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EVALUATION OF SOME OXIDATIVE STRESS MARKERS IN TYPE II *Diabetes mellitus* IN ILE-IFE, NIGERIA

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B. Tech. (Ogbomoso)

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APPROVAL

This thesis was supervised by us and approved in accordance with partial fulfilment ofthe requirements for the award of Masters of Science (M.Sc.) Degree in Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

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DEDICATION

This work is dedicated to

God Almighty

and to

my dear parents, Late Mr. M. A. Odewole and Mrs. L.O. Odewole

for their unending support.

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LIST OF ABBREVIATION

AGE	Advanced Glycation End product
CAT	Catalase
CVD	Cardiovascular Diseases
DAG	Diacylglycerol
DM	*Diabetes mellitus*
FBS	Fasting Blood sugar
GDM	Gestational *Diabetes mellitus*
GPAT	Glutamine-6-phosphate Amindotransferase
GLUTH	Glucose Transporter
GOD	Glucose Oxidase
GSH	Glutathione
GSH-px	Glutathione Peroxidase
GST	Glutathione-S-Transferase
HbAlc	Glycosylated Hemoglobin
IDF	International Diabetic Federation
LADA	Latent Autoimmune Diabetes of Adult
LDL	Low Density Lipoprotein
LED	Light Emitted Diode

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MRDM	Malnutrition-related Diabetes mellitus
NF-Kb	Nuclear Factor Kappa B
PD	Photodiode
PKC	Protein Kinase C
POD	Peroxidase
PPAR	Peroxisome – Proliferator Activated Receptor
RAGE	Advanced Glycated End product receptor
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
T2DM	Type II Diabetes mellitus
TGF	Transforming Growth factor
UCP	Uncoupling Protein
VEGF	Vascular Endothelial Growth Factor
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
TZD	Thiazolidinediones
CVD	Cardiovascular Disease
FPG	Fasting Plasma Glucose
SU	sulphonylurea

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ABSTRACT

This study investigated the levels of plasma fasting blood glucose, glycated heamoglobin (HbA1c), and oxidative stress enzyme markers: superoxide dismutase (SOD), catalase, glutathione-S-transferase (GST) in the erythrocyte of type II diabetic patients. This was with a view to knowing the levels of these enzymes in the patients and in healthy subjects.

One hundred and fifteen respondents were involved in this study. They comprised sixty five diabetic subjects recruited from the Diabetes Clinic of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, and fifty healthy controls who volunteered to be part of the study and were members of staff of Obafemi Awolowo University (OAU) and were age and sex matched with test subjects. Ethical approval was obtained from the Ethics and Research Committee of OAUTHC, Ile-Ife. All subjects read and signed the informed consent form. Seven milliliters of venous blood of the patients and controls were collected aseptically. Glycosylated hemoglobin was determined in whole blood using clover HbA1c kit while Plasma fasting blood glucose was determined using Randox kit. The erythrocyte was separated from the plasma by centrifugation at 4000 rpm and washed three times with 250 mM Tris HCl, pH 7.4. The erythrocytes were re-suspended in 1.0 ml of the same buffer and stored at 4°C. The washed erythrocyte was lysed using standard procedure. The hemolysate was centrifuged at 13500 rpm for 30 min. The supernatant was collected and used to assay for the antioxidant enzymes (catalase, superoxide dismutase and glutathione-S-transferase).

Significant differences were found when erythrocyte superoxide dismutase, catalase and glutathione-S-transferase specific activities 53.22±2.44, 48.20±2.69, and 1.35±0.09 units/mg protein in diabetic patients were compared with healthy controls 62.54±2.68, 63.22±1.83 and 3.47±0.11 units/mg protein respectively ($p< 0.05$). When diabetic subjects were grouped into

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four groups based on the presence of microvascular complications, the specific activity of antioxidant enzymes; superoxide dismutase, glutathione-S-transferase and catalase in diabetic patients with no complications were 59.45±4.94, 51.07±3.59 and 1.52±0.14 units/mg protein. Diabetic patients with one complication had 50.08±4.32, 46.87±6.41 and 1.19±0.13 units/mg protein and diabetic patients with two complications had 46.85±3.66, 47.45±5.58 and 1.0±0.13 units/mg protein respectively. No significant difference was found between these groups (p>0.05). Also when diabetic patients were grouped based on glycemic control, the specific activity of antioxidant enzymes; superoxide dismutase, glutathione-S-transferase and catalase in diabetic patients with poor glycemic control were 73.79±3.01, 51.96±7.38 and 1, 42±0.18 units/mg protein. Patients with fair glycemic control had 45.67±3.07, 45.33±3.84 and 1.28±0.16 units/mg protein while patients with good glycemic control had 55.54±4.12, 52.62±5.29 and 1.39±0.13 units/mg protein respectively. No significant difference was found (p>0.05).

This study concluded that the level of erythrocyte antioxidant enzymes activities of *Diabetes mellitus* patients in Ile-Ife was significantly low.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting either from defects in insulin secretion and insulin action or both. The chronic hyperglycemia is associated with long-term damage, dysfunction and failure of normal functioning of various organsespecially the eyes, kidneys, nerves, heart and blood vessels (Paneni *et al*., 2013). Diabetes specific microvascular disease is a leading cause of blindness, renal failure, and nerve damage (Cade, 2008). The prevalence of diabetes is rising all over the world due to population growth, aging, urbanization and the increase of obesity due to physical inactivity. All nations, rich and poor, are suffering the impact of the diabetes epidemic. The impact is worse in those countries that are socially and economically disadvantaged (International Diabetes Federation, 2011).

Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 (Wild*et al.,* 2004). This increasein prevalence is expected to be more in the Middle Easterncrescent, Sub-Saharan Africa and India (Wild *et al*., 2004).In Africa, the estimated prevalence of diabetes is 1% inrural areas, up to 7% in urban sub-Sahara Africa, andbetween 8-13% in more developed areas such as SouthAfrica and in population of Indian origin (Motala, 2002).The prevalence in Nigeria varies from 0.65% in ruralMangu (North) to 7.6% in urban Lagos (South) (Akinkugbe, 1997) and datafrom the World Health Organization (WHO) suggests thatNigeria has the greatest number of people living withdiabetes in Africa (World Health Organization, 1994).

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Hyperglycemia increases reactive oxygen species (ROS) levels, which are produced in normal physiology as a result of metabolic activity of the mitochondria,which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in *Diabetes mellitus* (Jaganjac *et al*., 2013). The involvement of ROS in the etiology and the development of late complications of *Diabetes mellitus* have also been addressed (Hunt *et al.,* 1998). During normal metabolic functions, highly reactive compounds called free radicals are generated in the body; however, they may also be introduced from the environment. These molecules are inherently unstable as they possess lone pair of electrons and hence become highly reactive. They react with cellular molecules such as proteins, lipids and carbohydrates, and denature them.As a result of this, vital cellular structures and functions are lost ultimately resulting in various pathological conditions.

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt the oxidizing chain reaction to minimize the damage caused by free radicals. For the past decade, many studies have been devoted to the beneficial effects of antioxidant enzymes (Halliwell*et al.,* 1999;Worthington Enzyme Manual, 2009). It has been found that a substantial link exists between free radicals and more than sixty different health conditions, including the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, our body's potential to reducing the risk of free radical related health problems is made more intense. Antioxidant enzymes are, therefore, absolutely critical for maintaining optimal cellular and systemic health and wellbeing (Worthington Enzyme Manual, 2009).

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1.1 Statement of Research Problem

Diabetesis a public health problem all over the world with its prevalence increasing steadily. Data on relationship between *Diabetes mellitus* and levels of antioxidant defense system is not well reported and paucity of the levels of oxidative stress marker enzymesin Ile-Ife, Nigeria. There is a need to further explore the relationship between antioxidant enzymes and the occurrence of *Diabetes mellitus*and its complications in this environment, hence this study.

1.2 Specific Objectives of Research:

The specific objectives of the research are to

(a) determine levels of oxidative stress enzyme markers: superoxide dismutase, catalase, glutathione-S-transferase in the erythrocyte of diabetic subjects compared with healthy controls;

(b) ascertain the relationship between the levels of these parameters and glycemic control; and

(c) ascertain the association between the levels of these parameters and the occurrence of Diabetes and its complications.

1.3 Contribution to Knowledge

It is expected that this study will provide information that will assist in expanding treatment options for diabetic patients and provide measures which may prevent or delay the development of complications.

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