EVALUATION OF THE EFFECT OF NON-ENZYMIC ANTIOXIDANT AND TRACE ELEMENT STATUS IN DIABETES MELLITUS PATIENTS IN ILE-IFE, OSUN STATE, NIGERIA

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B. Tech. (Ogbomoso)

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF SCIENCE, OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE (M.Sc.) DEGREE IN BIOCHEMISTRY

2015

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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Date

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.

ACKNOWLEDGEMENT

My utmost gratitude goes to God for the Divine support, strength and opportunity I have received from Him and for making this work a great success.

My sincere and honest appreciation goes to my efficient, prolific, tranquil and indefatigable supervisors; Dr. F. K. Agboola who is also the Head of Department, Department of Biochemistry and Dr. (Mrs.) B. O. Emma-Okon who is also the Head of Department, Department of Medical Biochemistry, for their love, financial support, thoroughness, understanding and advice in places I can never imagined. I cannot but thank you enough for the invaluable support during the course of my research work and for making me a better person. God bless you sir and ma.

My appreciation goes to all my Lecturers: Prof. O. O. Oyedapo, Dr. (Mrs.) A. Kuku, Dr. O. Babalola, Dr. E. M. Obuotor, Dr. R. O. Osoniyi, Dr. (Mrs) B. A. Akinpelu, Dr. R. E. Okonji, Dr. O. O. Odekanyin, Dr. (Mrs) K. F. Akinwumi, Dr. (Miss) O. O. Ogunruku and Miss. Z. Salimanu. Thank you God bless you indeed.

I am greatly indebted to Prof. B. A. Kolawole of the Department of Medicine, O. A. U, Ile-Ife, a father indeed who gave his time during the research work for the recruitment of the needed Diabetic patients and for scoring the various microvascular complications and Dr. O. K. Clorundare; a rare gem at the Department of Ophthalmology, Obafemi Awolowo University Teaching Hospital Complex who helped during the eye screening of the diabetic patients.

I wish to thank all the members of the non-teaching staff of the Department of Medical Biochemistry particularly Mr. M. O. Babatunde, the Chief Technologist, and Mr. E. A.

Ademigbuji, a Laboratory Assistant, who have contributed one way or the other to the success of this work.

I cannot thank enough my research partner: Odewole Titilayo. You are really indeed a blessing to me. Your hard work also made my work a success. To my laboratory mates: Adediran Tolulope, Adedeji Nelson, Quadri Hafsat, Ilesanmi Olutosin and the Industrial Training students, I must confess I had a great time working with you. God bless you indeed. To all the laboratory undergraduates, though I cannot mention names, but I thank you all. I also appreciate all my classmates particularly Olasantan Olasiji, Abulude Ibukun and Akintunde Akinlalu. God bless you all. I am also greatly indebted to Agunbiade Oluwadare, Ojopagogo Yetunde and Adefila Adenike. Thank you for individual and collective contributions towards this research work. This page will not be complete without mentioning my sweetheart; Adeyemi Adebisi. You are but a great person for me and this whole world.

Finally, I am immensely grateful and undoubtedly appreciative of my parents; Mr. & Mrs. S. O. Fagbohun and Prof. & Prof. (Mrs) A. T. Salami for their parental supports both physically and financially, in love and prayers. Your care and love is beyond proportion. It is my prayer that you will eat the fruits of your labour. The thought of you my siblings, Mr. Fagbohun Oladayo, Mr. Fagbohun Oladotun and Mr. Fagbohun Oladoyin and my family members: Mrs. Ladoye Olayinka, Ajuwon Olabanji, Ayo-Salami Ayobami, Ayo-Salami Oluwatooni and Ayo-Salami Ayomipo who made the difference and kept me going throughout the period of the study. Thank you very much. God bless you all.

Fisoye Oladapo FAGBOHUN

2014

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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:	Glycoletherdiaminotetracetic Acid
AVP:	Arginine Vasopressin
NDI:	Nephrogenic *Diabetes insipidus*
CDI:	Central *Diabetes insipidus*
ADH:	Antidiuretic Hormone
ATP:	Adenosine Triphosphate
GIP:	Glucose-like Insulinotropic Peptide
GLP-1:	Glucagon-like Peptide 1
DM:	*Diabetes mellitus*
GLUT:	Glucose Transporter
ADP:	Adenosine Diphosphate
SUR:	Sulfonylurea
HbA1c:	Glycosylated Hemoglobin
T1DM:	Type 1 *Diabetes mellitus*
T2DM:	Type 2 *Diabetes mellitus*
GDM:	Gestational *Diabetes mellitus*
IDDM:	Insulin Dependent *Diabetes mellitus*
NIDDM:	Non-insulin Dependent *Diabetes mellitus*
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:	Parathormone
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 A1c kit. Plasma aliquots were frozen at -80^{o}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 trichloracetic 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 hemoglobin (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 µmol/L, 19.07±8.96 µmol/L, 0.64±0.07 mmol/L, 77.79±24.98 µmol/L and 1.47±1.10 µmol/L respectively while those for the healthy control subjects were 4.77±0.45 mmol/L, 15.01±4.26 µmol/L, 16.89±6.25 µmol/L, 0.77±0.03 mmol/L, 72.68±15.33 µmol/L and 4.79±1.38 µmol/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 glycemic control (good, fair and poor glycemic 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 glycemic 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 (Praveeena *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 (Gavin *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, prostanoid 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 (Bonnefont *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 (Bonnefont *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; Paolisso *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