a profound decrease in serum TAC when compared to the control. This could be attributed to increased ROS generation by mechanisms such as glucose auto-oxidation, polyol pathway and PKC activation or over expression of antioxidant enzymes in response to glucose-induced oxidative stress. This agrees with the reports of [9] and [19] but disagrees with the study done by Turklap [24] who reported no significant difference between TAC of diabetic and control groups.

V. Conclusion

From our study, alloxan monohydrate administered at a dose of 150mg/kg body weight induced diabetes in the experimental animals. It may therefore be concluded that untreated or uncontrolled diabetes caused alterations in serum lipid profile and total antioxidant concentrations.

References


DOI: 10.9790/3008-1103038790 www.iosrjournals.org 89 | Page
Serum Lipids and Total Antioxidant Concentration in Alloxanized Diabetic Rats.


