

**EVALUATION OF THE EFFECT OF NON-ENZYMIC ANTIOXIDANT AND TRACE
ELEMENT STATUS IN DIABETES MELLITUS PATIENTS IN ILE-IFE, OSUN
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B. Tech (Ogbo moso)

SCP11/12/R/0146

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**A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF
SCIENCE, OBAFEMI AWOLowo UNIVERSITY, ILE-IFE, IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE (MSc.)
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APPROVAL

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

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DEDICATION

This work is dedicated to the

Almighty God

The Author and the Finisher of my Faith

The Beginning and the Ending

Your Greatness reaches beyond the Stars

And to the Fagbohuns

God bless you all.

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Fisoye

Oadapo

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LIST OF ABBREVIATIONS

OAUTHC	Obafemi Awolowo University Teaching Hospital Complex
WHO	World Health Organization
IDF	International Diabetes Federation
SPSS	Statistical Package for Social Sciences
GEDTA	Glycol ether diamino tetracetic Acid
AVP	Arginine Vasopressin
NDI	Nephrogenic <i>Diabetes insipidus</i>
CDI	Central <i>Diabetes insipidus</i>
ADH	Antidiuretic Hormone
ATP	Adenosine Triphosphate
GLP	Glucose-like Insulinotropic Peptide
GLP-1	Glucagon-like Peptide 1
DM	<i>Diabetes mellitus</i>
GLUT	Glucose Transporter
ADP	Adenosine Diphosphate
SUR	Sulfonyl urea
HbA1c	Glycosylated Hemoglobin
T1DM	Type 1 <i>Diabetes mellitus</i>
T2DM	Type 2 <i>Diabetes mellitus</i>
GDM	Gestational <i>Diabetes mellitus</i>
IDDM	Insulin Dependent <i>Diabetes mellitus</i>
NIDDM	Non-insulin Dependent <i>Diabetes mellitus</i>
HLA	Human Leukocyte Antigen

DAG	Diacylglycerol
FFA	Free Fatty Acid
ESRD	End Stage Renal Disease
USA	United States of America
ERC	Ethics and Research Committee
AGEs:	Advanced Glycation End-products
RAGE	Receptor for Advanced Glycation End-products
IUPAC	International Union of Pure and Applied Chemistry
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
PKC	Protein Kinase C
UHMWPE	Ultra High Molecular Weight Polyethylene
LDL	Low Density Lipoprotein
CTGF	Connective Tissue Growth Factor
SR-A	Scavenger Receptor Class A
FBS	Fasting Blood Glucose
OGTT	Oral Glucose Tolerance Test
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
RDA	Recommended Dietary Allowance
PTH	Parathyroid hormone
HDL	High Density Lipoprotein
ROS	Reactive Oxygen Species
CoA	Co-enzyme A
CuZnSOD	Copper-Zinc Superoxide Dismutase

ABSTRACT

This study investigated levels of the trace elements; zinc, copper and magnesium and vitamins C and E in diabetic patients at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife. This was with a view to understanding the relationship between the levels of these parameters and the presence of diabetes and its complications.

One hundred and fifteen subjects, comprising sixty five diabetic patients and fifty healthy controls were involved in this study. Ethical clearance was obtained from the Ethical and Research Committee of OAUTHC, Ile-Ife. All subjects signed informed consent forms. Fasting blood samples of the patients and controls were collected aseptically and the plasma was collected after centrifugation at 4000 revolutions per minute (rpm) for 20 minutes. Fasting blood glucose was determined using Randox kit while glycosylated haemoglobin was determined using Clover Alc kit. Plasma aliquots were frozen at -80°C and used to analyse vitamin C, vitamin E, zinc, copper and magnesium. Vitamin C was determined by redox titration with 2, 6-dichlorophenolindophenol against plasma aliquots acidified by trichloroacetic acid. Vitamin E was determined by a modified spectrophotometric method. Plasma levels of zinc, copper and magnesium were determined using standard procedures.

The mean values of fasting blood glucose, glycosylated haemoglobin (HbA1c), zinc, copper, magnesium, vitamin C and E for diabetic patients in this study were 9.12 ± 3.86 mmol/L, 8.83 ± 2.34 %, 6.61 ± 4.11 $\mu\text{mol/L}$, 19.07 ± 8.96 $\mu\text{mol/L}$, 0.64 ± 0.07 mmol/L, 77.79 ± 24.98 $\mu\text{mol/L}$ and 1.47 ± 1.10 $\mu\text{mol/L}$ respectively while those for the healthy control subjects were 4.77 ± 0.45 mmol/L, 15.01 ± 4.26 $\mu\text{mol/L}$, 16.89 ± 6.25 $\mu\text{mol/L}$, 0.77 ± 0.03 mmol/L, 72.68 ± 15.33 $\mu\text{mol/L}$ and 4.79 ± 1.38 $\mu\text{mol/L}$ respectively. Fasting blood glucose level was significantly higher ($p < 0.05$) in the diabetic group compared to the control group while levels of zinc, magnesium and vitamin E

were significantly lower in the diabetic group compared with the controls. When the mean values of the biochemical parameters of diabetic patients with no complications, those with one microvascular complication, those with two microvascular complications and those with three microvascular complications were compared, significant differences were found in the levels of vitamin E and zinc. No significant differences were found in the parameters when the diabetic patients were grouped on the basis of glycaemic control (good, fair and poor glycaemic control).

This study concluded that the pattern of alterations in antioxidant trace elements and vitamins of diabetic patients appeared to be a consequence of diabetes itself, and were not predicted by glycaemic status or the presence of microvascular complications.

CHAPTER ONE

INTRODUCTION

1.1 Overview

Diabetes mellitus is the most prevalent disease worldwide (Shaw *et al.*, 2010). It is a group of metabolic diseases characterized by high blood glucose levels resulting from either defects in insulin secretion or action (Praveena *et al.*, 2013). These high blood glucose levels otherwise known as hyperglycemia, is associated with long term damage, dysfunction and failure of various organs such as the eyes, kidneys, nerves, heart and blood vessels (Taylor *et al.*, 1995), and symptoms include polyuria, polydipsia, weight loss, polyphagia and blurred vision. There are several pathogenic processes involved in the development of diabetes ranging from autoimmune destruction of β -cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on target tissues which result from inadequate insulin secretion or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action (Cavin *et al.*, 2003).

Majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. Type 2 *Diabetes mellitus* affects a vast number of persons worldwide and according to the World Health Organization (WHO) and the International Diabetes Federation (IDF), this type of diabetes has reached an epidemic proportion and has become one of the most challenging health problems of the 21st century (Unwin *et al.*, 2010). The number of people with type 2 diabetes in the year 2010 is estimated to be 285 million, representing 7% of the adult world population and by the year 2030, an estimated 439 million individuals worldwide will have this disorder with the most marked increase projected for the population greater than 65 years of age (Shaw *et al.*, 2010; Unwin *et al.*, 2010). The African continent counts approximately 100 million people with type 2 diabetes with Nigeria having the highest proportion (approximately 12,180,000 people affected). Nigeria also has the highest number of people with impaired glucose tolerance with an estimated 4.85 million people (Shaw *et al.*, 2010).

Diabetes is also associated with an increased risk of long term complications (Feldman, 2003). Long term complications include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers etc. Complications however, have been associated with the presence of oxidative stress (Sinclair *et al.*, 1990). Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (free radical) and antioxidant defenses. Factors responsible for oxidative stress include polyol pathway, prostanoïd synthesis and protein glycation which disturbs the antioxidant defense system of the cell by increasing the functional activities of free radicals such as superoxide anion

(O_2^-), the hydroxyl radical (OH) and hydrogen peroxide (H_2O_2), others are hypochlorous acid ($HOCl$), reactive nitrogen species like nitric oxide (NO), nitrogen dioxide (NO_2) as well as non-radicals such as peroxynitrite ($ONOO$), nitrous oxide (HNO_2) and alkyl peroxynitrates ($RONOO$).

Antioxidant defense system of the body play a major role in scavenging free radicals produced in relation to the progression of *Diabetes mellitus*. Antioxidants are molecules that inhibit the oxidation of other molecules by terminating a chain oxidative reaction via the removal of free radical intermediates (Gavin *et al.*, 2003). There are two types of this complex system the enzymic antioxidant defense system which include glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase while vitamins A, C and E constitute the non-enzymic antioxidant system. Trace elements which are essential nutrients with regulatory, immunologic and antioxidant functions resulting from their action as essential components or cofactors of enzymes throughout metabolism also exist. Trace elements influence the pathogenesis of Diabetes and its complications, mainly through their involvement in peroxidation and inflammation (Bonney *et al.*, 2000; Robertson, 2004).

These antioxidant defense agents have been investigated as potential preventive and therapeutic agents for the complications of type 2 diabetes (Kelly, 1998). In particular, type 2 diabetes has been shown to be associated with abnormalities in the metabolism of zinc, chromium, copper, magnesium and manganese (Bonney *et al.*, 2000). These metals serve as cofactors for the enzymes; superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, which are the major antioxidant defense mechanisms of the body (Pidduck *et al.*, 1970). Alterations in the metabolism of several trace elements, including copper, zinc, manganese and the macro-element magnesium have been associated with impaired insulin release, insulin resistance and glucose intolerance in experimental animals and humans (Fields *et al.*, 1983; Park *et al.*, 1986; Padisso *et al.*, 1989). Other antioxidant agents include Vitamins C and E. These Vitamins are diet derived and detoxify free radicals directly. Vitamin E has been reported to protect membranes from lipid peroxidation and its deficiency is concurrent with increased peroxides and aldehydes in many tissues (Feher *et al.*, 1987). Ascorbic acid is known to reduce or neutralize reactive oxygen species such as hydrogen peroxide. It is also a substrate for the redox enzyme; ascorbate reductase (Padayatty *et al.*, 2003).

1.2 Statement of Research Problem

Diabetes and its complications constitute a public health issue in Nigeria. Data on antioxidant trace elements and vitamin status of diabetic patients in Ile-Ife is not readily