KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

NUTRIENTS INTAKE, ANTIOXIDANT MICRONUTRIENT STATUS AND VISION DISORDERS AMONG DIABETICS ATTENDING CAPE COAST TEACHING HOSPITAL IN THE CENTRAL REGION OF GHANA

THIS THESIS IS PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARDS OF MPhil DEGREE IN HUMAN NUTRITION AND DIETETICS

BY

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DECLARATION

I declare that I have wholly undertaken the study reported herein under the supervision of Dr. Reginald Annan, and that except portions where references have been duly cited, this dissertation is the outcome of my research.

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DEDICATION

I dedicate this work to the Almighty God for His Grace and Mercies. This thesis is also dedicated to my father Mr. Eric Otoo, and Dr. Henry Doku for their relentless financial, moral, spiritual and emotional support.

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ABSTRACT

Globally, complications related to diabetes, including diabetic retinopathy (DR), cataract, macular oedema and dry eye syndrome represent the chief causes of blindness in adults but good antioxidant status may delay or prevent these. Using a cross-sectional design, the study assessed the relationship between intake and serum status of antioxidant micronutrients and vision disorders among 100 outpatient diabetic patients in Cape Coast, Ghana. Body Mass Index, glycated haemoglobin (HbA1c), fasting plasma glucose (FBG), serum concentrations of vitamin A, vitamin C and total antioxidants were determined. Visual acuity (VA), intra-ocular pressure (IOP), cup-to-disc- ratio (CDR) and funduscopy were conducted on the participants. Twenty four (24) hour dietary recall and food frequency questionnaires were used to assess dietary intakes of antioxidant nutrients. Data was analyzed using IBMS-SSPS (version 23). Nineteen percent of the participants were males and 81% were females, while 90% of the participants had of type 2 diabetes. Average age of participants was 59.7 years with no gender difference. Mean BMI (30.8 kg/m^2) put the participants as obese, although males had low mean BMI (p=0.002). Systolic blood pressure was higher in the males than females (148.7 versus 132.7 mmHg, p=0.004). Majority of participants (86%) had high HbA1c, 64% had high FBG, 33% were overweight and 48% were obese. One in 2 participants had cataract (50%), 27% had dry eye syndrome, 20% had uncorrectable refractive error (UNRE), 15% had DR and 2% had Diabetes Macular Oedema (DME). Vision problems were not significantly associated with duration of diabetes. Majority of participants reported daily or 1-3 times per week consumption of antioxidant-rich foods such as leafy vegetables, fruits, oily fish and nuts. Intakes of vitamin A, vitamin E, and Zinc were inadequate by 16.8%, 41.7% and 65% respectively based on Recommended Daily Allowances (RDA) while vitamin C and selenium intakes were in excess by 5.7% and 100.9% respectively. Biochemically, serum vitamin A (0.023±0.01)µmol/L and vitamin C (0.26±0.1) µmol/L were low while twenty four percent (24%) of the subjects had low total antioxidant status (TAS) (mean=24.4±5.3 µmol/L). Participants with inadequate vitamin C intake were more likely to have cataracts (31 versus 13%, p=0.046) than those with high intake, those with inadequate selenium status were more likely to have DME (2% for low, versus 0 for normal and high intake, p=0.002) and those with inadequate Zinc status were more likely to have cataracts (44% for low, 1% for normal and 5% for high intake, p=0.0013) and DR (10% for low, 5% for normal and 0% for high intakes, p=0.028). On the other hand, high selenium intakes was associated with higher level of UNRE (2% for low, 5% for normal and 13% for high intakes, p=0.043) and cataracts (11% for low, 3% for normal and 36% for high intakes, p=0.041). Adequate dietary fibre intake showed to reduce the risk of diabetic vision by 2-fold. In conclusion, hyperglycemia, overweight/obesity and vision impairment were common among the diabetics studied. Significant proportions of the participants had inadequate intakes and serum status of antioxidant micronutrients and in addition to fibre were associated with vision impairment.

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LIST OF ABBREVIATION

DM	-	Diabetes mellitus
T2DM	-	Type 2 Diabetes Mellitus
T1DM	-	Type 1 Diabetes Mellitus
IDF	-	International Diabetes Federation
BP	-	blood pressure
HbA1c	-	Glycated Hemoglobin
FPG	-	Fasting Plasma Glucose
DR	-	diabetic retinopathy
DME	-	diabetic macular edema
WHO	-	World Health Organization
PDR	-	proliferative diabetic retinopathy
ETDRS	-	Early treatment Diabetic Retinopathy Study
SOD	-	superoxide dismutase
GPx	-	glutathione peroxidase
TAS	-	total antioxidant status
HDL	-	Hyperlipidemia
VI	-	visual impairment
UNRE	-	uncorrected refractive errors
DES	-	dry eye syndrome
CAT	-	Cataract
VEGF	-	vascular endothelial growth factor
SCL	-	soft contact lens
CCTHERC	-	Cape Coast Teaching Hospital Ethical Review Committee
IOP	-	Intra Ocular pressure

CDR	-	Cup-disc-ratio
FPG	-	fasting plasma glucose
NPDR	-	Non-Proliferative Diabetic Retinopathy
RDA	-	Recommended Daily Allowance
CDC	-	Centre for Disease Control
AGEs	-	Advanced Glycated End products
OS	-	Oxidative Stress
GPx	-	Glutathione Peroxidase
BMI	-	Body Mass Index
LDL	-	Low Density Lipoprotein
DDS	-	Dietary Diversity Score
FFQ	-	Food frequency Questionnaire
PVA	-	Pro-Vitamin A
CHO	-	Carbohydrate

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information on Diabetes and its complications

Diabetes mellitus (DM) is a systemic disease marked by the presence of hyperglycemia, which can lead to many complications over time (Costa & Soares, 2013). International Diabetes Federation (IDF) reported that the number of people living with diabetes was expected to increase from 415 million in 2015 to 642 million by the year 2040 worldwide (IDF, 2015). Globally, Diabetes mellitus affects about 382 million people resulting in considerable morbidity and mortality, this number is expected to rise to 592 million and in Sub-Saharan Africa, prevalence was to increase from 19.8 million in 2013 to 41.4 million by 2035 (Guariguata *et al.,* 2014). The effects of diabetes are catastrophic and well recorded. About 450,000 Ghanaians have been diagnosed with diabetes, with an urban population prevalence rate of about 6% (Ofori-Asenso & Garcia, 2016). Unfortunately, this disease is not curable but can only be managed, poor control of blood glucose in diabetics lead to both macro blood vessels and micro blood vessels of the body being attacked and this leads to non-injury amputation, blindness and visual impairments, cardiovascular diseases and end stage kidney disease in adults in both developed and developing countries. (Kowluru & Chan, 2007). In type 1 DM, beta-cells of the pancreas are destroyed resulting from a cellular-mediated autoimmune response which prevents insulin production while type 2 DM is a result of insulin resistance and/or inadequate insulin secretion (ADA, 2014). Type 2 DM leads to serious microvascular and macrovascular complications such as diabetic visual problems among which diabetic retinopathy is a prominent cause of vision loss among diabetic patients (Lam *et al.*, 2011).

Currently, it is not possible to predict who will develop retinopathy and other visual impairments from individual perspective, even though these conventional risk factors are monitored on regular basis in diabetic patients. There are inadequate biomarkers that are sensitive indicators for early stages of complication onset. Diabetes mellitus causes increase hyperglycemia which can lead to oxidative stress on endothelial tissue and blood. These reactive oxygen species contribute to pathological alteration, leading to diabetes complication such as diabetic retinopathy (Porasuphatana *et al.*, 2012).

In DM patients, elevated levels of oxidative stress and reduced antioxidant status are often found and these may play an essential role in the evolution of diabetic complications including retinopathy and other visual problems (Choi *et al.*, 2008; Will *et al*., 1999). Dietary antioxidants are emerging promising evidence of nutritional intervention for the prevention of diabetes complications. The manifestations of diabetes in the eye, notably diabetic retinopathy (DR) and diabetic macular edema (DME) are the most significant causes of visual impairment and blindness among diabetic patients. They are 25times more liable to become blind than the general healthy population (Williams *et al.*, 2004).

Globally, diabetic retinopathy (DR) accounts for 5% of all blindness, affecting 2 million people and it is the leading cause of blindness in people aged 15 – 64 years in industrialized countries (WHO, 2006). Other studies show that not only DR, but also cataract and other visual impairments such as uncorrected refractive error, dry eye syndrome, and glaucoma are recorded (Gill *et al*., 2009; Klein *et al*., 2009; Mohamed *et al.,* 2007). The diet of a diabetic is essential to controlling and achieving normoglycemia or near normal glycaemia which is the primary goal of diabetes management. In view of this, food and nutrition interventions including dietary intake of whole grains, fruits, vegetables, legumes and low fat diet are recommended to aid in reducing postprandial blood glucose (ADA, 2008).

Vitamins, minerals and other micronutrients have the potential of boosting antioxidant defense, serve as enzymatic cofactors for glucose homeostasis and as regulators of cell growth and differentiation. Currently, there has been interest in these nutrients due to their potential for preventing or treating several complications caused by diabetes (Kowluru & Zhong, 2011). Antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, vitamin A, vitamin C are substances that delay or inhibit oxidation and neutralize the oxygen free radicals. Increasing production of free radicals in diabetes mellitus over time may play a role in the development of diabetic retinopathy which is a significance complication of the disease (Gürler *et al*., 2000). A cross-sectional study conducted in Nigeria to evaluate the serum total antioxidant status (TAS) in T2DM patients discovered that serum level of TAS was low among the T2DM patients in the area (Sarah *et al*., 2014). Similarly, Mvitu-Muaka *et al.,* (2016), conducted a survey in Kinshasa-DR Congo, found that, lowest mean levels of serum selenium, magnesium, vitamin D, vitamin C, vitamin E, total antioxidant status was observed among type 2 diabetic mellitus (T2DM) patients with DR and recommended regular intake of foods, and supplements rich in antioxidants. Evidence suggests that antioxidant micronutrient especially vitamin C and E intake could improve cell defense system against oxidative stress and that further studies is warranted (Garcia-Medina, *et al.,*2011; Valdés-Ramos, *et al.,* 2015). This research assessed nutrient intake, antioxidant micronutrient status and how that is associated with vision outcomes among diabetic patients.

1.2 Problem Statement

According to IDF, (2015) diabetes mellitus has caused 4,790 deaths among adults in Ghana, and prevalence rate is about 6% (Ofori-Asenso & Garcia, 2016). Dietary intakes and lifestyles are associated with occurrence of diabetes and its complications. Foods such as whole grains, fruits and vegetables rich in antioxidant micronutrients have been showed to help reduce blood glucose and prevent risk of developing diabetic complications. The antioxidant properties of these micronutrient rich foods help to protect endothelial cells, blood vessels and nerves from oxidative damage, thus preventing/delaying the onset of diabetes-related complications. Evidence indicate that these antioxidant micronutrients especially vitamin C and E levels are decreased significantly in diabetics (Gupta & Chari, 2005). Therefore, antioxidant micronutrients have become a great public health concern because of their role in preventing diabetic complications, including retinopathy but very few studies have been conducted in Africa and for that matter Ghana. The relationship between dietary and antioxidant micronutrients status and vision problems in diabetes have not been established in Ghana. It is therefore essential to assess the dietary and serum level of antioxidant micronutrients and their protective effect against microvascular complications such as vision problems in people with diabetes mellitus.

1.3 General Objective

To determine the relationship between dietary nutrients intake, serum levels of antioxidant micronutrients, and prevalence of visual disorders among Diabetic patients.

1.4 Specific Objectives

- To determine the dietary nutrients intake of diabetic patients.
- To determine antioxidant micronutrients status of diabetic patients.
- To determine the prevalence of visual disorders among the diabetic patients

- To determine relationship between nutrients intake, antioxidant micronutrients status and the prevalence of visual disorders among diabetic patients.

1.5 Research Questions

- What are the nutrients intakes of diabetic patients?
- What is the serum antioxidant micronutrient status of diabetic patients?
- What is the prevalence of visual impairments among diabetics?
- What is the relationship between dietary patterns, serum antioxidant micronutrient status and the prevalence of visual impairment among the diabetic patients?

1.6 Justification

Assessing the relationship between the dietary patterns (including dietary antioxidant micronutrient intake), serum levels and the prevalence of vision problems will help to identify those with deficiencies and those at high risk. This will help to detect some vision problems and early treatment to prevent or delay irreversible vision problems. This will also provide information to health stakeholders to develop interventional strategies to improve diabetic health care specifically to prevent vision loss and delay permanent vision damage from diabetes. It will also add to existing knowledge in the arena of research.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Diabetic Eye complications

Diabetes Mellitus (DM) is described as a group of metabolic disorders which is marked by elevated blood glucose levels due to the destruction of insulin-producing pancreatic beta cells referred as type 1 (T1DM) or through insulin insensitivity to its target tissues like muscle and adipose also referred to as type 2 (T2DM)(Schwarz, & Tuomilehto, 2009). The prevalence rates of diabetes especially T2DM in both the developed and developing world are raising rapidly and these are attributed to the established risk factors, such as growing levels of obesity and physical inactivity and also increased numbers of older aged people (Aguiree *et al.*, 2013; Wild *et al*., 2004).

Diabetic eye disease represents the leading cause of blindness in adults under 75years of age in developed countries and known to be the leading cause of blindness among working aged adults around the world (Shaw *et al*., 2010). In 2010, over one-third of an estimated 285 million people worldwide showed signs of Diabetic Retinopathy(DR) and a third of these were burdened with vision-threatening retinopathy defined as severe non-proliferative DR or diabetic macular edema(DME) (Yau *et al.,* 2012). Moreover, proliferative diabetic retinopathy (PDR) has seen to be the most occurring vision-threatening lesion in type 1 diabetics (Simó & Hernández, 2009). The rate of DR has increased by 89% in the USA over the last decade, even though clinical trials show that better blood glucose control and blood pressure minimizes the risk of microvascular complications caused by diabetes (Jackson *et al*., 2012). This implies that blood glucose control alone has not been successful.

In Ghana, a study conducted by Amissah and Boateng at Ghana, (2014) on the prevalence of diabetic complications among people with T2DM attending Cape Coast Teaching Hospital

indicated, the prevalence of eye complications was found to be 58.6% (excluding blurred vision).

Unluckily, the eyes of a diabetic patient are not only at risk of retinopathy but also macular edema, cataract, glaucoma, dry eye syndrome, uncorrected refractive errors, poor corneal healing, optic and cranial neuropathies and retinal detachment caused by tissues scars which contract and make the retina to tear from the prime structure or root (Doherty, 2015). A study conducted by Donatella *et al.*, (2011) indicated that globally, cataract (33%) and uncorrected refractive errors (43%) are the chief causes of visual impairment and that cataract (51%) , glaucoma (1%) , age-related macular degeneration (5%), uncorrected refractive errors and trachoma (3%) and diabetic retinopathy (1%) are the major causes of blindness among diabetics.

The duration of diabetes is argued to strongly influence progression of diabetes and many other factors have been considered to also elevate the risk for the development and progression of retinopathy, which including hyperglycemia, hyperlipidemia, hypertension, nephropathy and pregnancy (Marathe *et al*., 2017). The United Kingdom Prospective Diabetes Study (UKPDS) discovered that systolic blood pressure and HbA1c among diabetics were the major risk factors for the incidence of diabetic retinopathy (Stratton *et al*., 2001). Also a reduction in every 1% of Hb1Ac decreased 40% of retinopathy, 25% need for retinal laser and 15% of blindness among people living with diabetes (Mohamed *et al*., 2007).

A cross-sectional study conducted by Selim, (2017) in Bangladesh found that majority of the T2DM patients were not able to achieve or maintain optimum glycemic control and had increased risk to DR. The mean HbA1c was 9.83% and HbA1c <7% was found in only 19.1 %(997), this implies poor glycaemic control. Increasing systolic blood pressure was found to be a risk factor for retinopathy among diabetics indicated by Raman *et al* in 2012. It was also

found that, a decrease in systolic pressure by every 10 mmHg reduced retinopathy and the need for retinal laser by 35% respectively and also decreased blindness by 50% (Klein *et al*., 2008: Klein *et al*., 2009)**.**

A cohort study of 1809 patients with T2DM conducted in Japan in 2016 indicated that high BMI and high weight category were significantly associated to diabetic retinopathy, as such more than half of the patients shows increased risks by approximately 1-4 folds for DR (Tanaka *et al*., 2016). Also, increased BMI >31kg/m^2 (men), BMI >32 kg/m^2 (women) and increased waist-hip ratio were associated with elevated risk of developing retinopathy (Klein *et al*.,1997). Hyperlipidemia has also been shown to be a risk factor; triglycerides level is associated with DR whilst LDL, high non-HDL cholesterol and HDL/LDL ratio are associated with DME (Raman *et al.*, 2010). Other studies suggest that puberty and pregnancy have roles to play in increasing the risk of DR and other visual impairments. It is indicated that the onset of any DR in post pubertal period is rapid (2years lesser) and has 30% increased risk of developing DR among diabetics than the pre-pubertal periods (Olsen *et al*., 2004). Pregnancy has also shown to increase the risk of DR and its progress by 2-3 folds and after delivery about 29% of the expectant mothers would have a reversion of the DR. The Early Treatment Diabetic Retinopathy Study aims to minimize visual disabilities in diabetics by early diagnosis and rapid treatment, which the Diabetic Retinopathy Study indicates that efficient treatment could reduce severe vision loss by 90% (Ting et al., 2016).

2.2 A review on the effects of antioxidant micronutrients on vision among diabetics.

Persistent hyperglycemia and insulin resistance are the hallmark of diabetes mellitus (Rafighi *et al.*, 2013) which enhance the occurrence of endothelial cell damage and elevated oxidative stress (King & Grant, 2016). Oxidative stress through the creation of reactive oxygen species as a result of heightened hyperglycemia can initiate diabetic complications (Doddigarla *et al.*,

2016). During oxidative stress, antioxidant capacity is decreased and it has been suggested that intakes of dietary antioxidant micronutrients can perform some function in oxidative stress reduction (Tabar, 2012). In spite of the numerous reviews and journal publications that had been done on diabetes mellitus and diabetic complications, very few data on antioxidant micronutrients in type 2 diabetes complications especially one pertaining to the eye or diabetic vision exist. Seemingly, the clinical importance of antioxidant micronutrients in diabetes particularly type 2 diabetes is still not fully understood which needs auxiliary evaluation (Mahdizadeh *et al.*, 2014).

A systematic investigation was conducted to select published studies from April, 2000 to March, 2016. The search assessed the defensive effects and efficacy of antioxidant micronutrients both intake and status (vitamins, minerals, phytochemicals and/or supplements) in the prevention and treatment of diabetic visual complications among type 2 diabetic patients. Google scholar, Pubmed, PMC, Plosone and Cochrane were the electronic databases used in the investigation. The search yielded in total of 21433 articles from the database with; Google scholar (17805), Pubmed (2026), PMC (1572), Plosone (20) and Cochrane (10). The search terms included "diabetic vision and nutrition", "antioxidants and diabetes", "antioxidants and diabetic complications", "antioxidants and risk of diabetic retinopathy", "antioxidant micronutrients and diabetic retinopathy", "dietary intakes", "macronutrients intakes", "antioxidant micronutrient intakes and diabetic retinopathy". The rest included "type 2 diabetes and visual impairment", "antioxidant micronutrient and diabetic cataract", "intakes of vitamin C and diabetic retinopathy", "vitamin A and diabetic eye disease", "serum vitamin C and diabetic visual complication", "serum vitamin C and risk of diabetic retinopathy", "serum vitamin A and diabetic vision", 'intakes of vitamin E and Zinc and diabetic vision", serum vitamin E/ α- tocopherol and diabetic retinopathy", "selenium and diabetic vision". After detailed evaluation of the searched articles, majority of

the articles were rejected due to duplicate articles, unrelated information, incomplete data and articles that did not meet the inclusion criterion. Following the removal of these articles, fourteen (14) articles comprising full text of publications were analyzed. Vitamin A, vitamin C, vitamin E, zinc, and selenium were searched. The main outcomes were decreased blood glucose (HbA1c or fasting plasma glucose (FPG), and/or reduced oxidative stress (lipid peroxidation/Malonialdehyde), improved antioxidant status, reduced risk of diabetic vision complication, and improvement in vision parameters (visual acuity, macular thickness, light sensitivity). The review added age groups in the search strategy, though most studies had age limit either early or late. Figure (1) presents the summary of the study selection process and result;

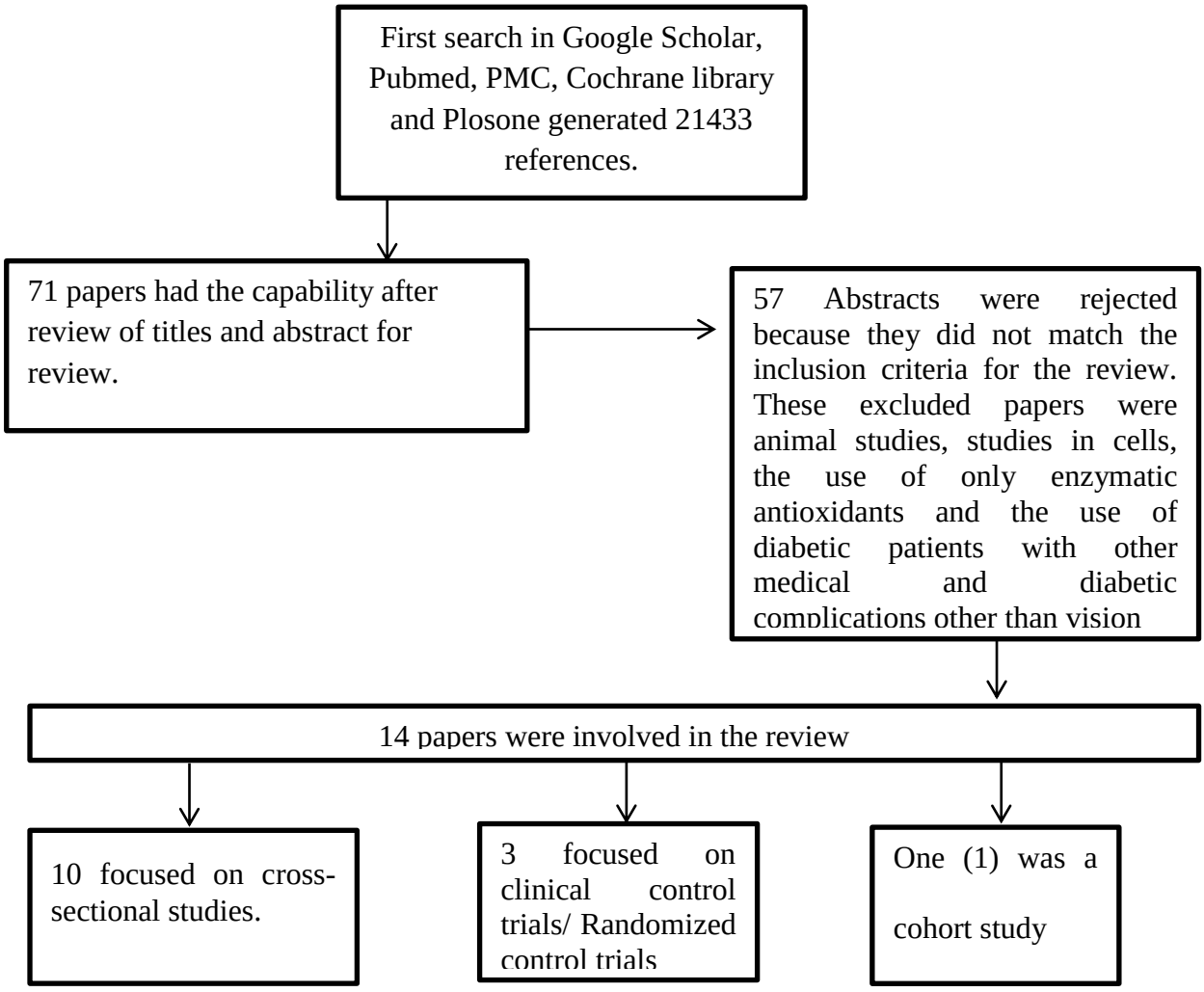


Figure 2.1: Summary of Search Strategy

2.2.1 Inclusion criteria

Study design: Cross-sectional, cohort, case-control, randomized clinical controlled trial.

Study population: Human samples aged ≥20 years and gender outcome.

2.2.2 Outcome measured

Antioxidant micronutrients status among type 2 diabetes mellitus patients (with vision complications), effects of dietary antioxidant micronutrient intakes (including supplements) on diabetic vision/retinopathy patients.

2.2.3 Exclusion criteria

Articles in Press

Animal studies

Review articles

Case reports that did not match the inclusion criteria

Studies on macrovascular complications

Type 1 and gestational diabetes mellitus patients.

2.2.4 Study design

Three study designs were identified in the 14 studies reviewed: Cross-sectional studies conducted focused on oxidative stress markers and antioxidants in diabetic vision, antioxidant micronutrient intake and status and the risk of diabetic retinopathy and carotenoids intake and serum status of diabetes mellitus patients (with or without retinopathy). Three (3) studies employed randomized clinical control trials where antioxidant micronutrient were supplemented in type 2 diabetes mellitus patients and healthy non-diabetics orally to determine its effect on treatment of DM vision complication especially DR and varying outcomes were also quantify after intervention.

2.2.5 Study population and country

Articles involved in the assessment had comparable population. Type 2 male and female diabetes mellitus patients and healthy controls within the age range from 20 and 80years were the subjects involved in the review. The dominant countries from search outcomes were India (3), United States of America (2) and China (2). Other studies from Turkey (1), Germany (1), Japan(1), Russia(1), Italy(1), Australia (1), and only one (1) from DR Congo in Africa. This indicates that very little studies on antioxidant micronutrients has been done in African since the past 17years especially pertaining to diabetic vision.

2.2.6 Antioxidant micronutrient involved in study

The principal antioxidant micronutrients taken into account were vitamin C (12 studies), vitamin A (2 studies), and vitamin E (7 studies). Nevertheless, other micronutrients such as zinc (2 studies), selenium (2 studies) and carotenoids (lutein/zeaxanthin, lycopene, α-carotene, β-carotene, β-cryptoxanthin (Ceriello, 2006) were also evaluated among type 2 diabetics with vision complications and compared to healthy non-diabetics.

Table 2.1: Summarizes the findings obtained from the studies reviewed

Study Design	Micronutrients	Main Findings	Knowledge Gap
Cross-sectional	Vitamins C, E, D, magnesium , zinc, selenium	Serum vitamin C, E, D, magnesium zinc and selenium were lowest in T2DM patients with diabetic retinopathy (DR) than controls. Significant higher SOD levels in T2DM with DR.	Supplementation and additional research is recommended for further evaluation
Randomized clinical control trial	Vitamins C,E,A, $B_{2,}$ lutein, zeaxanthin, rutin, zinc, selenium,	With the supplementation of antioxidant formula, best corrected visual acuity had significant decrease, increased macular thickness, and improved light sensitivity in DR groups	Serum biochemical markers were not assessed .
Randomized clinical control trial	Complex taurine and vitamin C	With the supplementation of C-taurine and vitamin C, there was a positive effect on carbohydrate and lipid metabolism in Type 2 diabetes mellitus patients with NPDR.	Duration was short and did not assess vision parameters for improvement.
Cross-sectional (Case-control)	Vitamin C	Higher mean HbA1c of T2DM patients with or without retinopathy, increased MDA levels in T2DM group with retinopathy and decreased serum vitamin C levels in the T2DM group.	Other antioxidant micronutrient should be measured apart from vitamin C.
Cohort	Vitamin C, E, beta- carotene	Increased fruit intake, increase intakes of energy and fat, HbA1C, BMI, triglycerides and systolic blood pressure and odd ratio of retinopathy with increased intakes of fruit shows decreased risk of 2-4 folds.	Further research on serum vitamin C, E and A should be assessed for further observation.
Randomized double-blind placebo controlled clinical control trial (RCT)	α – lipoic acid, genistein, vitamins C, E and B-complex	Statistically significant elevation in plasma antioxidant levels and full field electroretinogram (ERG) values improved.	Sample size was small and duration of treatment of short.
Cross-sectional	Ascorbic acid	Both HbA1c and FPG significantly correlated with DNA. DNA damage significantly and inversely associated with ascorbic acid and plasma antioxidant level	Correlation of the outcomes with prevalence of DR needs to be evaluated.

Cross-sectional	Lycopene	Decreased levels of lycopene in diabetic group, Significant lower lycopene levels in diabetics with PDR. HbA1c inversely correlated with lycopene levels.	Sample size was small
Cross-sectional	Carotenoids: Non-pro vitamin A (lycopene, lutein/zeaxanthin) and Pro- vitamin A (α-carotene, β-carotene and β-cryptoxanthin)	Lower levels of Non-pro vitamin A (lycopene, lutein/zeaxanthin) than Pro- vitamin A (α-carotene, β-carotene and β-cryptoxanthin) in the DR group compared to the non-DR group.	Much research to be done for further observation of these carotenoid and their effect on DR.
Cross-sectional	Vitamin E and vitamin C	Serum vitamin E and C	Total antioxidant status not assessed and sample size is small.
Cross-sectional	Vitamin C	Lipid peroxidation, Superoxide dismutase (SOD), MDA levels, Gluthathion peroxidase (GPx)	Dietary assessment data was not recorded.
Cross-sectional	Vitamin C and E	Intakes of vitamin C and C from food and supplement and DR incidence.	Correlation of serum nutrient data assessment was not recorded.
Cross-sectional (Case-control)	Ascorbic acid and α-tocopherol	Serum ascorbic acid and serum α-tocopherol	Further research into oxidative stress marker and the vitamins recommended.
Cross-sectional	vitamin C	Serum vitamin C	No dietary assessment data was recorded.

2.3 RESEARCH GAP

From the outcome of the systematic review, some studies (three) on antioxidant micronutrients in type 2 diabetics especially ones pertaining to diabetic visual complications had been inconclusive on the protective effect or efficacy of treatment of diabetic vision complications (delaying progression or improving for the better) and that needs additional

research or studies to assess such effects of antioxidant micronutrient on type 2 diabetes mellitus and its complications. Most of the studies did not include the intakes of dietary antioxidant micronutrients and to even conclude whether type 2 diabetic had sufficient intakes, to relate them to their serum levels, total antioxidant status and blood glucose levels. Again, very little studies on the effects of antioxidant micronutrients of vision of diabetic patients had been done in sub-Saharan Africa, with only one study in DR Congo.

2.4 DISCUSSION

The review conducted included ten (10) cross-sectional studies, three (3) randomized double-blind controlled clinical trials and one (1) control studies. The outcome of 5 cross-sectional studies focused on oxidative stress and antioxidants in T2DM with or without vision complications. The studies indicated that serum antioxidant micronutrients like vitamin C and E were significantly decreased in Type 2 diabetes and more so subjects with retinopathy compared to healthy controls. It was also indicated that levels of malonialdehye (MDA) as a marker of lipid peroxidation was elevated in all the 5 studies compared to controls and increase HbA1c and FPG were also increase in T2DM with or without vision complications (retinopathy). It was noted that MDA positively correlated with blood glucose markers (HbA1c and FPG) in type 2 diabetic patients which insinuates the functions of hyperglycemia in the production of reactive oxygen species (Gupta & Chari, 2005; Kumari *et al.,* 2008; Kundu *et al.,* 2014; Lam *et al.,* 2011). HbA1c had a positive correlation with serum vitamin C level (Kundu *et al.,* 2014). However, in a study by Gupta and Chari, (2005), there was no correlation between HbA1c and serum vitamin C levels and the remaining cross-sectional studies did not report on that. There was also inverse correlation between increased oxidative stress (Plasma MDA/lipid peroxidation/DNA damage) and serum vitamins (C and E) in type 2 diabetic patients with vision complications (DR) compared to controls clarifying the defensive utilization in oxidative stress dominating DR. This may be so, due to the

association of oxidative stress in the mechanism of action of diabetic retinopathy (Bertelsen *et al.,* 2001). It has been postulated that increase hyperglycemia may cause injury to the vascular endothelium and the retina by stimulating the production of reactive oxygen species which leads to elevated lipid peroxidation and degradation of antioxidants and consequently oxidative stress in type 2 diabetes mellitus patients (Kundu *et al.*, 2014).

Also, 3 other cross-sectional studies focused on antioxidant micronutrients such as vitamin C, vitamin A, vitamin E, zinc, and selenium and the incidence of diabetic retinopathy. There was high intakes of dietary vitamin C and vitamin E among type 2 diabetics but no relation was found between the intakes of vitamin C and E, and supplement with DR was reported (Millen *et al.,* 2003; Millen *et al.,* 2004). Increased odd ratio for DR among type 2 diabetics with high intakes of vitamin C and E from food and supplement was realized when DM treatment and glucose levels were adjusted. However, the odds ratio decreased for retinopathy among type 2 diabetics with long term intakes of vitamin C and E supplements compared to those who do not use supplements at all. Increased risks of DR was statistically significant among type 2 diabetics with high intakes of vitamin C and E from food and supplement combined among type 2 diabetics with poor glycemic control and this may explain the increased odds ratio of DR above (Millen *et al.,* 2004). The total odds ratio between type 2 diabetics with low serum ascorbic acid and high serum ascorbic acid did not vary (OR= 1.4, 95%Cl, 0.8-2.3). Serum ascorbic acid was not more directly related to retinopathy after vitamin C supplement user were eliminated and this may be due to the high intakes of ascorbic acid from food and supplement among type 2 diabetics. But the study indicated that type 2 diabetic subjects with increased serum ascorbic acid also had higher serum α-tocopherol and vitamin C intakes increased. However, the risk of retinopathy was direct among type 2 diabetic with high serum α-tocopherol than persons with low serum α-tocopherol and the bearing did not alter when all confounders were removed but the level of

association rose slightly and the relation then decreased when vitamin E supplement users were eliminated (Millen *et al.,* 2003). Additionally, there was a decreased mean serum level of vitamin C, E, zinc, selenium and total antioxidant status in type 2 diabetics with retinopathy (Mvitu-Muaka *et al.,* 2016).

The last two cross-sectional studies focused on carotenoids and DR among type 2 diabetic with and without DR. A significantly lower serum lycopene levels was observed in type 2 diabetic patients than in controls with the DR group having lower levels than non-DR group (OR=1.2, 95%Cl, 1.0-1.4) versus (OR = 1.6, 95%Cl, 1.4-1.7 respectively with P< 0.05) (Brazionis, *et al.,* 2009, Li *et al.,* 2010b). Within the DR group, a significant lower level of lycopene was showed in type 2 diabetics with proliferative diabetic retinopathy (PDR) compared to type 2 diabetic with non-proliferative diabetic retinopathy (NPDR) or without diabetic retinopathy (DR) at all. Also, a multivariate amendment indicated an inverse correlation(r = -0.345, P= 0.007) between HbA1c and serum lycopene level which implies an increase in blood glucose causes a fall in serum lycopene levels in type 2 diabetics. However, it was revealed that age and HbA1c level were important contributing factors of serum lycopene level (Li *et al.,* 2010b). Additionally, a decrease odds ratio was found for non-pro vitamin A (non-PVA) carotenoids (lutein/zeaxanthin) concentrations compared to pro-vitamin A (PVA) carotenoids (α- carotene, β-carotene and β-cryptoxanthin) in type 2 diabetics with DR than the non-DR group. This could be that, the risk of DR is decreased with an increase intake of non-PVA (OR= 0.33, 95%Cl, 0.12-0.95, P = 0.039). On the other hand, an increased level of PVA carotenoids were related to higher odds of DR when confounders were adjusted (Brazionis *et al.,* 2009).

The results obtained from the cohort study focused on fruit intakes, related nutrients and incidence of DR. Tanaka *et al.,* (2016), indicated that within a period of 8 years follow up, mean fruit consumption in quartiles varied from 23 to 253g/day with rising movement of vitamin C, vitamin E, carotene, retinol equivalent, dietary fiber, potassium and sodium along the quartiles of fruit consumed for vitamins among the type 2 diabetic patients. Also, average calories consumed extended from 1644 to 1863 kcal/day and nearly 25% fat was consumed among the type 2 diabetic patients. Glycated hemoglobin (HbA1c), BMI, triglycerides and systolic blood pressure had a good control. It was noted that, the prevalence of DR among the type 2 diabetics extended from 83%, and declining to 74%, 69% and finally 59%. As such, high intakes of fruits and vegetables decreases the chances of DR in type 2 diabetes mellitus patients.

The outcome obtained from the three (3) randomized controlled-clinical placebo trials emphasized some of the positive effects of antioxidant micronutrients (vitamin C, A, E, B-complex) combined with other antioxidants such as zinc, selenium, α-lipoic acid, lutein/zeaxanthin, genistein and rutin in a supplement form for treatment of type 2 diabetics with retinopathy and other vision complications (Lekishvili, 2014; Moshetova *et al.,* 2014; Nebbioso *et al*., 2012). According to Nebbioso *et al.,* (2012), 400mg/day treatment of α-lipoic acid combined with genistein and vitamins given to pre-retinopathic type 2 diabetic patients for 30 days reduced oxidative stress markers and improved retinal components using electroretinogram figures compared to the controls who received a placebo. Similarly, Moshetova *et al.,* (2014) indicated a significant increase in visual acuity, decrease in macular thickness and an improvement in light sensitivity in type 2 diabetic patients with DR receiving 240mg/day supplement containing lutein, zeaxanthin, vitamin C, vitamin E, vitamin A, vitamin B_2, zinc, selenium, rutin and bilberry extract for 2 months.

However, the result by Lekishvili, (2014) did not show vision parameters but biochemical markers. It found that, a significant impact was seen in carbohydrates and lipid metabolism, and hepatoprotective features in type 2 diabetics with NPDR after receiving taurine and vitamin C combined supplement for 42 days. These beneficial effects of antioxidant micronutrients through supplementation in type 2 diabetic patient with DR agrees with studies conducted by Millen *et al.,* (2003) and Millen *et al.,* (2004). It also supports the endorsement of the use of food supplements by many nutritionist and dietitians recently due to the depletion of soils, pesticides use, processing and alteration of vegetables, preservation, storage as well as cooking methods decrease the initial antioxidants present in food (Toshihiko & Yoji, 2006).

2.5 CONCLUSION

The evaluation of outcomes of the reviewed studies suggests adequate evidence on the role antioxidant micronutrients from food (fruits and vegetables) and supplements play in the pathogenesis of diabetic vision. It has shown to reduce oxidative stress, and also increases total antioxidants status (TAS) in type 2 diabetics with or without vision complication. Nevertheless, there were significant decrease in serum concentrations of antioxidant micronutrients among diabetics and more so those with vision complication. The randomized controlled trial (RCT) studies analyzed showed that a combined supplementation of vitamin C, A, E, alone or combined with other micronutrients improved vision parameters and metabolism of carbohydrates and lipids among diabetics.

2.6 Antioxidant micronutrients and diabetic vision

Over the past two decades, studies have clarified significant details on the systematic influence of oxidative stress (OS) in the occurrence of DM complications (Baynes & Thorpe, 1999; Giacco & Brownlee, 2010). In oxidative stress state, antioxidants are transferred in a

good turn of oxidants. Nonetheless, if antioxidants defenses are inadequate, then protein, lipids, and DNA will be destroyed, bringing about a change in biochemical function and eventually apoptosis (Birben *et al.,* 2012). This supports the thinking that improvement of OS with antioxidants would have a therapeutic outcome in preventing or delaying the commencement of DM complications including the ones that affect vision (Maritim *et al.,* 2003; Soufi *et al.,* 2012).

2.6.1 Vitamin A

Vitamin A is a fat soluble vitamin with antioxidant activity. Vitamin A does this by helping to upregulate the body's enzymatic antioxidant activities, in their action to scavenge free radicals caused by oxidation (Ali *et al.,* 2017). Also, the body requires vitamin A, in a form of retinal, for normal vision and functioning of the eye (Chehade *et al.,* 2009). Vitamin A is obtained from food sources as retinol esters, and become hydrolyzed to retinol in the intestine. The remaining retinol esters bind to retinol binding protein, and are transported into circulation to target organs such as eye, liver and adipose tissue (Goodman, 1975). In fatty tissue, the retinoic acid stimulates insulin signaling gene, glucose transporter 4 and activate PPARγ. This induces the expression of insulin gene, which affects glucose and lipid metabolism, by improving insulin action (Berry & Noy, 2009). However, in diabetes mellitus, the presence of uncontrolled high blood glucose is associated with imbalance in retinol concentration and retinol binding proteins, impairing their physiological role in vision (Lu *et al.,* 2000). A study by Basualdo *et al,* (1997) showed reduced available of retinol levels in diabetics. A study by Campoy *et al,* (2002) found significant reduced concentration of serum vitamin A in type 1 diabetics (1.03 ±0.03 µg/dL) compared to healthy controls (1.17±0.06 µg/dL, $p< 0.05$). Basu *et al,* (1997), reported that, factors involved in serum vitamin A concentration may be that the occurrence of diabetes mellitus might neither affect dietary intake or absorption of vitamin A, but deficiency in serum vitamin A could be related

to impaired transportation to target organs. As this occurs, there can be structural and functional alteration in retinal vasculature, which is related to diabetic retinopathy. An impaired retinal vasculature may lead to widened retinal arteriolar, which have been suggested as early physiological outcome of microvascular impairment, progressing to diabetic retinopathy (Cheung *et al.,* 2010).

2.6.2 Vitamin C

In human plasma, ascorbic acid popularly known as vitamin C exists as an important hydrophilic antioxidant. According to Badawi *et al.,* (2013), vitamin C has a defensive role in immune function, anti-inflammatory and scavenging of reactive oxygen species brought forth by oxidative stress. In the diabetic eye, vitamin C has been proved to defend the lens, cornea, vitreous humor and retina from oxidative stress (Bendich *et al.,* 1986). It also serves as a standby antioxidant and avoids indirect injury from free radicals (Song *et al.,* 2009). Nonetheless, vitamin C may display as a pro-oxidant and glycated proteins occasionally (Lee *et al.,* 2004). In diabetes mellitus, especially type 2, the need for vitamin C consumption is raised because of the stimulation of oxidation stress caused by hyperglycemia (Valdés-Ramos *et al.,* 2015). This is may be the reason why diabetes mellitus patients have low levels of serum or plasma vitamin C (Lee *et al.,* 2004). Carter *et al.,(*2013) as well as Mazloom *et al.,* (2011) indicated an inverse correlation between HbA1c, Fasting plasma glucose and Plasma vitamin C in type 2 diabetes mellitus patients. Also a negative correlation was indicated with serum/plasma vitamin C and diabetic retinopathy (Gurler *et al.,* 2000, Gupta and Chari, 2005). It is therefore essential to increase intake of vitamin-rich foods provide dietary with purpose of stimulating plasma concentration of total antioxidant capacity (Zhou *et al.,* 2016).

2.6.3 Vitamin E

Tocopherol and tocotrienol are the composite form of vitamin E. A member of the tocopherol family is the alpha (α) tocopherol which is the highly prevalent form in the human plasma with advantages to health (Valdes-Ramos *et al.*, 2015). It is a fat soluble antioxidant known for its defensive mechanism on lipid membrane; thus inhibiting lipid peroxidation (Goud *et al.,* 2016).Vitamin E ceases breeding chain reactions of peroxyl radical formed in cells (Goldenstein *et al.*, 2013). In the diabetic eye, vitamin E has been proved to decrease epoxides of retinal cells and shields them away from oxidative injury (Sparrow *et al.*, 2003). A negative correlation between serum vitamin E status and DR has been reported (Rema *et al.*, 1995).

2.6.4 Zinc

Zinc induces glucose metabolism by enhancing sensitivity of insulin (Devi *et al.*, 2016) by taking part in the production, discharging and stowing of insulin in the pancreas (Pujar *et al.*, 2014). Its acts as a cofactor for several enzymes throughout carbohydrates, proteins and lipid metabolisms (Olaniyan *et al.*, 2012) and also for elimination of free radicals from biomolecules and alleviating lipid membrane from lipid peroxidation, by enzymes like catalase, peroxidase and superoxide dismutase (Puri *et al.*, 2013). In the eye, Zinc exists in increased levels, mainly in the retina and choroid. However, Zinc insufficiency has been proved to affect ocular growth, cataracts, age-related macular degeneration, and diabetic retinopathy (Miao *et al.*, 2013). Zinc insufficiency in humans leads to weak acclimatization and night blindness (Afridi *et al.*, 2011). This is because adequate zinc improves the activity of the enzyme retinol dehydrogenase and can be hindered if there is a deficiency of zinc (Huber & Gershoff, 1975). Furthermore, zinc has been proved to defend the retina from lipid peroxidation levels stimulated by diabetes and reduce level of glutathione by alleviating the membranes in rats (Moustafa, 2004). Mahdizadeh *et al.*, (2014) and Lee *et al.*, (2016) found

that changed zinc levels in type 2 diabetics may be due to the raised urinary defecation in type 2 diabetes patients with increased blood glucose or exogenous decrease intakes from diet.

2.6.5 Selenium

Selenium is an important part of an amount of selenoproteins entrapped in vital enzymatic roles like duplication, redox homeostasis, immunity and thyroid hormone metabolism (Burk, 2002) due to its antioxidant properties. Intakes of selenium from the diet ranges significantly among countries and regions mostly because of the different selenium content of soil which affects selenium levels of food and animal feed (Rayman, 2008) Recently, the intakes of selenium are set on enhancing plasma glutathione peroxidase which are boosted at increased consumption of 55mcg/day (Ross *et al.,* 2011). An inverse association has been indicated between serum selenium levels and HbA1c (Ruiz *et al*., 1998). An adequate serum selenium level is required to confirm an accurate activity of the selenodependent glutathione peroxidase enzyme which cleanses hydro peroxides produced by lipid peroxidation at functional location (Evans & Halliwell, 2001). It is significant that selenium, vitamin C and alpha tocopherol work interdependently (Chaudière & Ferrari-Iliou, 1999).

2.7 Definition of Study Terms

2.7.1 Evaluation of Blood Glucose

In blood circulation, carbohydrates results in glucose which is either reduced to supply energy or transformed to glycogen (Wei *et al.,* 2003). Nevertheless, when glucose in the blood becomes excessive, it leads to a breakdown in the body initiating diabetes mellitus. This is the reason why monitoring blood glucose is significant clinically (Traore *et al.,* 2013). According to the American Diabetes Association (ADA, 2013), fasting plasma glucose is described as blood glucose <126 mg/dL (< 7.0 mmol/L) as normoglycemia and ≥ 126 mg/dL

as hyperglycemia (≥ 7.0 mmol/L) and HbA1c < 6.5% is described as normoglycemia and HbA1c ≥ 6.5% referred as hyperglycemia.

2.7.2 Body Mass Index (BMI)

This is described as weight- for- height which is aimed at categorizing underweight, overweight, and obesity in adults. An adult's BMI can be used to categorize underweight, a healthy weight, overweight or obese based. BMI is estimated by dividing weight in kilograms (kg) by the square of height (m^2) (National Health and Medical Research Council, 2013). Future health outcomes and functional status of people can be envisaged using the BMI (Grzegorzewska *et al.,* 2016). On the other hand, BMI use is limited to lean mass and body fat (Zhu *et al.,* 2014) along with age, sex and people's culture (Grezegorzewska *et al.,* 2016). Obesity is associated to raise health problems worldwide such as type 2 diabetes and its complications.

Table 2.2: The World Health Organization classification of adult underweight, overweight, and obesity, according to BMI.

Category	**BMI (kg/m^2)**
Underweight	≤18.5
Healthy	18.5-24.9
Overweight (Pre-obese)	25-29.9
Obese class 1 (moderately obese)	30-34.9
Obese class 2 (severely obese)	35-39.9
Obese class 3 (morbidly obese)	≥ 40

Adopted from WHO, (2006).

2.7.3 Dietary Assessment Methods

The usual personal dietary evaluation tools used for the collection of food consumed by individuals are the 24-hour dietary recall, dietary records, and food frequency questionnaire (Shim *et al.,* 2014).

2.7.4 Twenty-Four Hour Dietary Recall

This gives an evaluation of food and beverages eaten in the previous day, starting from morning to evening. The 24-hour dietary recall shows information on detailed account, portion sizes, and methods of food preparation and where and when food was eaten. It's a tool that supplies both exact and acceptable information on people's energy and nutrients intakes. Nevertheless, it depends on respondents' memory during recall and so poses risk of under or overestimating of nutrients intakes has the chances in under-rating or over-rating nutrients (Salvador Castell *et al.*, 2015).

2.7.5 Food Frequency Questionnaire (FFQ)

It measures regular food intakes of carefully chosen list of foods in terms of the quantity and frequency of ingestion in a stipulated time. FFQ largely emphasizes on dietary consumption of specified nutrients which is linked to particular diseases (Rodrigo *et al.*, 2015). It is beneficial in supplying common dietary intake over an extended time (Yanagisawa *et al.*, 2016).

2.7.6 Dietary Reference intakes of Antioxidant Micronutrients and other nutrients.

It is a set of values for the dietary nutrient intakes of healthy people. These values are used for planning and assessing diets. Average daily intakes of vitamin A, vitamin C, vitamin E, zinc, selenium, carbohydrate, protein and fibre were measured using the Dietary Reference Intakes values.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design

A cross-sectional study design was used to assess the relationship between dietary patterns, serum levels of antioxidant micronutrients and the prevalence of visual impairments among outpatient diabetics attending Cape Coast Teaching Hospital. The study took place from December, 2016 to May, 2017.

3.2 Study Site

The location for the study was the Cape Coast Teaching Hospital. Currently, it is the largest hospital in the central region of Ghana and Referral Centre for Central and Western regions. The Hospital is well-equipped and has a well-organized diabetic unit situated within the Department of medicine.

3.3 Study Population

The study used outpatients diabetics (both type 1 and type 2) aged 35 to 85years. The prevalence rate was 6% and Cape Coast Teaching Hospital has 2100 registered diabetic patients (males = 600, females = 1500; making 71% females and 28% males) who visit the clinic regularly (Cape Coast Teaching Hospital Annual Report, 2016).

3.4 Inclusion Criteria

Participants who had been clinically diagnosed of diabetes mellitus for a 1 year and above by a physician. Both Outpatient type 1 and type 2 diabetic patients within age range 35 to 85 years who gave their consent to undertake the study and/or Diabetic patients with co-morbidities such as hypertension, nephropathy and neuropathy who attended diabetic clinic at Cape Coast Teaching Hospital were recruited for the study.

3.5 Exclusion Criteria

Participants with gestational diabetes and diabetic patients who are critically ill, blind or have any communicable disease such as Tuberculosis were excluded from the study. Also Diabetic patients who were less than age 35 or above 85years were excluded.

3.6 Ethical Consideration

Ethical approval was sought and obtained from the Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC) which is affiliated to University of Cape Coast, School of Medical Sciences for the commencement of this research. A written permission to conduct the research was obtained from the CCTHERC to the various units (Diabetic clinic, Eye clinic and biomedical laboratory) of the hospital for the commencement of data collection. Verbal and written consent was also sought from each diabetic patient and from caregivers or relatives who were with them at the clinic for voluntary participation.

3.7 Sampling Technique and Sample size

The purposive sampling technique was used to select participants from a list of diabetic patients and those who met the criteria set for the study was provided by the Diabetic clinic nurses at the Cape Coast Teaching Hospital. After which, the study participants was randomly sampled from this list by selecting every third name after the previous.

3.7.1 Sample size Determination

The study used outpatient diabetic patients, with an urban population prevalence rate of 6.0 % in Ghana *(Ofori-Asenso, 2016)*

The sample size is calculated using the Cochrane formula below, (Cochran, 2007);

$N= Z^2 p (1-p)/d^2$

N = sample size

Z= confidence level of 95 % (standard value for Z-score= 1.96)

p = Estimated prevalence of study population = 6% = 0.06

d = marginal error

$N = z^2 p (1- p) / d^2$

$N= 1.96^2 * 0.06 (1-0.06)/0.05^2$

N= 3.8416 * 0.06 (0.94)/0.0025

N= 86.7= 87 = 100

The minimum sample is 87; however, the study used sample size of 100.

3.8 Data Collection

Information obtained from respondents using a structured questionnaire during the study was categorized into six (6) sections. These were socio-demographic data, Diabetic medical history, and diet management, awareness of diabetic visual impairments, anthropometrics, and biochemical.

A pretested structured questionnaire comprising of open and close ended questions was administered to respondents who reported on any of the 3 diabetic clinic days. The demographic data covered the ages, sex, educational background, marital status, religion, ethnicity, occupation, and residence (rural and urban), other information such as type of diabetes, duration, treatment regimen (medication or diet and physical activity), and type of medications (oral or insulin) were also retrieved.

3.8.1 Anthropometric Assessment

The height, weight, and body mass index of the study participants were assessed using a body composition analyzer (OMRON). Also blood pressure (BP) was checked using a digital Bp monitor (OMRON).

3.8.2 Dietary Assessment

A food frequency questionnaire which contains foods that are rich sources of the antioxidant micronutrients of interest for the study was used to assess the frequency of intake of these foods by subjects. These were foods that had been eaten within the period of three (3) months. The frequency of intake of the nutrient rich foods was assessed on daily, one-three times weekly, monthly basis, occasionally and never.

A Three (3) day 24 Hour recall method was used to assess the daily macronutrients and antioxidant micronutrients intake of diabetic outpatients. During the interview, respondents were asked to recall foods and beverage intake of the previous 24 hours for two week days and one weekend day. The portions of foods and beverages consumed were estimated using common household handy measures and food models. However, particular considerations were given to recall days and intervals between recall days. Samples of commonly consumed foods by respondents were bought from food vendors and weighed with a balance. The quantity of foods consumed by respondents was estimated using the food conversion table from the handy measures and food models into grams.

A Nutrient Analysis Template (University of Ghana, Food Science and Nutrition Department, 2010) and the West African Food Composition Table (2012) were used to determine Antioxidant micronutrient contents and macronutrient (carbohydrates) foods consumed by respondents. This template provided the nutrient contents of raw foods, convenient foods and combined dishes usually consumed in Ghana. Nutrient content of raw and cooked food ingredients were provided using the West African Food Composition Table. Subjects were categorized as having high, low, and moderate/normal intake using the 24 Hour recall. Those whose intake were <50% of the RDA were categorized as having low intake, those whose intake were 50%-74.9% of the RDA were categorized as having moderate intake and those

with intake ≥75% of the RDA were classified as having high intake (Rocourt & Cheng, 2013). The Pattern of intake of Antioxidant micronutrient rich foods on the list was determined using the food frequency questionnaire.

Household Handy Measures Used For Dietary Assessment.

Figure 3.1: Household handy measures used in estimating the quantity of foods consumed by respondents.

- Label A and label B – Teaspoon (5ml) and Dessert spoon (10ml). These were used in estimating or quantifying the quantities of sugar, milk, oil, and grounded pepper.
- Label C – Stewing Spoon/ladle. This was used to estimate quantities of rice, stews consumed by respondents.
- Label D - Soup Spoon/ladle. This was used to estimate the quantities of soups, porridges, gari and cut vegetables consumed by subjects.
- Label E - Sardine tin. The sardine tin was used to estimate the bread, yam and cassava sizes.

- Label F – Match box. The match box was used to quantify respondents' intake of fish, chicken and meat.
- Label G – Orange ball. This was used to estimate the quantities of fufu, banku, kenkey, akple, rice balls, tuo zaafi, doughnuts and konkonte consumed by respondents.
- Labels H, I and J –200ml, 250ml and 550mls Cups respectively. These cups were used to estimate respondents' intake of porridges and beverages.

3.9 Ocular Assessment

A detail ocular examination was conducted on each study participant. Visual acuity (V/A) measurements; ocular alignment and motility; pupil reactivity and function; cup-to-disc ratio(CDR), tonometry, intraocular pressure (IOP), Slit lamp examination of the cornea, iris, lens, and vitreous; and dilated fundus examination were some of the ocular examinations assessed. Two experienced ophthalmologists, an optometrist and two ophthalmic nurses were involved in conducting the assessment.

3.9.1 Visual acuity testing and refraction

Visual acuity was measured at six (6) meters with the use of Snelling's chart and finger counts. For each eye, visual acuity was measured separately and defined in accordance with the lowest line on the chart for which majority of the letters were read correctly. Study participants with eye glasses had their visual acuity measured twice with their typical distance correction and with the full required distance correction as established by their refraction data. The visual acuity in the best eye with full distance correction was elucidated as the best corrected visual acuity. Subjects eyes were refracted with Retinoscope and trial lenses.

Cataract was assessed by defining dilated lens using the lens opacities classification system (iii) grading as nuclear (≥ 4), cortical (≥ 2) and posterior subscapular (≥2) cataracts. For statistical analysis, Aphakic and Pseudophakic eyes were included as operated cataracts.

At best possible mydriasis, a detailed fundus examination was conducted after the pupils of subjects have been dilated with Phenylephrine Hydrochloride Ophthalmic solution USP (2.5%) and Tropicamide (1%) or one percent (1%) Cyclopentolate Hydrochloride Ophthalmic solution USP (Cyclogyl 1%) and with the aid of direct ophthalmoscope were used to conduct funduscopy. When a subject had a minimum of one microaneurysm in any field as well as exhibiting hemorrhage (dot, blot, or flamed shaped) and maculopathy (with or without clinically significant macula oedema) was diagnosed of Diabetic Retinopathy (DR) or Diabetic Maculopathy or macular oedema (DME). The presence of hemorrhages, microaneurysms, cotton wool spots, neovascularization, cataract, glaucoma, retinal detachment, maculopathy in both eyes of each study participant were recorded on an ocular assessment form for the categorization of ocular complications. Retinal photographs were excluded from the study as a result of large number of subjects and limited resources.

A

B

C

Figure 3.2: Eye examination Procedures used to assess the prevalence of visual problems among the study participants.

Label A- assessing visual acuity of study participants using the Snellings Chart.

Label B- assessing visual acuity of study participants using finger counts.

Label – the use the Slit-lamp to assess the lens of the eye.

3.10 Biochemical Assessment

At recruitment, a thumb of the subjects was pricked with a lancet and fasting blood sugar (FBS) checked using a glucometer after which 5ml of venous blood was collected from subject into an EDTA tubes and serum tubes by the phlebotomists. The blood samples in the EDTA was stored at room temperature for about 3hours until ready for analysis for glycated hemoglobin and the blood samples in the serum tubes were centrifuged for 5minutes for separation of serum from plasma. The serum samples were frozen at -20C until they were ready to be analyzed. Glycated hemoglobin was analyzed using SD A1cCare Test Kits and Uniten HbA1c Analyzer at the Biochemical Laboratory of the Cape Coast Teaching Hospital. Vitamin A, C, and E were analyzed using the Human Vitamin ELISA Kits Partly at the Clinical Analysis Laboratory and Central Laboratory of KNUST respectively. The Total Antioxidant Capacity (TAC) of subjects was analyzed at the Biochemistry laboratory of the university of Cape Coast.

3.10.1 Glycated Hemoglobin (HBA1c) Analysis

The Uniten HBA1c analyzer, Pipette, wipes gloves, R1 reagent, R2 reagent, cartridge, pipette tips, 5ul capillary tube and using SD A1cCare Test Kits which was kept at room temperature. Uniten Analyzer was switched on and calibrated. An EDTA tubes containing a blood samples each at room temperature was mixed by shaking the test tube, 5ul capillary tube was used to collect the blood sample (Plasma) and placed into an R1 reagent test tube and mixed thoroughly. The mixture is then incubated for two (2) minutes and shake R1 reagent test tube again to mix thoroughly. A 2.5ul of the R1 reagent mixed with blood sample was extracted using pipette and then transferred into the cartridge and then 2.5ul of the R2 reagent using a pipette was taken and added to the cartridge in placed in the analyzer for reading.

3.10.2 Serum Retinol (Vitamin A) Analysis

The Thermo-Scientific Well Washer and Thermo-Scientific Multiscan Plate Reader, Human Vitamin A (VA) ELISA Kit was used for the serum retinol analysis.

Procedure

3.10.3 Dilution of Standards

Ten wells were set for standards in a microelisa strip plate. In Well 1 and Well 2, 100µl standard solution and 50µl Standard Dilution buffer were added and mixed well. In Well 3 and Well 4, 100µl solution from Well 1 and Well 2 were added respectively. Then 50µl Standard Dilution buffer was added and mixed well. A 50µl solution was discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 µl solutions from Well 3 and Well 4 were added respectively. Then 50 µl Standard Dilution buffer was added and mixed well. In Well 7 and Well 8, 50µl solution from Well 5 and Well 6 were added respectively. Then 50µl Standard Dilution buffer was added and mixed well. In Well 9 and Well 10, 50µl solution from Well 7 and Well 8 were added respectively. Then 50 µl Standard Dilution buffer was added and mixed well. 50µl solution was discarded from Well 9 and Well 10. After dilution, the total volume in all the Wells were 50 µl and the concentrations were 1500 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml and 125 pg/ml, respectively.

In the Microelisa strip plate, a well was left empty as blank control. In sample wells, 40µl sample dilution buffer and 10µl sample were added (dilution factor is 5). Samples were loaded onto the bottom without touching the well wall and mixed well with gentle shaking. It was then incubated for 30minutes at 37^0C after it was sealed with Closure plate membrane. The concentrated washing buffer was diluted with distilled water (30 times for 96T and 20 times for 48T). Washing was done by carefully peeling off closure plate membrane, aspirated and refilled with the wash solution. The wash solution was discarded after resting for 30

seconds and washing procedure repeated for 5 times. Then, 50µl HRP-Conjugate reagent was added to each well except the blank control well. Incubation as described in step 3 and washing as described in step 5. 50µl Chromogen Solution A and 50µl Chromogen Solution B was added to each well mixed with gently shaking and incubated at 37^0C for 15minutes. Light was avoided during coloring. Then 50µl stop solution was added to each well to terminate the reaction and the color in the well changed from blue to yellow. Absorbance O.D was read at 450nm using a Microtiter Plate Reader (Thermo-Scientific Plate Reader). The O.D value of the blank control well was set as zero. The Assay was carried out within 15minutes after adding stop solution.

Calculation of Results

Known concentration of Human Vitamin A (VA) standard and its corresponding reading Optical Density (OD) was plotted on log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human Vitamin A (VA) in sample was determined by plotting the sample's O.D on the Y-axis. The original concentration is calculation by multiplying the dilution factor.

3.10.4 Serum Vitamin C Analysis

The Thermo Scientific Well Washer and Thermo Scientific Multiscan Plate Reader, Human Vitamin C (VC) ELISA Kit was used for the serum Vitamin C analysis.

Procedure

3.10.5 Dilution of Standards

Ten wells were set for standards in a microelisa strip plate. In Well 1 and Well 2, 100µl standard solution and 50µl Standard Dilution buffer were added and mixed well. In Well 3 and Well 4, 100µl solution from Well 1 and Well 2 were added respectively. Then 50µl Standard Dilution buffer was added and mixed well. 50µl solution was discarded from Well 3

and Well 4. In Well 5 and Well 6, 50µl solution from Well 3 and Well 4 were added respectively. Then 50µl Standard Dilution buffer was added and mixed well. In Well 7 and Well 8, 50µl solution from Well 5 and Well 6 were added respectively. Then 50µl Standard Dilution buffer was added and mixed well. In Well 9 and Well 10, 50µl solution from Well 7 and Well 8 were added respectively. Then 50µl Standard Dilution buffer was added and mixed well. 50µl solution was discarded from Well 9 and Well 10. After dilution, the total volume in all the Wells were 50µl and the concentrations were 30ng/ml, 20ng/ml, 10ng/ml, 5ng/ml and 2.5ng/ml, respectively.

In the Microelisa strip plate, a well was left empty as blank control. In sample wells, 40µl sample dilution buffer and 10 µl sample were added (dilution factor is 5). Samples were loaded onto the bottom without touching the well wall and mixed well with gentle shaking. It was then incubated for 30 minutes at 37^0C after it was sealed with Closure plate membrane. The concentrated washing buffer was diluted with distilled water (30 times for 96T and 20 times for 48T). Washing was done by carefully peeling off Closure plate membrane, aspirated and refilled with the wash solution. The wash solution was discarded after resting for 30 seconds and washing procedure repeated for 5 times. Then, 50µl HRP-Conjugate reagent was added to each well except the blank control well. Incubation as described in step 3 and washing as described in step 5. A 50 µl Chromogen Solution A and 50µl Chromogen Solution B was added to each well mixed with gently shaking and incubated at 37^0C for 15minutes. Light was avoided during coloring. Then 50µl stop solution was added to each well to terminate the reaction and the color in the well changed from blue to yellow. Absorbance O.D was read at 450 nm using a Micro titer Plate Reader (Thermo-Scientific Plate Reader). The O.D value of the blank control well was set as zero. The Assay was carried out within 15minutes after adding stop solution.

Calculation of Results

Known concentration of Human Vitamin C (VC) standard and its corresponding reading Optical Density (OD) was plotted on log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human Vitamin C (VC) in sample was determined by plotting the sample's O.D on the Y-axis. The original concentration is calculation by multiplying the dilution factor.

3.10.6 Serum Total Antioxidant Capacity (TAC) Analysis

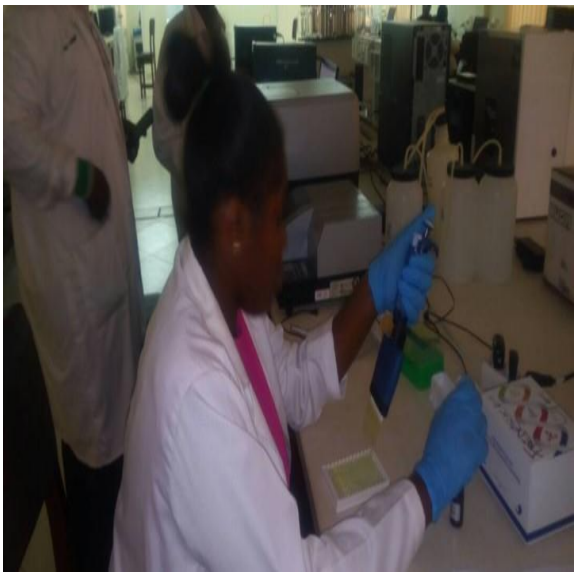
The Spectro UV-VIS Double Beam (Labomed Spectro UVD3200) Reader, glass test tubes, Hot water bath, micropippettes and Pippette tips, reagent bottle, beakers and Human serum was used for the serum total antioxidant capacity analysis(Tac) using Phosphomolybdenum reduction method with slight modifications (Boampong *et al*., 2015).

Procedure

The reagent solution prepared contained Ammonium molybdate (4 mmol/L), Sodium phosphate (28 mmol/L), Sulfuric (0.06 mol/L) which was mixed in 1:1:1 ratio respectively. Accurately, 0.15ml of the various concentrations of the ascorbic acid (20-200 µg/ml) and aqueous (20-200µg/ml) were mixed with 1.5ml of the reagent solution. The mixture was incubated for 60 minutes at 80^0C after which the absorbance of the green phosphomolybdenum complex formed was measured at 695 nm against a reagent blank (0.15 mL distilled water and 0.15mL reagent solution). The results were expressed as ascorbic acid equivalents.

E

F



G

H

Figure 3.3: Various biochemical analyses conducted to assess glycated haemoglobin, serum vitamin A and vitamin C.

Label D- analyzing blood sample for glycated haemoglobin.

Label E- Pippetting of reagent to blood sample during analysis of vitamin A and vitamin C analyses.

Labe F – fixing the well plate in the microtitier reader

Label G- The microtitier plate reader ejecting the well plate after it has been read

3.11 Data Analysis

Data was entered using SPSS version 23, tested for normality and exported to Microsoft Excel 2010. Data analysis was done with SPSS version 23. Results were presented in tables, bar graphs, pie charts and reported as frequencies, means and percentages. Continuous variables were summarized as means with their standard deviations. Associations between the categorical variables were determined using the Independent- Sample T-test analyzed at 95% confidence interval. The Chi square was used to determine the significant association between categorical variables. The Pearson Correlation was used to determine relationships between continuous variables. A binary logistic regression analysis was used to estimate the prevalence of risk between nutrient intake, serum level of antioxidant micronutrients and the prevalence of vision problems among diabetic patients. Nutrients intake were analyzed using Nutrient Analysis Template. The average intake was compared with Recommended Dietary Allowance. All analysis less than 0.05 will be considered as statistically significant.

3.12 Exposures (Antioxidant Micronutrients Assessed)

Vitamin A, vitamin C and total antioxidant capacity(TAC)

3.13 Ocular Screening (Parameters)

Visual acuity (VA), Intra Ocular pressure (IOP) and Cup-disc-ratio (CDR)

3.14 Visual Impairments Assessed

- Refractive Error, Dry eye syndrome, Cataract, Diabetic Retinopathy, and Diabetic macular Oedema.

CHAPTER FOUR

4.0 RESULTS

4.1 Introduction

This chapter presents the results of the study which are presented in frequency tables, pie-charts and bar graphs.

4.2 Socio-demographic and clinical characteristics of respondents.

The socio-demographic characteristics of respondents are presented in table 4.1. One hundred (100) diabetic outpatients were reported for the study. Eighty one percent (81%) of the respondents were females and nineteen percent (19%) were males. Most (40%) of the respondents fell within age groups 56-65years with a mean age of 59.7± 10.5 years. The minimum and maximum ages were 35 years and 85 years respectively. With regards to the type of diabetes, majority (90%) of the study subjects had type 2 diabetes. Seventy nine percent (79%) of the study participants were taking oral medications. Sixty five percent (65%) of respondents consume alcohol and majority (95%) of the respondents was aware of the fact that diabetes affects vision.

Table 4.1 Socio-demographic, Diabetic and Clinical Characteristics of Respondents

Parameters	Frequencies (%)	Parameters	Frequencies (%)
Age			
35-45	8(8)	**Type of Diabetes**	
46-55	29(29)	Type 1	10(10)
56-65	40(40)	Type 2	90(90)
66-75	19(19)	**Duration of Diabetes**	
76-85	4(4)	1-5yr	30(30)
Gender		6-10yr	33(33)
Males	19(19)	11-15yr	23(23)
Female	81(81)	16-20yr	7(7)
Educational level		>21yr	7(7)
None	15(15)	**Medication type**	
Primary	21(21)	Oral	79(79)
Junior high	37(37)	Insulin	5(5)
Senior High	21(21)	Both	16(16)
Tertiary	6(6)	**Alcohol Intake**	
Marital Status		Yes	35(35)
Single	3(3)	No	65(65)
Married	59(59)		
Widowed	21(21)	**Vision awareness**	
Divorced	17(17)	Yes	95(95)
Occupation		No	5(5)
Employed	7(7)		
Self-employed	56(56)		
Unemployed	21(21)		
Retired	16(16)		

Categorical data are presented as percentages. Respondents who are self-employed represent those trading, hairdressing, dressmaking and farming and employed respondents represents government workers.

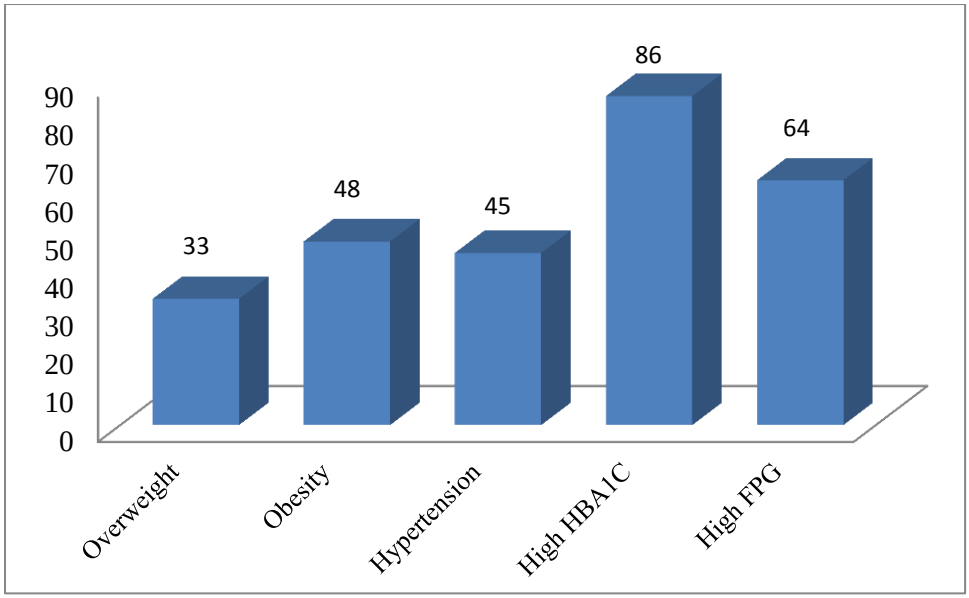


Figure 4.1 Prevalence of morbidities

4.2.1 Prevalence of morbidities among the study population

Other morbidities were found among the study participants, these are presented in Fig 4.1 above and frequencies are presented in percentages. Overweight and obesity levels were 33% and 48% of the study population respectively, 45% of the respondent had hypertension (Bp >140/90), majority (86%) of the study population recorded higher glycated hemoglobin (HBA1c) and 64% for fasting plasma glucose.

4.2.2 Mean Age, Blood pressure, Anthropometric and biochemical indices of the study population.

Table 4.4 compares some parameters between the male and female participants. The mean age, FBG, and HbA1c were not different between males and females. However, BMI of females was higher than males while systolic blood pressure of males was higher than that of females. From the table, it is also obvious that, there are some significant differences between mean BMI and systolic values of male and female respondents, also it could also be seen that there were no significant difference between the means of ages, fasting plasma glucose and glycated haemoglobin according to sexes.

Table 4.2 Comparison of mean Age, Blood pressure, Anthropometric and biochemical indices of the study population

Parameters	Male(Means±SD)	Female(Means±SD)	Overall mean	P-value
Age (Years)	60.8±12.1[a]	59.4±10.2[a]	59.7±10.5	0.644
BMI (Kg/m^2)	26.5±4.5[b]	32.2±7.1[b]	30.8±7.8	**0.002**
Systolic (mmHg)	148.7±19.8[b]	132.7±19.2[b]	135.8±20.2	**0.004**
Diastolic (mmHg)	85.0±11.8[a]	80.5±12.5[a]	81.4±12.4	0.155
FPG (mmol/L)	8.5±2.3[a]	8.4±3.6[a]	8.4±3.4	0.935
HbA1c (%)	13.4±7.5[a]	10.8±4.7[a]	11.3±5.4	0.162

Data presented are ages, anthropometric and biochemical indices according to gender. Continuous data were presents as means. Continuous data were compared using unpaired t-test: means with the superscript "a" (alphabets) are not statistically different, $p>0.05$ while means with the superscript "b" (alphabets) are statistically significant, $p<0.05$.

4.3 Mean Nutrient intake of the Study Population compared with Dietary Reference Intakes (DRIs) and by Gender.

Table 4.4 compares nutrients intake by gender and Dietary Reference Intakes (DRIs). There was a difference in the mean intake for protein (62.59± 15.46 g) for males than females (53.28± 17.70 g). Mean intake of carbohydrate was higher (248.92± 65.18 g) than the female respondents (199.21± 64.68 g). Mean intake of Fibre was higher (25.0± 11.34 g) for males the females (19.22± 6.62 g). The mean intake of Selenium was high for males (110.5± 27.5mcg) than the females (85.6±30.6 mcg). These were statistically significant.

The table also compares the percentage DRI of the means intake of the nutrients to the DRI standards. For the macronutrients, protein (111.7%) and carbohydrate (191%) intakes were in excess while fibre intake (83%) was adequate. Mean intake of micronutrients, thus, calcium (29%) was inadequate, and thiamin (100.8%), riboflavin (76%), and magnesium (78%) intakes were adequate. However, mean intakes of iron (156%), niacin (136%), and vitamin B12 (246%) were in excess. Mean intakes of antioxidant micronutrients; vitamin A (16.8%),

and vitamin E (41.7%) were inadequate while vitamin C (105.7%) and selenium (200.9%) were in excess. On the other hand, zinc intake (65%) was inadequate. Table 4.7.1 shows the means intake of nutrients and RDA percentage for females study participants. For the macronutrients, protein (115.8%) and carbohydrate (153%) intakes were in excess while fibre intake (91.5%) was adequate. Mean intake of micronutrients, thus, calcium (25%) was inadequate, and thiamin (96%), riboflavin (86%), and magnesium (90%) intakes were adequate. However, mean intakes of iron (133.8%), niacin (143%), and vitamin B12 (172.9%) were in excess. Mean intakes of antioxidant micronutrients; vitamin A (26%), and vitamin E (35%) were inadequate while vitamin C (100.8%) and selenium (155.7.9%) were in excess. On the other hand, zinc intake (82%) was adequate.

Table 4.3 Mean Intake of Nutrients Compared with Gender and DRIs.

Nutrients	DRIs	Males (Mean±SD)	% of RDA	Interpretation	Females (Mean±SD)	% of RDA	Interpretation	p-value
Protein (g)	56, 46	62.59 ± 15.46	111.7	Excess intake	53.28 ± 17.70	115.8	Excess Intake	**0.029**
CHO(g)	130	248.92 ± 65.18	191	Excess intake	199.21 ± 64.68	153	Excess Intake	**0.006**
Fibre (g)	30, 21	25 ± 11.34	83	Adequate	19.22 ± 6.62	91.5	Adequate	**0.045**
Calcium(mg)	1200	349.68 ± 121.89	29	Inadequate	305.77 ± 140.24	25	Inadequate	0.18
Iron(mg)	8	12.57 ± 4.40	156	Excess intake	10.71 ± 4.27	133.8	Excess Intake	0.107
Magnesium(mg)	420, 320	329.56 ± 91.19	78	Adequate	288.10 ± 100.24	90	Adequate	0.091
Zinc (mg)	11, 8	7.16 ± 2.13	65	Inadequate	6.57 ± 3.14	82	Adequate	0.329
Selenium (mcg)	55	110.53 ± 27.49	200.9	Excess intake	85.64 ± 30.59	155.7	Excess Intake	**0.002**
Vitamin C	90, 75	95.11 ± 51.99	105.7	Excess intake	75.61 ± 33.95	100.8	Adequate	0.134
Thiamin(mg)	1.2, 1.1	1.21 ± 0.43	100.8	Adequate	1.06 ± 0.61	96	Adequate	0.233
Riboflavin (mg)	1.3, 1.1	0.99 ± 0.37	76	Adequate	0.95 ± 0.58	86	Adequate	0.708
Nacin (mg)	16, 14	21.83 ± 6.99	136	Excess intake	20.03 ± 11.01	143	Excess Intake	0.377
VitB12(mcg)	2.4	5.91 ± 3.36	246	Excess intake	4.15 ± 2.49	172.9	Excess Intake	**0.043**
VitA (mcg)	900, 700	151.91 ± 73.40	16.8	Inadequate	183.01 ± 172.09	26	Inadequate	0.226
VIT E (mg)	15	6.26 ± 3.10	41.7	Inadequate	5.25 ± 2.88	35	Inadequate	0.207

Data presented are the mean intakes of antioxidant micronutrients compared with the DRIs according to gender. Continuous data were presented as means ±SD. Continuous data were compared using unpaired t-test: means with high-lightened p-values are considered statistically significant ($p<0.05$).

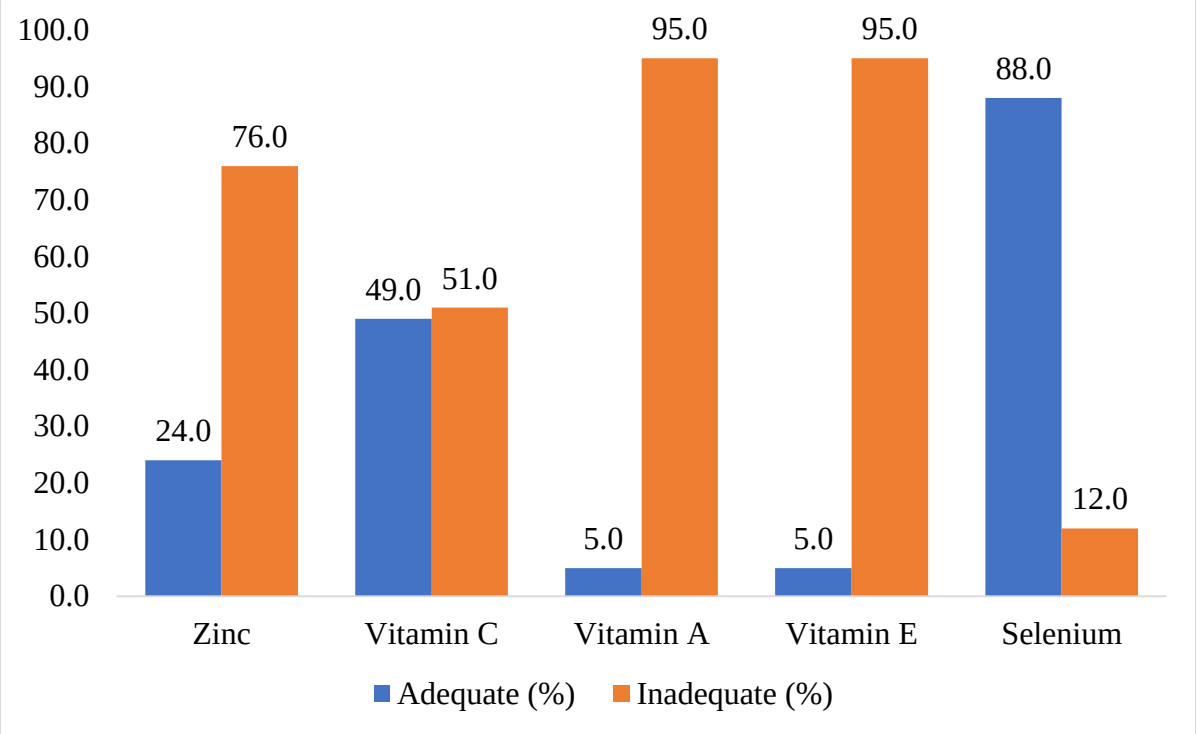


Figure 4.2 Dietary Antioxidant Micronutrients Intake Among the study Participants

4.3.1 Comparison of Dietary Antioxidant Micronutrients intake of the Study Population with Daily Recommended Intake (DRIs).

The figure above shows that the intake of Zinc was inadequate recording 76% of the population. Inadequate intakes of vitamin A and vitamin E were seen, with both recording 95% of the study population. Vitamin C intake was also slightly inadequate and as such these antioxidant micronutrients did not meet the standards of the Dietary Reference Intakes (DRIs). However, adequate intake of Selenium was seen recording 88% of the study population which was able to meet the DRIs.

4.3.2 Food intake Pattern of the study population using Food frequencies.

Table 4.8 displays the food intake pattern of selected food groups by diabetic patients in a period of three (3) months. Majority (68%) of the respondents consumed fruits 1-3 times weekly while Thirty-five percent (35%) consumed non-green leafy vegetables daily and 65% 1-3 times weekly. Sixty five (65%) percent ate green leafy vegetables 1-3 times weekly.

However, fifty two (52%) percent of the diabetics never consumed nuts and good oils for the past 3months. Forty-six percent (46%) of the subjects ate oily fishes daily, and 47% 1-3 times a week. Forty-one percent (41%) of the respondents never consumed red meat in the past 3 months, 32% ate red meat occasionally and 16% ate 1-3 times weekly. Thirty-eight percent (38%) of diabetics ate poultry occasionally, 21% ate poultry monthly, and 15% ate poultry 1-3times weekly. Fifteen percent (15%) of the respondents took vitamin supplement daily, 24% took vitamin supplement 1-3 times weekly, 19% took vitamin supplement monthly, 22% took vitamin supplement occasionally and 20% never took vitamin supplement.

Table 4.4 Dietary Intake Pattern of the study population

	FREQUENCY OF INTAKE				
Food Groups	**Daily (%)**	**1-3 times weekly (%)**	**Monthly (%)**	**Occasionally (%)**	**Never (%)**
Fruit	19	68	9	3	1
Non-green leafy vegetables	35	65	0	0	0
Green Leafy Vegetables	18	65	11	4	2
Nuts and Oils	12	22	2	12	52
Oily Fishes	46	47	4	2	1
Meat	0	16	11	32	41
Poultry	2	15	21	38	24
Vitamin Supplement	15	24	19	22	20

Data presented are the frequency (%) of food intake by participants. Green leafy vegetables (Kontonmire, ayoyo, cabbage, alefu, dandelion); Non-green leafy vegetables (tomatoes, garden eggs, pepper, onion, carrot); Nuts and oils (corn, soy, canola, coconut, sunflower oils and tiger nut, groundnut); Fruits (mango, pear, banana, watermelon, orange, pineapple, pawpaw).

4.4 Antioxidant Micronutrient Status of the Study Participants

4.4.1 Description of Serum Antioxidant Micronutrient levels among the study population

Table 4.9 shows a description of serum antioxidant micronutrient levels among diabetics. The maximum serum level of vitamin A was 0.04 µmol/L and vitamin C was 0.94 µmol/L. The mean serum levels of vitamin A and C were 0.023± 0.01 and 0.256± 0.1. For total antioxidant capacity (TAC), the minimum serum level was 7.1 µmol/L and maximum serum level 38.6 µmol/L. The mean serum level of total antioxidant capacity (TAC) was 24.4± 5.3 µmol/L.

Table 4.5 Description of antioxidant status

Nutrients	Minimum	Maximum	Means±SD	Units
Vitamin A	0.01	0.04	0.023±0.01	µmol/L
Vitamin C	0.05	0.94	0.256±0.10	µmol/L
TAC	7.10	38.60	24.4±5.30	µmol/L

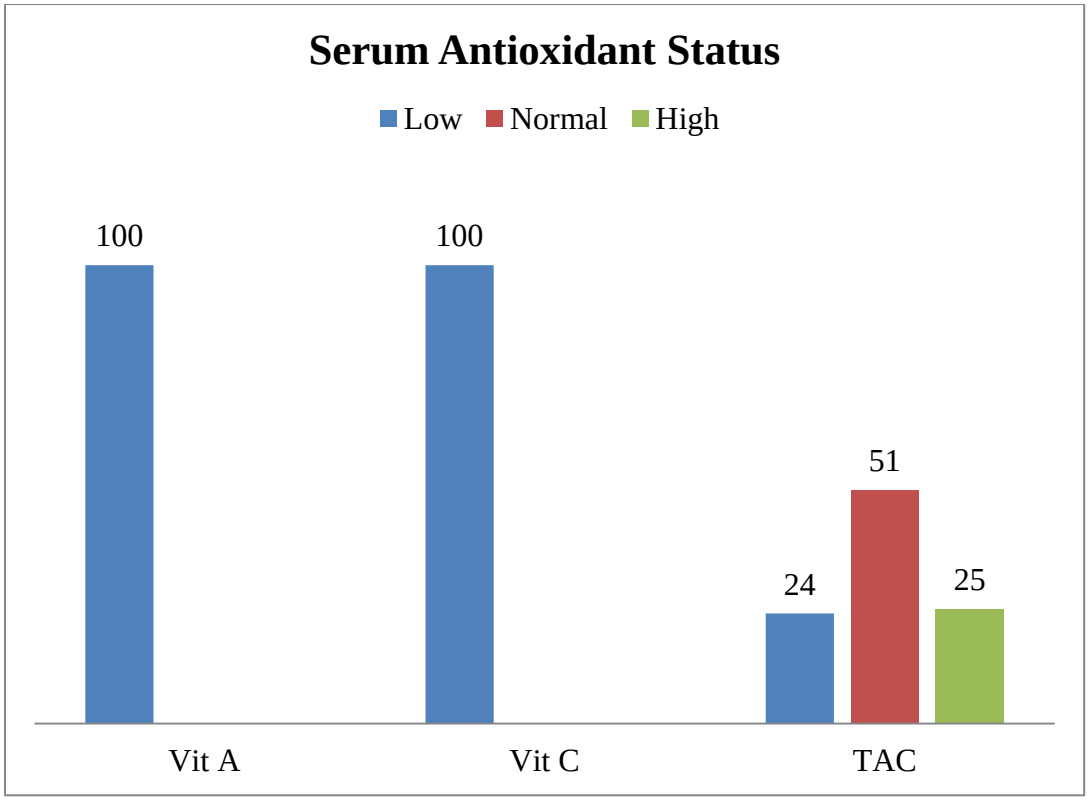


Figure 4.3 Serum Antioxidant Micronutrient Status of Diabetics

4.4.2 Serum Antioxidant Micronutrient Status of Diabetics

The graph (Figure 4.2) shows that all the participants had low serum levels of vitamin A and vitamin C. However, majority (51%) of the study participants had normal level of total antioxidant capacity (status), even though they had low vitamin A and vitamin C status. Study participants who have low TAC were 24%.

4.4.3 Pearson's correlation between HBA1c, FPG and Serum Antioxidant Status

In table 4.10, a summary of the associations between blood glucose levels using glycated haemoglobin (HBA1c and FPG) and serum antioxidant status of the study population have been presented. There were weak positive correlations between HBA1c, FPG and serum vitamin C and total antioxidant capacity (TAC). This implies that, an increase in blood glucose levels directly influences serum vitamin C and TAC. Also, weak negative correlations were found between HBA1c, FPG and serum vitamin A and C. This indicates

that, an increase in blood glucose levels decreases serum levels of vitamin A and C. However, the associations were insignificant because the p-values for the correlation coefficients were above 0.05.

Table 4.6 Pearson's correlation between HBA1c, FPG and Serum Antioxidant Status

Blood glucose parameter	Vitamin A r (p)	Vitamin C r (p)	TAC r (p)
FPG	-0.142(0.158)	0.069(0.497)	0.172(0.086)
HBA1c	-0.015(0.884)	-0.005(0.957)	0.168(0.095)

Data presented are correlation coefficients and p-values (in parenthesis) of associations between blood glucose levels (HBA1c and FPG) and serum antioxidant status (vitamin A, C and TAC) with confidence interval of 95%.

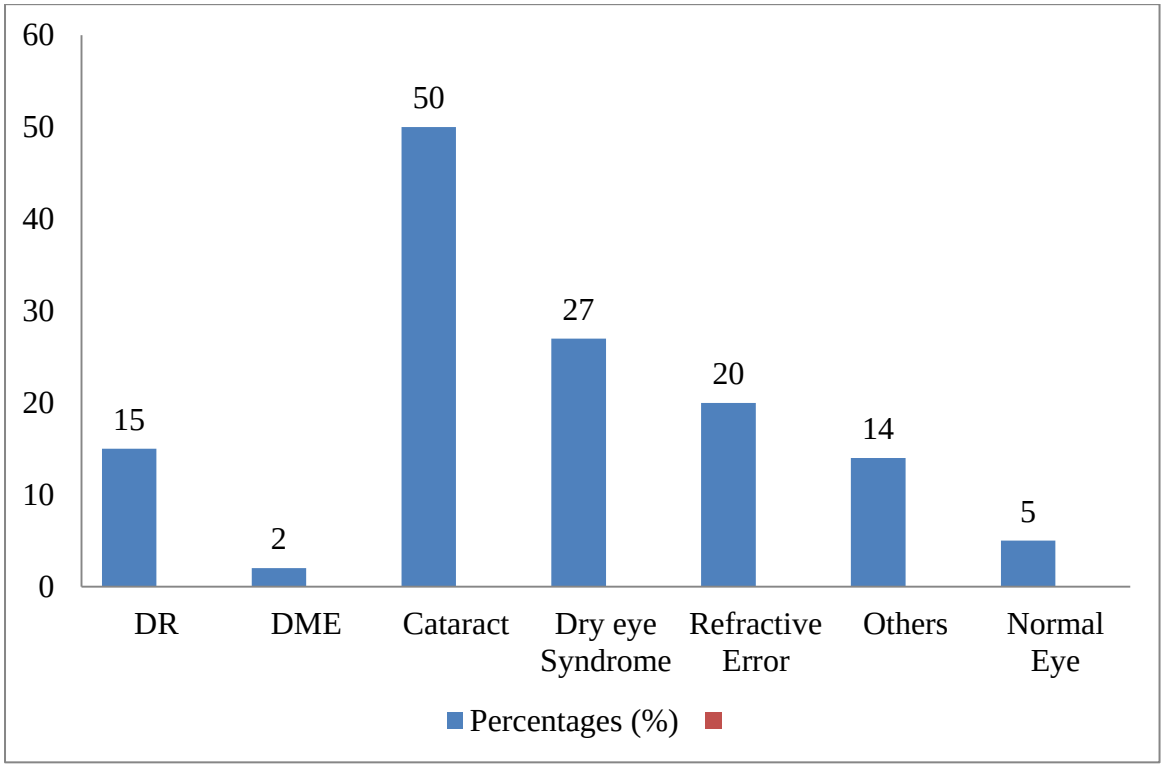


Figure 4.4 Prevalence of Diabetic Visual Disorders

4.5 Prevalence of Diabetic Visual Impairments

The prevalence of some diabetic visual problems accounted for in the study is presented in Fig4.1. Fifty percent (50%) of the respondents had cataract (both mature and immature), 27% had dry eye syndrome, 20% had uncorrected refractive errors, 15% had diabetic retinopathy (both mild-to moderate and severe NPDR), 2% for diabetic macular edema, and 14% had other visual disorders which were not included in the study. Five percent (5%) of the respondents had normal eyes without any visual disorder.

4.5.1 Association of Disease duration to the Prevalence of Vision Problems among Diabetics.

Table 4.11 presents the relation of diabetes duration to the prevalence of vision problems among diabetics. The prevalence (9%) of uncorrected refractive error was high in the 1-5years duration of the disease group while DES (8%) and DR (4%) were high in the 11-15years duration. Also, 1% each recorded in 11-15years and 1-5years durations for DME.

Table 4.7 Association of prevalence of visual impairment to disease duration among the study population

Visual Impairmen	1-5y	6-10y	11-15y	16-20y	>21y	P-value
UNRE	9	4	5	1	1	0.501
DES	6	7	8	3	3	0.367
CAT	14	16	15	2	3	0.649
DR	3	3	4	2	1	0.836
DME	1	0	1	0	0	0.775

Data are presented using chi square. The data presented show association between duration of diabetes and visual problems with confidence interval of 95%. Durations are in years (1-5y) and vision problems in abbreviated on the left column side of the table.

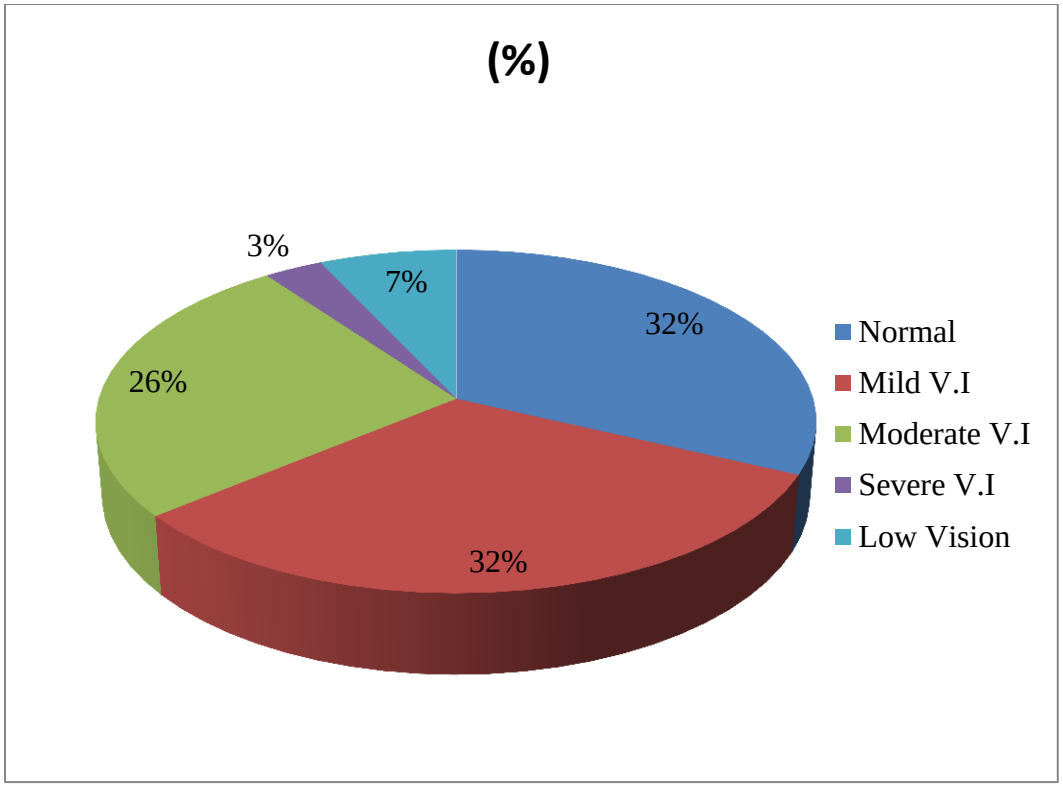


Figure 4.5 Prevalence of visual Impairment (Visual Acuity) of the study Population.

4.5.2 Prevalence of visual Impairment (Visual Acuity) among the study Population (Diabetics).

The prevalence of visual impairment among diabetics are displayed in figure 4.4. Using the World Health Organization (WHO) definition and revision of classification, visual acuity was categorized into "normal" (6/6-6/9), "mild" (<6/9-6/18), "moderate"(<6/18-≥6/60), "severe"(<6/60-≥3/60) and (<6/18-≥3/60) as "low vision" (WHO,2004;Dandona & Dandona, 2006). Thirty-two percent (32%) of the diabetic patients had normal vision (visual acuity) and 32% also had mild visual impairment. Diabetic patients who had moderate visual impairment were found to be 26%, 7% had low vision and 3% of the diabetics had severe visual impairment.

4.5.3 Comparison of the effect of Age and Gender on the Prevalence of DM visual Problems

The prevalence of diabetic visual problems as compared to sexes and age ranges are presented in Table 4.3. Respondents who had uncorrected refractive errors were approximately 11% (10.5) males and 22.2 % females. Within the age groups, 50% was recorded in 35-45 age group, 23.3% for 46-55 age group, 10% for 56-65 age group, 26.3% for 66-75 age group and nil for respondents within 76-85 age group all recording a P-value (0.770). Respondents who had dry eye syndrome were approximately 11% (10.5) males and 31% (30.9%) females. In age group 35-45, 37.5% was recorded, 23.3% for 46-56 age group, 27.5% for 56-65 age group, respondents in 66-75 age group were 31.6% and nil for respondents in 76-85 age group all with P-value (0.741).

For cataract, majority 57.9% approximately (58%) was males and 48% was females, in between their age groups, majority (73.7) approximately 74% in age group 66-75 had cataract. Respondents in 56-65 age group recorded 55%, 33.3% for 46-55 age group, 12.5% for 35-45 age group and those within age group 76-85 recorded 100%, all with a p-value (0.004).

With regards to diabetic retinopathy (DR), respondents who had mild to moderate non-proliferative diabetic retinopathy (NPDR) were 15.8% approximately 16% males and 12.3% females. Within their age groups, respondents who fell within 35-45 age group were 25%, 21% was recorded for 66-75 age group, 16.7 for 46-55 age group, 5% for age group 56-65 and nil was recorded for respondents within 76-85 age group, and also respondents who had severe non-proliferative diabetic retinopathy (NPDR) were all males recording 10.5%. Within the age groups, nil was recorded for 35-45years, 46-55years, 66-75years, and 76-85 age groups but 56-65 age groups recorded 5% respectively, all recording p-value (0.440). Lastly,

study subjects who had diabetic macular edema were 2.5% all females recording 33.3% in age group 76-85 and 2.5% for 56-65 age groups all with p-value of (0.003) respectively.

Table 4.8 Effects of Age, Gender on Prevalence of DM Vision Problems

Visual Impairments	Gender	%	p-value	35-45y	46-56y %	57-67y	68-78y	79-85y	p-value
UNRE	Male	10.5	0.251	50	24.1	10	26.3	0	0.067
	Female	22.2							
DES	Male	10.5	0.072	37.5	24.1	27.5	31.6	0	0.689
	Female	30.9							
CAT	Male	57.9	0.444	12.5	31	55	73.7	100	**0.002**
	Female	48.1							
NPDR (Mild-moderate)	Male	15.8	**0.011**	25	17.2	5	21.1	0	0.420
	Female	12.3							
DR(Severe)	Male	10.5	**0.011**	0	0	5	0	0	0.420
	Female	0							
DME	Male	0	0.489	0	0	2.5	0	25	**0.017**
	Female	2.5							

Data presented are the results of prevalence of visual problems related by age groups gender. Variables were described using chi-square with a standard p-value (<0.05). Highlighted p-values in the table are those less than (<0.05) which is described as significant.

4.5.4 Association of Glycated Haemoglobin, and Fasting Plasma glucose to Vision disorders among Diabetics.

The results of the association of glycated haemoglobin (HBA1c) and fasting plasma glucose (FPG) to vision problems among diabetics are present in table 4.13. Study subjects with normal A1c who had uncorrected refractive errors (RE) were 35.7% and 17.4% for high A1c. 22.2% of the respondents had RE with normal FPG and 18.8% with high FPG. The percentage of respondent with high FPG who had dry eye syndrome were 31.2% , 19.4% had normal FPG, 28% had normal A1c and 26.7% had high A1c levels. With regards to cataract,

50% of the respondents had normal A1c and FPG and 50% also recorded for high A1c and FPG levels. For DR, 14.3% of respondents with normal A1C and 12% with high HbA1c levels had mild-moderate NPDR while 13.9% with normal FPG levels, 12.5% with high FPG had NPDR. Subjects with high HbA1c who had DME were 2.3%, 2.8% with normal FPG and 1.6% with high FPG also had DME.

Table 4.9 Association of HBA1c, FPG and Vision disorders among the study population

Visual Impairment	**Normal (%)**	**High (%)**	**p-value**	**Normal (%)**	**High (%)**	**p-value**
UNRE						
Yes	35.7	17.4	0.113	22.2	18.8	0.677
No	64.3	82.6		77.8	81.2	
Dry Eyes Syndrome						
Yes	28.6	26.7	0.886	19.4	31.2	0.202
No	71.4	73.3		80.6	68.8	
Cataract						
Yes	50	50	1	50	50	1
No	50	50		50	50	
NPDR(Mild-moderate)						
Yes	14.3	12.8	0.841	13.9	12.5	0.895
No	85.7	87.2		86.1	87.5	
NPDR(Severe)						
Yes	0	2.3	0.841	2.8	1.6	0.895
No	100	97.7		97.2	98.4	
DME						
Yes	0	2.3	0.564	2.8	1.6	0.677
No	100	97.7		97.2	98.4	

Data presented are the results of the association of blood glucose levels (HBA1c and FPG) to the prevalence of visual problems related. Variables were described using chi-square with a standard p-value (<0.05). P-values in the table are not statistically different because they are greater than (>0.05) which is described as insignificant.

4.5.5 Comparison of mean Antioxidant micronutrients intake and status among subjects with or without cataract.

Table 4.16 shows the comparison of the means of antioxidant micronutrients intake and status among diabetic patients with and without cataract. The mean (standard error of the mean) of vitamin A was 149.4± (129.2) [a] mcg for subjects with diabetic cataract and 204.8± (179.9) [a] mcg for diabetics without. For vitamin C, diabetic with cataract had a mean of 72.9± (33.9) [a] mg and 85.7± (41.9) [a] mg for diabetics without cataract. 4.8± (2.4) [b] mg was the mean intake of vitamin E for diabetics with cataract and those without had 6.1± (3.2) [b] mg. The mean intake of selenium was 84.0± (28.7) [b] mcg and 96.7± (33.1) [b] mcg for diabetics with and without cataract. Mean zinc intake was 5.9± (2.6) [b] mg for diabetic patients with cataract and 7.4± (3.2) [b] mg for those without. The mean status of vitamin A and vitamin C for diabetics with cataract was 0.024± (0.005) [a] µmol/L and 0.24± (0.1) [a] µmol/L while diabetics without cataract had 0.022± (0.01) [a] µmol/L and 0.3± (0.2) [a] µmol/L . Regarding the total antioxidant capacity, the mean status for diabetic with cataract was 24.7± (5.7) [a] µmol/L and 24.2± (5.0) [a] µmol/L for those without. It is evident from the table that, some relation between the antioxidant micronutrients intake and status and cataract had some to be statistically different with a superscript (b) and p-value <0.05 while others were not statistically different with a superscript (a) and p-value >0.05.

Table 4.10 Comparison of Mean Antioxidant Micronutrients intake and status among subjects with or without cataract.

Nutrients	Cataract (Means±SD)	No Cataract (Means±SD)	p-value
Vitamin A (mcg)	149.4±129.2	204.8±179.9	0.080
Vitamin C (mg)	72.9±33.9	85.7±41.9	1.000
Vitamin E (mg)	4.8±2.4	6.1±3.2	**0.019**
Selenium(mcg)	84.0±28.7	96.7±33.1	**0.044**
Zinc (mg)	5.9±2.6	7.4±3.2	**0.017**
Serum Vitamin A(µmol/L)	0.024±0.005	0.022±0.01	0.119
Serum Vitamin C(µmol/L)	0.24±0.1	0.3±0.2	0.380
TAC(µmol/L)	24.7±5.7	24.2±5.0	0.671

Data presented are the results of two 24hour recalls and serum levels. Continuous data were presents as means and was compared using unpaired T-test: High lightened p-value means they are statistically different, p>0.05.

4.5.6 Association of Antioxidant micronutrients intake to Visual Disorders among the study population.

The association of antioxidant micronutrients intake to vision problems among the study population is presented in table 4.17. Twelve percent (12%) of diabetics with low and 8% with high vitamin C intake had uncorrected refractive errors (UNRE). It was noticed that 17% of diabetic patients who had low vitamin C intake and 9% who had high vitamin C intake were found to have dry eye syndrome (DES). Also, 31% with low vitamin C intake and 13% with high intake had cataract (either mature or immature) and this was significant (P-value: 0.046). With regards to diabetic retinopathy (DR), subjects with low vitamin C intake were 8% and subjects with high were 7%. Two percent (2%) of the subjects with high vitamin C intake had diabetic macular edema (DME) and 4% of diabetics without any visual problem had low vitamin C intake. Thirteen percent (13%) of diabetics with high and 5%

with normal selenium intake had uncorrected refractive errors (UNRE) and this was significant (p-value: 0.043). It was noticed that 19% of diabetic patients who had high selenium intake, 5% who had low and 3% who had normal selenium intake were found to have dry eye syndrome (DES). Also, 36% with high selenium intake, 11% with low and 3% with normal intake had cataract (either mature or immature) and this was significant (P-value: 0.041). With regards to diabetic retinopathy (DR), subjects with high selenium intake were 13% and subjects with low selenium intake who had diabetic macular edema (DME) were 2% which was significant (p-value:0.002) and 5% of diabetics with high selenium intake had DME.

Thirteen percent (13%) of diabetics with low and 5% with normal zinc intake had uncorrected refractive errors (UNRE). It was noticed that 22% of diabetic patients who had low zinc intake and 3% who had normal zinc intake were found to have dry eye syndrome (DES). Also, 44% with low zinc intake and 5% with high intake had cataract (either mature or immature) and this was significant (P-value: 0.013). With regards to diabetic retinopathy (DR), subjects with low zinc intake were 10% and subjects with normal were 5%and this was significant (p-value:0.028). Two percent (2%) of the subjects with low zinc intake had diabetic macular edema (DME) and 4% of diabetics without any visual problem had low zinc intake.

Table 4.11 Association of Antioxidant micronutrients intake to Visual Problems among the study population.

Variables	Vitamin C (%)				Selenium (%)			
Visual Impairment	**Low**	**Normal**	**High**	**P-value**	**Low**	**Normal**	**High**	**P-value**
RE	12	0	8	0.337	2	5	13	**0.043**
DES	17	1	9	0.493	5	3	19	0.691
CAT	31	6	13	**0.046**	11	3	36	**0.041**
DR	8	0	7	0.758	1	1	13	0.850
DME	0	0	2	0.176	2	0	0	**0.002**
NORMAL	4	0	1	0.651	0	0	5	0.75
	Zinc (%)				**Vitamin A (%)**			
Visual Impairment	**Low**	**Normal**	**High**	**P-value**	**Low**	**Normal**	**High**	**P-value**
RE	13	5	2	0.081	18	2	0	0.277
DES	22	3	2	0.782	25	1	1	0.255
CAT	44	1	5	**0.013**	49	0	1	0.078
DR	10	5	0	**0.028**	15	0	0	0.920
DME	2	0	0	0.750	2	0	0	0.948
NORMAL	4	0	1	0.518	5	0	0	0.312
	Vitamin E(%)							
Visual Impairment	**Low**	**Normal**	**High**	**P-value**				
RE	20	0	0	1				
DES	26	0	0	0.469				
CAT	49	1	0	1				
DR	15	0	0	0.835				
DME	2	0	0	1				
NORMAL	5	0	0	0.787				

Categorical Data was presented as frequencies. The results of association of antioxidant micronutrient intake to diabetic vision problems were described using Fisher's exact test and chi-square with a standard p-value (<0.05). Highlighted p-values in the table are those less than (<0.05) which is described as significant.

Table 4.18 is a summary of the relationships between diabetic vision and nutrient intakes, antioxidant status and dietary diversity scores. The table provides a reflection of the relationship between nutrient intake, antioxidant status, dietary diversity and diabetic vision.

The first odd ratio is placed with inadequate intake and 1 is the standard odds ratio (95%Cl). It was observed that, carbohydrates intake did not show any relationship with diabetic vision. However, adequate protein intake increases the risk of diabetic visual impairment more than 3-fold (OR= 2.7; 95%Cl, 0.7-10.2). The risk of developing diabetic vision problem is decreased less than 1-fold (OR= 0.2; 95%Cl, 0.1-0.8) with adequate intake of fibre (P = 0.024). Adequate intake of vitamin C has decreased risk of less than 1-fold (OR= 0.6; 95%Cl, 0.2-1.8). Vitamin E intake showed no relationship with diabetic vision while adequate zinc intake has decreased risk of less than one fold (OR=0.8; 95%Cl, 0.2-3.1). Adequate intake of selenium by diabetics had (OR= 0.3; 95%Cl, 0.1-2.9). With regards to dietary diversity score, diabetics who had normal dietary diversity score had a decreased risk of less than one fold (OR= 0.6; 95%Cl, 0.2-2.7) to diabetic vision problems while those had high dietary diversity score had more one fold increased risk to diabetic vision problems (OR= 1.2; 95%Cl, 0.3-6.4). Total antioxidant status had a strong influence on diabetic vision, in that, diabetics had normal total antioxidant status (TAS) had an increased risk of more than one fold (OR= 1.1; 95%Cl, 0.2-4.0) while those with high TAS showed a decreased risk of less than 1 fold to diabetic vision problems (OR= 0.8; 95%Cl, 0.1-3.4).

Table 4.12 Relationship between Diabetic Vision and nutrient intake, dietary diversity scores, antioxidant status.

Nutrient intake	OR(95% Cl)	P-value	Nutrient	OR(95% Cl)	P-value
Carbohydrates			**Vitamin E**		
Inadequate	1		Inadequate	1	
Adequate	0	0.999	Adequate	-	-
Proteins			**Zinc**		
Inadequate	1		Inadequate	1	
Adequate	2.7 (0.7-10.2)	0.140	Adequate	0.8 (0.2-3.1)	0.855
Fibre			**Selenium**		
Inadequate	1		Inadequate	1	
Adequate	0.2 (0.1-0.8)	**0.024**	Adequate	0.3(0.0-2.9)	0.329
Vitamin C			**TAC**		
Inadequate	1		Low	1	
Adequate	0.6(0.2-1.8)	0.417	Normal	1.1(0.2-4.0)	0.914
			High	0.8 (0.1-3.4)	0.764

Data presented are the results of 24hour recalls and antioxidant status. Continuous data were presents as odds ratios (95% Cl) and compared using binary logistic regression. The relationship between the dependent variable and the covariate are not statistically different, $p>0.05$.

CHAPTER FIVE

5.0 DISCUSSIONS

Diabetes Mellitus results in considerable chronic complications causing morbidities among populations. Recognizing diabetic visual disorders, its mechanism and nutrients to ameliorate this complication in diabetic patients is essential due to the disability it confers on DM patients which affects their quality of life.

5.1 Background Characteristics of Participants

The basic and socio-demographic and clinical features of subjects cannot be undermined. In the total population, one hundred (100) subjects were recruited to go through the study processes. Female subjects (81%) were categorically more than male subjects (19%). This is because more female patients report to the clinic often than their male counterpart and also the most prevalent age group was 56-65 (40%). This result is in agreement with Amissah and Amoako-Boateng, (2014), who also reported in that female (392± (71.5%) subjects were more than the males (156± (28.5%) and similar age groups (50-59 and 60-69) in their study at the same hospital where this present study was conducted.

In this present study, higher educational level (junior/middle school) with majority (56%) of the subjects was engaged in self-generating income activities (petty trading) and duration of diabetes was 6-10years. This was also similar to Amissah and Amoako-boateng, (2014), who reported 30% of their subjects being self-employed (petty trading) and duration of diabetes where majority (41%) fell between 1-5years and 6-10years. other studies such as Genet *et al.,* (2013), Mvitu-Muaka *et al.,* (2011) and Ovenseri-Ogbomo *et al.,* (2013), Padmaja *et al.,(*2017) Kim and Kim, (2006) were all in agreement with above results.

Majority (90%) of the subjects had T2DM compared to T1DM. This study also looked at the treatment regimen given of which most (79%) of them were on oral glucose lowering

medications, this was consistent with Glover *et al.,* (2014). Knowledge of diabetic complications generally and diabetic eye disorders was high among the subjects. However, knowledge on diabetic visual impairment in this study is on the contrary to Ovenseri-Ogbomo *et al.,* (2013) study at the Korle-bu Teaching Hospital in Ghana which reported that knowledge of ocular manifestations among diabetic patients was poor.

Other co-morbidities which are also biomarkers of diabetic complication including diabetic eye disorders were prevalent among the study population. These are also known traditional risk factors to diabetic complications. The principal aim of managing diabetes is achieving near normal glycaemia. The American diabetes Association has endorsed HbA1c <7% as goal of good glycemic control (ADA, 2013). Selim *et al.,* (2017) indicated the significance of glycemic control in diabetes to prevent complications such as retinopathy. He found the majority of the T2DM patients were not able to achieve or maintain optimum glycemic control. In this present study, the prevalence of hyperglycemia was higher (86% and 64%) using both HbA1c and fasting plasma glucose (FPG) respectively with overall means of 8.5±(3.4)mmol for FPG and 11.3±(5.4)% for HbA1c. These results show that glycaemia is poorly controlled among the study population and this is consistent with Selim *et al.,*2017 and Al-halaweh *et al.,* 2017 from the literature who found that glycemic control in terms of HbA1c and FPG assessed were all associated to diabetic complications. In Selim et al study, the mean HbA1c was 9.83% and HbA1c <7% was found in only 19.1% (997), which was high and denoted as poor glycaemia.

Selim *et al.,* (2017) also suggest that hypertension was significantly associated with poor glycemic control in diabetics. Raman *et al.,* (2012) showed increasing systolic blood pressure to be a risk factor for retinopathy among diabetics with odd ratio (OR= 1.06, P =0.001), however, the findings of this present study showed that hypertension (Bp >140/90) which is

also a known risk factor and a co-morbid condition among diabetic patients was fairly controlled. Only 48% which is less than half of the study participants had Bp >140/90mmHg. The overall mean systolic blood pressure was high (135.8± (20.2) mmHg than the normal (<120 mmHg) and the diastolic blood pressure slightly high. These also depict that systolic Bp is not well controlled among the subjects even though it may fluctuate. Also, there was a significance (P-value; 0.004) in comparing both sexes where males had a higher mean systolic value than female subject. The findings above also agree with Klein *et al., (*2008) and Klein *et al.,* (2009) from the literature.

High BMI (overweight and obesity) is implicated in diabetes and its complications. From literature, Tanaka *et al.,* (2016) concluded that obesity was associated with increased risks of microvascular complications in T2DM. They indicated that high BMI and high weight category were significantly associated to diabetic retinopathy. Similarly, the findings of this study indicated that, BMI >25kg/m^2 was found among the study subjects with overweight (35%) and obesity (48%) and a total mean of 30.8±(7.8)kg/m^2 of which female participants were found to have a higher BMI than their male counterparts with P-value (0.002) which was significant. This may be due to inadequate/lack of physical activity as most of the female subjects were petty traders. Also, Increased BMI >31kg/m^2 (men), BMI >32 kg/m^2 (women) and increased waist-hip ratio were associated to the elevated risk of developing retinopathy (Klein *et al.,* 1997).

5.2 Dietary Assessment of Study Population

Data collected with a three (3) days 24hour recall question was to estimate the total daily intakes of subjects and compared with daily recommended intakes for adults (Otten *et al*., 2006; Table & Table, 2001). The findings of this study showed that mean intakes of carbohydrates and proteins were high compared with the DRI's for both male and female

subjects this depicts an increased intake of proteins and carbohydrates among subjects. Conversely, subjects recorded low mean intakes of Fibre and did not meet the DRI's. This may be the cause of poor glycemic control observed in the study as high low carbohydrate diets decrease plasma glucose levels as indicated by Boden *et al.,* (2005). Also fibre intakes are and also fibre has shown to decrease blood glucose levels (HbA1c and fasting plasma glucose) (Giacco *et al.,* 2000). However, some antioxidant micronutrients such as vitamin A , vitamin E, zinc and other micronutrient like magnesium, calcium, riboflavin, recorded lower means intakes for both male and female subjects as they did not meet the DRI's and its denotes insufficient intakes of these nutrients. On the contrary to the above, selenium and niacin showed high mean intakes for male and female subject respectively depicting over intakes of these nutrients which can cause toxicity.

The means selenium for male and female subject were (110.5± 17.2) and (85.6±30.0) respectively, suggesting higher intakes in the total population. Selenium is an important trace element due it antioxidant properties and role in enzymatic process (Rayman, 2008). According to literature, it usual acquired via diet and its content in foods differs greatly depending on the content in the soil where the food was planted or animal was tend or fed (Rayman, 2008). Hurst *et al.,* (2013), indicated that Ghana was included in a range of countries which are likely to have dietary selenium present here of about 82 to 260µg/day. Additionally, Adotey *et al.,* (2011) found high level of selenium which was over the DRI for selenium intakes was consumed by three orphanages in the southern part of Ghana. This is consistent with the finding of the present study as the study was conducted at the southern part of Ghana and this could be the reason for increase intakes of selenium by study subjects. Whereas mean vitamin C intakes was adequate compared with the DRI's. In addition, there were some statistical differences between the means of both sexes showing an increase or decrease across the sexes.

Also, the findings of this study show some correlations between nutrient intakes and blood glucose levels (both HbA1c and FPG). The higher intakes of calories (kcal), protein, carbohydrates, fat and selenium, the higher the blood glucose level, this may be the reason why glycemia was poorly controlled. Again, as intakes of fibre, zinc, vitamin C, vitamin A, vitamin E increases, blood glucose level decreases. Kundu *et al.,* (2014) and Millen *et al.,* (2004) also agrees with these findings.

The means intakes of nutrients were observed in the age groups. Higher mean intakes of carbohydrates (224.2± (64.0), zinc (7.1± (3.4) was observed in age group 56-65years. This might be due to Mean intakes of kcal (1562.7± (451), protein (65.0± (22.9), selenium (111.7± (40.9), vitamin C (92.4± (31.3) and vitamin E (6.4± (3.3) was high in the 35-45 age group as this age group is the adult youth and require more energy for their activity. Mean intakes of vitamin A was high in the 66-75 age group whereas high intake of Fibre was observed in the age groups 35-45years and 46-55years simultaneously. This may be because the above age groups have their nutrients requirements separately and at different given times.

Majority (68%) do not consume fruit and (65%) green leafy vegetables as suggested by "five a day program" suggesting inadequate intake of fruits and vegetables in the study population and disagreeing with the adequate intakes of vitamin C and agreeing with low intakes of other micronutrients. This is not in agreement with Tanaka *et al.,* (2016) who indicated in his study that the risk of retinopathy is reduced with increase intakes of fruit and vegetables. Oily fishes were consumed more 1-3 times weekly than daily contributing to low intakes of vitamin E. There was general low intake pattern of meat and poultry and also vitamin supplement (24%) weekly suggesting low intakes of zinc and the other nutrients.

Adequate intakes of variety of foods to provide carbohydrates, proteins, essential fatty acids, fibre, vitamins, minerals and phytochemicals required for maintaining body processes and removing body waste are recommended by the World Health Organization (WHO), worldwide nutrition associations (Example; American Dietetic Association and The Canadian Dietetic Association) and the regenerative health and nutrition program under the Ghana Ministry of Health (Byrd-Bredbenner *et al.,* 2014).

Pearson correlations showed some positive associations between the different dietary nutrient intakes and blood glucose levels. This implies that, the intakes of a variety of foods by diabetics will provide them with all the essential nutrients they need and increase intakes of these nutrients will directly influence their blood glucose levels. The effects of these associations are in harmony with the rationale within which the creation of MyPlate by the United States Department of Agriculture (USDA, 2011) are established. These add to the declaration that, there is no particular food needed for a decent nutrition and that every food provides some nutrients but supplies inadequate amount of at least a single essential nutrient; as such no one food group supplies all essential nutrients in sufficient amount and that each food group forms a significant, distinguishing contribution to nutritional intake; and also foods within a group may differ widely with regards to energy and nutrients(Byrd-Bredbenner *et al.,* 2014). It is therefore essential that diabetic patients keep to dietary recommendations concerning dietary diversification to ensure proper glycemic control, normal lipids and optimum antioxidant micronutrient status so as to prevent or decrease the rate of diabetic vision complications.

5.3 Serum Antioxidant Micronutrient Status of Study Population.

Vitamin C has a defensive role in immune function, anti-inflammatory and scavenging of reactive oxygen species brought forth by oxidative stress. In the diabetic eye, defense of the

lens, cornea, vitreous humor and retina from oxidative stress is carried out by vitamin C (Bendich *et al.,* 1986). However, in this current study, a decrease in serum vitamin C was observed among the diabetics with an overall mean (0.023± 0.01) µmol/L. This mean of vitamin C level was not consistent with the (third NHANES Survey 1988-1994) as stated by Schleicher *et al.,(*2009) who establish vitamin C deficiency to be (serum concentrations < 11.4µmol/L) as all the subjects in this present study did not meet this criteria which implies a deficiency in vitamin C in the study population .However, the study consistent with Kundu et al.,(2014) where the means serum levels of vitamin C in diabetics groups were lower (0.70±0.24 and 0.87±0.29 mg/dl) compare to healthy subjects(1.25±0.27 mg/dl). Also, Kumari *et al.,* (2008), Gurler *et al.,* (2000) and Gupta and Chari, (2005), are all in agreement the above.

For serum vitamin A, a decrease was also observed with a total mean (0.256± 0.1) which did not meet the WHO, (2011) standards, who established vitamin A deficiency to be (serum concentration ≤ 0.70µmol/L). This implies a deficiency in vitamin A and it is explainable since intakes vitamin A too was low and did not meet DRI. Basualdo *et al.,* (1997) showed reduced bioavailability of retinol levels in diabetics. Additionally, Campoy *et al.,* (2002) found significant reduced concentration of serum vitamin A in type 1 diabetics (1.03 ±0.03 µg/dL) compared to healthy, control (1.17±0.06 µg/dL, p< 0.05). The findings of this study are in agreement with Basualdo *et al.,* (1997) and Campoy *et al.,*(2002)

In diabetes mellitus, level of antioxidant status systems in defending against oxidative process is significant as alterations in energy metabolism, change in sorbitol pathway mechanisms and the stage of inflammatory intermediaries' results in metabolic stress (Baynes, 1991). It is therefore important to assess the status of antioxidant due to its role in oxidative stress. The findings of the study showed that about half of the subjects fell within

the second quartile of total antioxidant status comparing the first and third quartiles with a total mean of 24.4± (5.3) µmol/L. However, Rodriquez-carrizalez *et al.,* (2013), showed a significant decrease in total antioxidant status in diabetics with lowest possible degree in subjects with severe DR.

Also, the findings of the study showed correlations between serum antioxidant micronutrients and blood glucose level. As such, an increase in blood glucose, increases total antioxidant status (TAS). Nevertheless, an inverse correlation shows that an increase in blood glucose levels decreases serum vitamin A and vitamin C which implies that as blood glucose increases, the serum vitamin A and vitamin C reduces. This is contradictory to a study by Kundu *et al.,* (2014), who showed positive correlation between HbA1c and serum vitamin C. However, Gupta and Charis, (2005), did not find any association with serum vitamin C and HbA1c.

5.4 Prevalence of diabetic visual problems of the study population

Diabetic mellitus causes significant ocular manifestations among its sufferers. These ocular manifestation leads to visual impairment and eventually blindness which causes disability and poor quality living among diabetic populations. This current study determined the prevalence of ocular manifestations among the diabetic patients by taking them through eye examinations and due to this, subjects had treatment when it was necessary. In this study, it was revealed that cataract (50% out of 100) was the highest ocular manifestation among the diabetics, followed by dry eye disease, uncorrected refractive, DR had (15%) which was also alarming, others and DME with lowest prevalence (2%). Coincidentally, the prevalence of cataract (50%) in this current study reflects the same prevalence of a study conducted by Kim and Kim, (2006), which disclosed the prevalence of cataract to be 50% in their diabetic population. Again, this report is similar to that of Amissah and Amoako-Boateng, (2014),

who also recorded a high prevalence (24.4%) of cataract and a lower prevalence of DR (1.9%) with 57% of their subjects having blurry vision (which not included in their analysis and discussion) using 214 respondents suffering from eye complications. Also Mvitu-Muaka *et al.,* (2011) reported the prevalence of cataract including operated eyes being 46.7%.

In their study and this present study, cataract was commoner among diabetics than DR. Dry eye syndrome also had a significant percentage (27%) among the diabetic subjects and its prevalence was higher than DR. Najafi *et al.,(*2013) reported that dry eye syndrome is common in T2DM patients and more especially those with diabetic retinopathy. A significant correlation (OR= 2.29, Cl= 1.16-4.52, P= 0.016) was between dry eye and DR which also in turn had a correlation with HbA1c. Also, more males (57.9) had cataract compared their female (48.1) counterpart which was significant comparing both sexes and duration to the prevalence of DM vision disorders. This report agrees with Mvitu-Muaka *et al.,* (2011).

Prevalence of visual impairment using visual acuity was displayed in this study. Using the World Health Organization (WHO) definition and revision of classification, visual acuity (<6/9 to 6/18) was found to be (32%) prevalent among the subjects showing a mild visual impairment (VI) and fewer with severe VI (3%) and low vision (7%). This is in contrast with Padmaja *et al.,* (2017) in his study there was scarcity where only 4% were visually impaired with mild occurring in 3.6% unlike our current study reporting on 32%. Also severe VI was low (0.4%) and 0.1% were blind.

Furthermore, duration of DM was related to the prevalence of visual impairments/ocular manifestations. It was observed that the early part of the disease thus 1-10years had more prevalence of ocular disorders than the longer duration. This then may debate with other studies who contend that visual complications may occur due to longer of diabetes. On a second note, this may agree with several studies (Al-Zuabi *et al.,* 2005; Bała *et al.,* 2009; Di

Pietro *et al.,* 2007; Harzallah *et al.,* 2006; Olivarius *et al.,* 2001; Wahab *et al.,* 2008) who suggested that, in patients who are newly diagnosed with diabetes, the prevalence of microvascular complication has been discovered to fluctuate from 5%-35% in different population and as such this rise may be attributed to the delay between the onset of DM and the diagnosis of the disease. Evidence suggest that the disease has a silent phases (4-7years) in which its asymptomatic before clinical diagnosis and this results in the development of chronic complications causing morbidity such as disability, lack of fitness to work, and Poor quality of life (Piechowsiki-Jozwaik *et al.,* 2005, Spijkerman *et al.,* 2004).

5.5 Relationship of Diabetic vision and nutrient intakes, dietary diversity score and antioxidant status.

Over the past two decades, few studies have been conducted in humans diabetics to investigate the relation between antioxidant micronutrient and diabetic vision problems or eye diseases but more evidence stands for experimental studies. A chi square and fisher's exact test showed a significant association of vitamin C intakes with cataract ($P = 0.046$, $P<0.05$), selenium intakes were found to be significantly associated with uncorrected refractive error ($P = 0.043$, $P< 0.05$), cataract ($P = 0.041$, $P< 0.05$) and diabetic macular edema ($P = 0.002$, $P< 0.05$). Additionally, a significant association was indicated between zinc intakes and cataract ($P = 0.013$, $P<0.05$) and DR ($P = 0.028$, $P<0.05$).

Additionally, using binary regression analysis, the findings of this study show the relationship of antioxidant micronutrient intakes, dietary diversity score and antioxidant status with the presence of diabetic eye diseases or vision problems. It was observed that adequate intakes of protein increase the risk of visual problems among the diabetic (OR=2.7, 95%Cl, 0.7-10.2). However, a reduced risk of ocular disorders was observed in this study with adequate intake of Fibre (OR= 0.2, 95%Cl, 0.1-0.8);($P = 0.024$) among people living with diabetes as fiber

has some positive effect on blood glucose which is a known risk factor for ocular complications in diabetics as indicated by Giacco *et al.,* (2000). However, carbohydrates, vitamin A, vitamin E showed no relationship with diabetic vision problems. Also, adequate intake of vitamin C showed a decreased risk of ocular disorders with odds ratio and 95% confidence interval (OR= 0.6, 95%Cl, 0.2-1.8). Even, excess level of the above reduces the risk drastically. However, the odds ratio of vitamin C was inconsistent with Millen *et al.,* (2004) who indicated that there was no association between vitamin C but agrees with vitamin E intakes not having any influence on diabetic eye disorders. Zinc and selenium intakes also showed to reduce the risk of diabetic vision disorders by odds ratio and 95% confidence interval (OR= 0.8, 95%Cl, 0.2-3.1) and (OR= 0.3, 95%Cl, 0.1-2.9).

Furthermore, the findings of this study, showed that the second quartile of total antioxidant status (TAS) compared with the first and third quartile increases the risk of DM ocular complications with odd ratio and 95% confidence interval (OR= 1.1, 95%Cl, 0.2-4.0) while the third quartile of TAS compared with the first and second showed a reduced risk. This implies that even adequate TAS of diabetics is not enough to protect them from visual complications as oxidative stress increase; a poor antioxidant defense system may not shield diabetics from metabolic stress.

Antioxidant micronutrients may play a role in preventing diabetic complications including visual disorders as the findings of the study indicate. Even though the study was not able to re-examine the diabetic eyes for possible progression or retrogression of ocular manifestations, the study agrees with Moshetova *et al.*, (2015) who found an improvement in best corrected visual acuity, macular thickness and light sensitivity, treating T2DM patients with antioxidant and angioprotective therapy (240mg per day).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

In conclusion, the prevalent ocular manifestation was cataract (50%), followed by dry eyes syndrome (27%), uncorrected refractive error (20%), diabetic retinopathy (15%), other ocular disease including glaucoma (14) and diabetic macular edema (2%) recording the lowest prevalence among the study population. Also dietary intakes of vitamin A (95%), vitamin E (95%), zinc (76%) and fibre were found lower among the diabetic patients by the DRIs. High intake of carbohydrates (75%) and selenium (88%) was also observed among the study subjects. There was a decrease in serum vitamin A and serum vitamin C and was associated with increase blood glucose and again, high antioxidant status (TAS) reduces the risk of diabetic vision complications. Ultimately, poor dietary and serum antioxidant micronutrients with risk factors of visual impairment may predispose diabetics especially type 2 diabetics to vision complications which leads to visual impairment and subsequently blindness causing disability and poor quality of life among diabetics.

- **Limitations of the Study**

Some subjects who participated by drawing their blood samples for serum analysis did not fill their questionnaires, some also had problem with recollecting their intakes of daily meals and quantities and this could influence the results of the study. Also, due to the hospital policies, eye examinations were not done same day as when other data was collected and as a result subjects had to go home n report to the eye clinic at another date. Again, some subjects left a wrong contact and could not be contacted for eye screening schedules so we had to drop them reducing the sample. A more efficient dietary assessment tool like the weighed food records could be used in a repeated study to accurately measure the dietary intakes of

diabetics and to eliminate the short falls (the flat slope syndrome) associated with the 24hr recall. Serum vitamin E, zinc, and selenium could not be determined due to limited resources. Other indicators of oxidation like serum malondialdehyde could be assessed in a repeated study to know the actual state of oxidation in diabetic

6.2 RECOMMENDATIONS

In view of the findings of this study, It is suggested that:

- Further studies should assess dietary antioxidants intakes and status of healthy adults and contrast with type 2 diabetes patients.
- Additional studies should examine oxidative stress markers and antioxidant micronutrients in type 2 diabetics with vision complication such as cataract.
- Nutrition education on antioxidant micronutrient rich foods also should be included in diet therapy and counseling of diabetic patients by dietician and other health professionals.
- Lastly, studies on the effect of antioxidant micronutrient on diabetic vision in humans remain inconsistent. Further observational and experimental studies will emphasize on it better.

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APPENDICES

APPENDIX A

RESULTS

Summarizes the Results obtained from the systematic review

Principal Author and Country	Year	Title	Main Objective	Study population
Mvitu-Muaka *et al.,* (DR Congo)	2016	Determination of retinopathy prevalence between non-diabetics and type 2 diabetic patients in Central Africa: Effects of vegetables intakes, nutrients and antioxidants.	To investigate the association of between established risks factors of retinopathy prevalence and DR patients with lack of Funbwa leaves, antioxidants, TAOS and incidence of retinopathy in non-diabetics and T2DM.	208
Moshetova *et al.,* (Russia)	2014	A study on outcome of the use of antioxidant and angioprotective mediators in T2DM patients with DR and age-related macular degeneration	To examine the change in clinical function and morphological parameter of the retina of T2DM before and after a course of antioxidant and angioprotectors in a therapy form.	180
Kundu *et al.,* (India)	2014	A study on oxidative stress in diabetic patients with retinopathy	To investigate and correlate oxidative stress marker (MDA) and antioxidant accompanied by blood glucose (HbA1c and FPG)in DR patients and patients without DR.	150

Lekishvili, 2014, (Germany)	2014	A study on the effects of C-taurine antioxidant complex on biochemical blood markers in the process of treatment of patients with T2DM with NPDR.	To examine the influence of C-taurine complex on blood biochemical markers in treating T2DM patients with DR.	68
Tanaka *et al.*, (Japan)	2013	A study on the intake of fruit and incident of DR withT2DM.	To investigate the relation between fruit intake and related nutrients and the occurrence of DR.	978
Nebbioso *et al.*, (Italy-Rome)	2012	A study on oxidative stress in preretinopathic diabetes subjects and antioxidants.	To assess the effectiveness of an oral antioxidant treatment in decreasing oxidative stress and diminishing damage to retinal neurons.	32
Lam *et al.*, (China)	2011	A study on the relationship among DR, antioxidants and glycemic control.	To ascertain a biomarker outline that will detect risk of DR or those in the primary stages.	420
Li *et al.*, (China)	2010	A study on serum lycopene levels in patients with DR.	To examine the change in serum lycopene between TDM patients with or without DR.	94
Brazionis *et al.*, (Australia)	2009	A study on plasma carotenoids and DR.	To assess the correlation between plasma carotenoids and DR.	111
Kumari *et al.*, (India)	2008	A study on Plasma MDA and antioxidant vitamins in DR.	To assess the oxidative stress status and serum vitamin antioxidant levels in DR cases and their severity.	86

Gupta and Chari, (India)	2005	A study on lipid peroxidation and antioxidant status in T2DM patients with DR.	To assess the role of oxidative stress and its relation to hyperglycemia and duration inT2DM with or without DR.	132
Millen *et al.*, (USA)	2004	A study on the association between intake of vitamin C and E and risk of DR in atherosclerosis risk in communities study.	To investigate the association between the occurrence of DR and intake of vitamin C and E.	1353
Millen *et al.*, (USA)	2003	A study on the relations of serum ascorbic acid and α-tocopherol to DR in the Third National Health and Nutrition Examination Survey.	To study the association between serum ascorbic acid and α-tocopherol concentration and incidence of DR.	988
Gurler *et al.*, (Turkey)	2000	A study on the role of oxidative stress in DR.	To examine the function of oxidative stress in the development of DR.	85

APPENDIX B

QUESTIONNAIRE

QUESTIONNAIRE FOR DIABETIC PATIENTS AT CAPE COAST TEACHING HOSPITAL

I am Fredericka Serwaa Otoo, a student of Kwame Nkrumah University of Science and Technology; I am conducting a research on the topic **"Nutrient Intake, Antioxidant Micronutrient Status and Vision Disorders among Diabetics in cape coast: central region of Ghana"** The information obtained from this questionnaire is solely for research purposes and confidential. Please kindly provide answers to all the questions to the best of your ability.

A. Personal/socio-demographic data

1. Date of interview _____ 2. Name; _____ 3. Sex; M ( ) F ( )

4. Code of participant:_____ 5. Age: _____

6. Education: _____

None ( ) Primary ( ) Junior High ( ) Senior High ( )

7. Marital status: _____

Single ( ) Married ( ) Widowed ( ) Divorced ( )

8. Religion: _____

9. Ethnic origin: _____

Ashanti ( ) Dagomba ( ) Fante ( ) Ga ( )

Other (specify) …….

11. Occupation

Farming ( ) Trading (Business) ( ) Hairdresser ( ) Tailoring ( )

Student ( ) Other Specify ……

12. Residential Address: _____ 12a.Contact number:_____

12b. Where is your house close to (any landmarks)? ……

Tick in box

B. Medical History

13. How long have you had diabetes? 1-5 ☐ 6-1 ☐ 11-15 ☐ 16-20 ☐ 21 above ☐

14. What type of diabetes mellitus do you have?

Type 1 ☐ Type 2 ☐

15. Which type of treatment regimen are you on?

Medication ☐ Diet and physical activity ☐

16. If you are on medication, which type do you use?

Oral ☐ Insulin ☐ Both ☐

17. Did you take any herbal medications? Yes ☐ No ☐

a. If yes, for how long ……. 1-2 ☐ 2-5 ☐ 5-7 ☐ 7-10 ☐

b. Did the herbal medication help in reducing the blood sugar? Yes ☐ No ☐ not really ☐

18. Do u do any physical activity? Yes ☐ No ☐

a. If yes, which type of physical activity? Aerobic ☐ Resistance ☐ strengthening ☐

b. How many times does u do it in a week? ☐ 1 time ☐ 2 times ☐ 3 times ☐ 4 times ☐ other… (Specify)

Tick in box ☐

- **Diet management**

18. Do you take fruit in your daily meals? ☐ Yes ☐ No

19. How many times do you eat fruit(s) per day? ☐ 1 time ☐ 2 times ☐ 3 times ☐ 4 times ☐other… (Specify)

20. How often do you take fruits? ☐ Daily ☐weekly ☐Monthly ☐Occasionally ☐ never

20. Do you add vegetables in your daily meals? ☐ Yes ☐ No

21. How many times do you eat vegetables a day? ☐ 1 time ☐ 2 times ☐ 3 times ☐ 4 times ☐ other… (Specify)

22. How often do you consume vegetables? ☐ Daily ☐ weekly ☐ Monthly ☐ Occasionally ☐ never

23. Do you take alcoholic drink? ☐ Yes ☐ No

Level of awareness of Retinopathy

24. Do know diabetes can affect your vision? ☐ Yes ☐ No

25. Have you had diabetes education on diabetic visual impairment before? ☐ Yes ☐ No

26. Do you know diabetes patient is at risk of visual impairment? ☐ Yes ☐ No

26. Do you know any of the following visual impairment among diabetics?

Blurred vision ☐ low visual acuity ☐ blindness

27. What do you think is the cause of diabetic visual impairment? (You can select more than one)

☐ Hereditary ☐ Aging ☐ diabetes ☐ diabetes medication ☐ low intake of fruits and vegetables ☐ I don't know

28. Do you think consumption of fruit and vegetables can reduce your risk of retinopathy? ☐ Yes ☐ No ☐ I don't know.

Section C: Anthropometrics

Weight(kg)	
Height(m)	
BMI(kg/m^2)	
BP(mmHg)	

Section 3: Biochemical Assessment

Blood glucose levels and Antioxidant Assessment

Blood markers	**Serum Concentration**
Glycated haemoglobin (HbA1c)	
Fasting blood sugar (FBS)	
Micronutrient	
Vitamin A	
Vitamin C	
Total Antioxidant Status	

Section 4: Dietary Assessment

Three (3) days 24 hour recall

Name			
Identification number & Date			
DIRECTIONS: The questionnaire is a 24-hour dietary recall to assess current dietary intake of foods**.**			
Day 1: Menu and Time	Description of Food and beverages	Estimated Portion size	Weight (Kg) or Volume (mL)
Breakfast Time:			
Mid-morning snack Time:			
Lunch Time:			
Mid-afternoon snack Time			
Supper Time:			
Bedtime snack Time			
Day 2: Menu and Time	Description of Food and beverages	Estimated Portion size	Weight (Kg) or Volume (mL)
Breakfast Time:			
Mid-morning snack Time:			
Lunch Time:			
Mid-afternoon snack Time			

Supper Time:			
Bedtime snack Time			
Day 3: Menu and Time	Description of Food and beverages	Estimated Portion size	Weight (Kg) or Volume (mL)
Breakfast Time:			
Mid-morning snack Time:			
Lunch Time:			
Mid-afternoon snack Time:			
Supper Time:			
Bedtime snack Time:			

Section 5: Dietary Assessment

Food Frequency Questionnaire

Foods consumed	Frequency of consumption				
	[1] Daily	[2] 1-3 times/ week	[3] Monthly	[4] Occasionally	[5] Not at all
Fruits					
Green leafy vegetables(alefu,Kontonmire, ayoyo, bokoboko					
Non-green leafy vegetables(carrots, tomatoes, garden eggs)					
Legumes (such as beans, nuts) and Oils (coconut oil, soy oil, sunflower oil, olive oil, groundnut oil , flax seed oil and palmnut oil)					
Oily fishes					
Meat (beef, shevon, cowleg, game)					
Poultry					
Vitamin supplement					

THANK YOU!!!

APPENDIX C

OCULAR EXAMINATION FORM FOR DIABETIC PATIENTS

I am Fredericka Serwaa Otoo, a student of Kwame Nkrumah University of science and Technology. I am conducting a research on the topic **"Nutrient Intake, Antioxidant Micronutrient Status and Vision Disorders among Diabetics in cape coast: central region of Ghana"** The information obtained from this examination is solely for research purposes and confidential.

Patient's name ……. ID Code …….

Age ……. Sex …….

Type of Diabetes (tick) Type 1 ☐ Type ☐ Gestational ☐ Others ☐

Duration of Diabetes ……. Visual Acuity ……

Diabetic Visual Impairments	**Right eye**	**Left eye**
Refractive Error		
Cataract		
Dry eye syndrome		
Diabetic Retinopathy and its Grades		
R0 – No retinopathy		
R1- Mild and moderate NPDR		
R2 – Severe NPDR		
R3 – Proliferative diabetic retinopathy		
Diabetic Maculopathy and Diabetic macular edema		
M0 – No Maculopathy		
M1- Maculopathy Centre involved		
U - Unobtainable/Ungradable		

Other Findings ……

……

……

Signature ……..

APPENDIX C

Consent Form Used For the Study

Participant Information Leaflet and Consent Form

This leaflet must be given to all prospective participants to enable them know enough about the research before deciding to or not to participate

Title of Research: Nutrient Intake, Antioxidant Micronutrient Status and Vision among Diabetics in attending Cape Coast Teaching Hospital: central region of Ghana

Name(s) and affiliation(s) of researcher(s): This study is being conducted by Dr. Reginald Annan and Fredericka Serwaa Otoo of the Kwame Nkrumah University of Science and technology, Kumasi, Ghana

Background (Please explain simply and briefly what the study is about):

Diabetes mellitus is a chronic challenging disease in the healthcare system of Ghana. Uncontrolled Diabetes mellitus can lead to complications which form major cause of morbidities resulting in poor quality of life and death among people with diabetes. Diabetes is known to affect visual function before the onset of retinopathy (diabetic retinopathy (DR). Vision impairment such as cataract, dry eye syndrome, refractive errors, low visual acuity, retinopathy and others are found in diabetic patients. Good glycaemic control and dietary antioxidants micronutrients such as vitamin A, C and E, are growing evidences of nutritional intervention for prevention of diabetes complications such as retinopathy and other diabetic eye diseases. The study seeks determine relationship between dietary patterns, serum level of antioxidant micronutrients and the prevalence of visual impairments among diabetics attending Cape Coast teaching hospital.

Purpose(s) of research: The purpose of this research is to form part of the assessment leading to the award of MPhil Human Nutrition and Dietetics.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

The research will randomly select both Type 1 and type 2 diabetic patients from Diabetic Clinic at Cape Coast Teaching Hospital. Each participant will undergo questionnaire interview after giving consent to partake in the study. The participants will have their weight and height using weighing a scale and stadiometer respectively, blood pressure(Bp) using a Bp apparatus , and fasting blood sugar (FBS) using a glucometer, all checked at the diabetic clinic of Cape Coast Teaching Hospital. Venous blood sample will be taken from each participant at biochemistry laboratory at Cape Coast Teaching Hospital for clinical analyses of glycated haemoglobin, serum vitamin A, C and E, and serum total antioxidant capacity (TAC). Again, an eye examination (visual acuity, dilation of pupil and funduscopy) will be conducted to screen for the various visual impairments. In total, the study will recruit 120 participants from Cape Coast Teaching Hospital.

Risk(s): There are no serious risks if participant(s) exclude(s) or partake in the study.

Participants may experience a little pain when blood is being drawn and eyes are being checked.

Benefit(s): Participants will receive nutritional education and counseling.
Results obtained will be used by healthcare professionals to improve on nutritional intervention strategies of diabetes which participants will benefit from. Participant will also know their vision status and any anomalies found will be treated.

Confidentiality: Identification number will be used to replace participants' names. The results obtained from the study will be held private. The names of the participants will be anonymous to the public if the study is published.

Voluntariness: Participation in this study is voluntary and participants are free to suspend their participation when they wish to. However, they would be advised to fully be involved since this is a relevant study and their health.

Alternatives to participation: If you choose not to participate, this will not prevent you from receiving treatment and care in this hospital

Withdrawal from the research: You may choose to withdraw from the research at any time without having to explain yourself.

Consequence of Withdrawal: There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study.

Costs/Compensation: For your time and inconvenience we will compensate you with white handkerchief and Fruit (apples) snacks to show appreciation for your participation.

Contacts: If you have any question concerning this study, please do not hesitate to contact Dr. Annan and Miss. Fredericka Serwaa Otoo on 0201237169 and 0546529090 respectively.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman

Committee on Human Research and Publication Ethics

Kumasi

Tel: 03220 63248 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to _____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

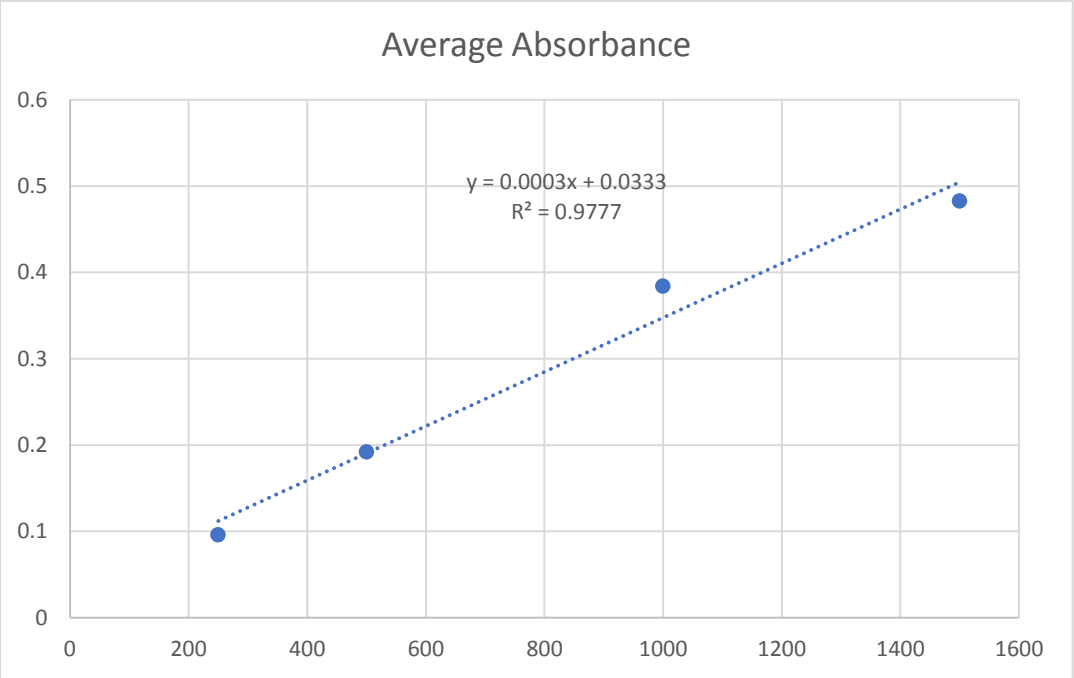
MOTHER'S SIGNATURE (maintain if participant is under 18 years): _____

MOTHER'S NAME: _____

FATHER'S SIGNATURE (maintain if participant is under 18 years): _____

FATHER'S NAME: _____

Calibration curve for serum vitamin A using Elisa kits.



Calibration curve for serum vitamin C using Elisa kits

Cross-Tabulation of Glycemic Control by Gender and Age Ranges

Parameters	Normal (%)	High (%)	P-value
Gender			0.726
Male	15.8	84.2	
Female	13.6	86.4	
Age Ranges			0.184
35-45	12.5	.87.5	
46-55	16.7	83.3	
56-65	5	95	
66-75	26.3	73.7	
76-85	33.3	66.7	

Categorical data are presented as percentages. The table above compares glycaemic control by gender and age groups of respondents. "Normal" and "High" represents blood sugar levels.

Correlation between dietary nutrients intake and Blood glucose levels

	HbA1c	FBG
Nutrients	**r (P-value)**	**r (P-value)**
Kcal	0.032 (0.749)	0.011(0.916)
Protein (g)	0.058 (0.57)	0.130 (0.197)
Fat (g)	0.032 (0.754)	0.018 (0.857)
CHO (g)	0.066 (0.511)	0.005 (0.96)
Sugar (g)	0.064 (0.524)	0.108 (0.286)
Fibre (g)	0.034 (0.736)	0.005(0.964)
Zinc (g)	0.100 (0.322)	0.071(0.486)
Selenium (g)	0.121 (0.231)	0.176 (0.079)
vitamin C	0.053 (0.603)	0.038 (0.71)
Vitamin A	0.078 (0.44)	0.104 (0.303)
Vitamin E	0.012 (0.903)	0.085 (0.402)

Mean intake of Macronutrients and Dietary antioxidant Micronutrients according to age groups.

Nutrients	35-45y	46-55y	56-65y	66-75y	76-85y	Pvalue
Kcal	1562.65±450.3	1443.24±522.8	1520.2±451.9	1308.1±460.9	1239.2±267.8	0.460
Protein (g)	65.01 ± 22.9	55.3 ± 16.6	55.3 ± 18.3	52.2 ±16.0	48.0 ± 10.2	0.474
Fat (g)	50.4±15.7	47.7 ± 26.3	47.3 ± 24.2	37.4 ±18.0	26.1 ± 5.0	0.266
CHO(g)	218.8 ± 66	199.7 ± 65.9	224.2 ± 64.0	188.3 ± 76.0	210.6±61.8	0.340
Fibre (g)	21.1±6.2	21.7±9.0	20.5 ± 8.6	18.0 ± 6.2	19.9±5.5	0.659
Sugar (g)	32.6 ± 16.0	32.7±19.9	45.3±25.0	34.5±21.9	51.1± 21.1	0.110
Zinc (mg)	6.9±2.3	6.9±3.0	7.1 ± 3.4	5.6±2.1	5.1±2.0	0.382
Selenium(mcg)	111.7±40.9	86.7±27.1	92.2±32.5	85.3±30.6	88.3±31.0	0.320
Vitamin C(mg)	92.4±31.3	84.1± 47.9	79.3±36.6	67.7±27.8	88.9±29.1	0.516
VitA (mcg)	160.8±55.9	186.3± 169.4	202.2±196.0	129.8±54.8	146.6±42.8	0.568
VIT E (mg)	6.4±3.3	5.7±3.1	5.7±3.2	4.4±1.8	3.9±1.4	0.339

Data presented are the results of two 24hour recalls. Continuous data were presented as means. Continuous data were compared using Anova.

Comparison of nutrients status and glycaemic control.

Nutrients	HbA1c		
Protein	**Normal (%)**	**High (%)**	**P-value**
Inadequate	35.7	8.1	0.899
Adequate	64.3	91.9	
CHO			
Inadequate	7.1	39.5	0.786
Adequate	92.9	60.5	
Fibre			
Inadequate	76.9	68.6	0.543
Adequate	23.1	31.4	
Zinc			
Inadequate	78.6	80.2	0.885
Adequate	21.4	19.8	
Selenium			
Inadequate	0	16.3	0.104
Adequate	100	83.7	
Vitamin C			
Inadequate	50	57	0.626
Adequate	50	43	
Vitamin A			
Inadequate	100	95.3	0.410
Adequate	0	4.7	
Vitamin E			
Inadequate	100	100	-
Adequate	100	100	-

ETHICAL LETTER

CAPE COAST TEACHING HOSPITAL
ETHICAL REVIEW COMMITTEE

In case of reply the reference number and the date of this Letter should be quoted

Our Ref.: CCTH

Your Ref.:

P. O. Box CT.1363
CapeCoast
Tel: 03321-34010-14
Fax: 03321-34016
Website: www.ccthghana.org
email: info@ccthghana.com

7th February 2017

Fredericka Serwaa Otoo
Department of Biochemistry
KNUST
Kumasi

Dear Ms. Otoo,

ETHICAL CLEARANCE – REF: CCTHERC/RS/EC/2017/1

The Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC) is glad to inform you that you have been granted provisional approval for implementation of your research protocol titled, "**Assessing Relationship Between Dietary Patterns, Serum Levels of Antioxidant Micronutrients and the Prevalence of Retinopathy Among Diabetics**".

The ERC requires that you submit periodic review of the protocol and a final full review to the ERC on completion of the research. The CCTHERC may observe or cause to be observed procedures and records of the research during and after implementation.

Please note that any modification of the project must be submitted to the CCTHERC for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the CCTHERC within fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithfully,

DR. AARON OFFEI
CHAIRMAN