# UNIVERSITY OF GHANA
# COLLEGE OF HEALTH SCIENCES
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## LEVELS OF ANTI-INSULIN ANTIBODIES IN PATIENTS WITH DIABETIC RETINOPATHY ATTENDING NATIONAL DIABETES RESEARCH AND MANAGEMENT CENTRE IN ACCRA

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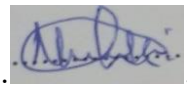
## DECLARATION

I, Bismark Nluki Mohammed, hereby declare that this work was carried out by me under the supervision of Dr. Seth Amanquah, Dr. Amissah Arthur. No previous submission on the topic has been made to this University or any other institution. Related work by others has been duly acknowledged by references to others.



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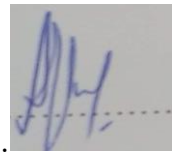

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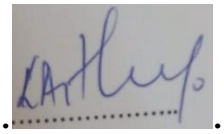

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Dr. Amissah Arthur) Date

(Supervisor)

## DEDICATION

This thesis is dedicated first and foremost to the Almighty God for the strength, health and wisdom bestowed on me. I also dedicated the work to my lovely supervisors for the care andlove they have for me and all my family.

## ACKNOWLEDGEMENT

The success of this thesis is based on the contribution of many people who contributed in many ways and deserve to be acknowledged. Without the involvement of the Most High, the success of this study would have been a mirage. My profound gratitude goes to my supervisors who with patience and love shared part of their productive to be with me to carry out the research, the correction of the errors of the work and to say to it all the material support given me. Indeed I owe them a huge dept of gratitude. I owe Dr. Emmanuel K. Ofori, Prof. Henry Asare-Anane, thestaff of the department of chemical pathology an appreciation for the wisdom, and rich knowledge they impacted onto me. My appreciation will be incomplete without extending it to friends and course mates: Christian Major Doran, Dr. Botweh and others.

## ABSTRACT

**Background:** Diabetes mellitus (DM) is one of the most common chronic diseases worldwide, and has attained epidemic status the past few decades especially in middle and low income countries. Diabetic retinopathy (DR) has become the most common microvascular complication of diabetes, and remains a leading cause of visual impairment and blindness in the working-age population in the developing world. Anti-insulin antibodies are glycoprotein molecules produced by the immune system and play a significant role in the body‘s defense against pathogens. These immuno-globulins, especially IgE, may be responsible for allergies by increasing the porosity of the blood retinal barrier causing leakage, pericyte and endoththelial cell loss and retinal neovascularization. Immunoglobulin G (IgG) may also be responsible for insulin resistance by upregulation of inflammatory molecule expressions, promoting leakostasis and increasing vascular permeability in retina.

**Aim**: The aim of the study was to evaluate the relationship between anti-insulin antibodies and subjects with diabetic retinopathy.

**Methodology:** This was a case-control study involving 90 individuals – forty (40) diagnosed with diabetic retinopathy and twenty-five (25) each of individuals with diabetes mellitus and apparently healthy non-diabetics serving as controls recruited at the National Diabetic Research Centre and the eye unit of the Korle-bu Teaching Hospital (KBTH). These individuals were interviewed using a standard questionnaire. Five milliliters (5mls) of venous blood was taken from participants, following standard procedures, and transported to the Department of ChemicalPathology Research Laboratory for biochemical analysis. With the aid of the Statistical Products and Services Solutions (SPSS), version 25 software, the data obtained were summarized using descriptive statistics (means, standard deviations, and proportions) and further analyzed at a 0.05 alpha level using one-

way between-groups analysis of variance (ANOVA). Associations between variables were determined using Pearson‘s product-moment correlations.

**Results:** In general, with the exception of the control group, which had a higher proportion of its participants being males (60%, $n$ = 15), most of the participants were females in both the retinopathy (65%, $n$ = 26) and diabetes (76%, $n$ = 19) groups. Higher levels of IgE and IgG concentrations were observed to be higher in diabetic retinopathy subjects than those of the diabetic without retinopathy group ($p< 0.05$). Furthermore, the factors that had significant associations with anti-insulin IgE and IgG antibodies were: fasting blood glucose, occupation, age, gender, and being on medications ($p< 0.05$ respectively). Being on nifedipine medication ($r = -0.32$, $p = 0.04$) had a significant negative correlation with the levels of anti-insulin IgE antibodies, whiles being on metformin medication ($r = 0.32$, $p = 0.04$) had a significant positive relationship with the levels of anti-insulin IgG antibodies.

**Conclusion:** Among the study participants sampled, neither diabetes nor diabetic retinopathy influenced the levels of anti-insulin IgE and IgG antibodies. Furthermore, the factors that had significant associations with anti-insulin IgE and IgG antibodies were: fasting blood glucose, occupation, age, gender, and being on tropicamide, methyldopa, and Phenylephrine medications.

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## LIST OFABBREVIATION

DM ..... Diabetes Mellitus
DR ..... Diabetic Retinopathy
Ig ..... Immunoglobulin
IgE ..... Immunoglobulin E
IgG ..... Immunoglobulin G
AIA ..... Anti-Insulin Antibody
KBTH ..... Korle-Bu Teaching Hospital
SPSS ..... Statistical Products and Service Solution
ANOVA ..... Analysis Of Variance
LADA ..... Latent Autoimmune Diabetes of Adults
MODY ..... Maturity Onset Diabetes Of The Young
GDA ..... Global Diabetes Association
ADA ..... American Diabetes Association
MHC ..... Major Histocompactibility Complex
HLA ..... Human Leucocyte Antigen
WHO ..... World Health Organization
HP ..... Human Placental Lactogen
USD ..... United State Dollar
AACC ..... American Association For Clinical Chemistry
TFS ..... Thermo Fishers Scientific
GDC ..... Global Diabetes Community
WLA ..... Wiki Loves Africa
IRI ..... Immune Reactive Insulin
BRB ..... Blood Retina Barrier
GAD ..... Glutamic Acid Decarboxylase
ICA ..... Islet Cell Antibody
DMICC ..... Diebetes Mellituus Interagency Coordinating Committee
DKA ..... Diabetic Keto Acidosis
HONK ..... Hyperosmolar Non Ketotic Coma
MFMER ..... Mayo Foundation for Medical Education and Research

HHS..... Hyperosmolar Hyperglycaemic State

NPDR.....Non Proliferative Diabetic Retinopathy

PDR..... Proliferative Diabetic Retinopathy

DME.....Diabetic Macular Edema

BMI..... Body Mass Index

BP..... Blood Pressure

FPG ..... Fasting Plasma Glucose

GOX..... Glucose Oxidase

POD.....Peroxidase

ELISA .....Enzyme Linked Immunosorbent Assay

HRP.....Horseradish Peroxidase

mmol/ L.....Millimol per litre

mL..... Milliliter

SD .....Standard Deviation

SBP ..... Systolic Blood Pressure

DBP.....Diastolic blood pressure

µL.....Microliters

Kg/m2 .....Kilogram per meter square

IDA ..... International Diabetes Association

GLU ..... Glucose

GLUT 4..... Glucose Transporters 4

FDA..... Food and Drug Administration

NDMRC..... National Diabetic Management and Research Centre

Ab..... Antibody

Nm.....Nanometer

Fab..... Fragment Antigen-Binding

mmHg .....millimeter mercury

cm.....centimeter

β-cell .....Beta Cell

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Diabetes mellitus (DM) is a syndrome or constellation of disorders characterized by hyperglycemia either immune-mediated (type 1diabetes), insulin resistance (type 2), gestation, infections and certain drugs (Baynes, 2015). Pouya *et al*. (2019) estimated that 578 million people will have diabetes by the year 2030. Diabetes mellitus is classified into three major types, namely primary diabetes mellitus, gestation diabetes, and secondary diabetes (Baynes, 2015). The primary diabetes mellitus consists of type 1 DM, latent autoimmune diabetes of adults (LADA), type 2 DM, and maturity onset diabetes of the young (MODY) (Kerner *et al.,* 2014; American Diabetes Association (ADA), 2014; WHO, 2019; Baynes, 2015).

Type 1 DM is caused by autoimmune destruction of insulin-producing cells in the pancreas by CD4+ and CD8+ T cells and macrophages infiltrating the islets (American Diabetes Association (ADA), 2014; Baynes, 2015 and Nora, 2017). The molecular features of type 1 diabetes mellitus as an autoimmune disease results in immuno-competent and accessory cells in infiltrated pancreatic islets, association of susceptibility to disease with the class II (immune response) genes of the major histocompatibility complex (MHC), human leucocyte antigens (HLA), and presence of islet cell-specific autoantibodies (Baynes, 2015).

Latent autoimmune diabetes of adults (LADA) involves autoimmune destruction of insulin-producing cells, but the process occurs so slowly that insulinopenia does not appear until the patient is middle-aged (ADA, 2014; Baynes, 2015; Nora, 2017; WHO, 2019).

Type 2 DM accounts for 80–85% of all cases of DM worldwide (Ryran *et al*., 2010; Baynes, 2015; Nora, 2017), and is the fastest growing group, primarily because of its association with

obesity, wrong dietary habits, alcohol consumption, and lack of exercise (ADA,2014; Kerner *et al*., 2014; WHO, 2019). Diminished tissue sensitivity and impaired B-cell function have been suggested as aetiological factors.

Maturity onset diabetes of the young (MODY) is a monogenic disorder characterized by a diabetes that occurs in late childhood or before the age of 25 as a result of a partial defect in glucose-induced insulin released ( American Diabetes Association (ADA), 2014; Baynes, 2015; Nora, 2017; WHO, 2019).

Gestation diabetes is diabetes that appears during pregnancy; it accounts for 2–4% of all cases of diabetes, and occurs in about 5% of pregnancies (ADA, 2014; Kerner *et al*., 2014; Nora, 2017). Pregnancy for non-diabetic women is diabetogenic, hormones produced by the placenta innormal pregnancy, especially human placental lactogen (HPL) and oestrogens induce a degree ofinsulin resistance to make relatively more glucose available to the developing foetus (Kerner *et al.*, 2014; Baynes, 2015; Nora, 2017).Pregnant women who cannot mobilize enough additional insulin to overcome the insulin resistance develop gestation diabetes. Gestation diabetes causes high blood sugar that can affect pregnancy and the baby`s health (ADA, 2014; Kerner *et al*., 2014; Baynes, 2015).

Secondary diabetes is diabetes caused by other diseases, such as pancreatic disease, endocrinopathies, drugs, and infections (Kerner *et al*., 2014; Baynes, 2015; Nora, 2017; WHO, 2019). Diabetes mellitus is a long-standing condition that can result in several aggravations over a period (Shashikala *et al.*, 2014; WHO, 2019). These aggravation complications are microangiopathy and macroangiopathy (Shashikala *et al.*, 2014). Many of these aggravations produce no signs and symptoms in the initial stages and most can be avoided or reduced with a

combination of standard medical care and regular blood glucose monitoring (Shashikala *et al.*, 2017).

Diabetic retinopathy (DR) has become the most common microvascular complication of diabetes mellitus, and it continues to be a leading cause of blindness and visual deterioration in the working-age population in developing countries (Baynes. 2015; Nora, 2017; Shashikala *et al*., 2017). Diabetic retinopathy arises as a result of injury to the microvasculature of the retina due tochronic exposure to the metabolic changes caused by diabetes (Jackson *et al.*, 2012; ADA, 2014; Shashikala *et al*., 2014). The two main types of DR are the less-severe form, non-proliferative DR, and the severe form, proliferative DR (PDR). Non proliferative DR is characterized by microaneurysms, superficial and deep retinal hemorrhages, hard exudates, and macular edema (ADA, 2014). Subsequent PDR involves the formation of new blood vessels (angiogenesis) in the retina that may cause scarification of the retina and vitreous (ADA, 2014; Nora, 2017).

Recent data suggest that the prevalence of DR may be reducing in the United States (ADA,2010; Burges *et al*., 2013). The launch of the VISION 2020 initiative on 18$^{th}$ February, 1999 from the Geneva Press Club in Switzerland, the free surveillance programs, intensified risk- factor control in response to the results of randomized controlled trials, and continuing improvement in health care systems have been implicated in the decreasing rates of DR in some developed countries (Burges *et al.*, 2013). However, in developing countries, such as Ghana, the launch of the VISION 2020 initiative has not been fully implemented. Although there are several articles relating to prevalence of diabetes mellitus and retinopathy, studies from Africa especiallyin Ghana are limited and do not mirror this findings.

An antibody (Ab) is immunoglobulin (Ig), Y-shaped proteins mainly secreted by plasma cells also known as differentiated B cell. These antibodies are used by the adaptive part of the immunesystem to neutralize, agglutinate, opsonize and also promote the release of inflammatory molecules to destroy pathogens such as pathogenic bacteria, virus and parasites (Ghose, 2020). Antibody recognizes a unique molecule of the pathogen called an antigen, through the fragment antigen-binding (Fab) of the variable region. Antibody paratope is specific for one particular epitope on an antigen, allowing these two structures to bind together with precision. Mechanism of binding, allows antibody to tag a microbe or an infected cell for attack by other parts of the immune system or opsonize, neutralize or agglutinate its target directly (Ghose, 2020). The present study sought to evaluate levels of anti-insulin antibodies among subjects with diabetic retinopathy. Findings may contribute to the formulation of new management policies on diabetes and associated complications such as retinopathy in Ghana.

### 1.2 Problem statement

Diabetic retinopathy is the most common complication of diabetes and the leading cause of blindness among the working-age population in Africa (Gatimu *et al.*, 2016). Worldwide, the prevalence of diabetes and DR is increasing at an alarming proportion, and Ghana is no exception (Pouya *et al.*, 2019). It is a priority area for the VISION 2020 programme –the right to sight is designed to eliminate avoidable and treatable blindness by the year 2020‖ (WHO, 2016). The increasing prevalence of DR in Africa is ranged between 7.0 and 62.4% (Burgess *et al.*, 2013). Screening and prompt treatment of diabetic retinopathy are not top priorities in many countries of the world, because the impacts of other causes of preventable blindness remain an issue (WH0, 2016). In Ghana, there is an increase in visual complications and blindness burden due to diabetic retinopathy (Akpalu *et al.*, 2011). The National Health Insurance Scheme spends

an average 2,500–5000 USD per annum on diabetes management, and the cost is expected to be much higher for DR (Akpalu *et al.*, 2011). The few treatment facilities for DR are in the urban centers, and are expensive. The cost of laser treatment is 100 America dollars per eye at Korle- Bu Teaching Hospital Eye- Unit price list, 2019. Diabetic retinopathy brings about substantial economic loss to people with diabetes and their families and to health systems and national economies through direct medical costs and loss of work and wages (WHO, 2016).

Quality of social life of individuals living with diabetic retinopathy is reduced due to their poor participation in social gatherings, such as conferences, meetings, seminars and income generation. Changes in anti-insulin antibodies levels may be implicated in diabetic complications, and this further reduces productivity, and may even increase mortality rates (Rodriguez-Segade *et al*., 1996). Elevated levels of some antibodies, which may serve as early signs in the aetiogenesis of diabetic complications have however not been demonstrated in Ghana.

### 1.3 Justification

Prevalence of diabetic retinopathy in sub-Saharan Africa is very high (7.0 to 62.4%) according to a prior study by Burges *et al.* (2013). There is a need to reduce complications and contribute to the reduction of the burden of diabetes-related morbidity and mortality in Africa. Anti-insulin antibodies affect the efficacy of insulin when bound to the insulin receptor. The binding of anti-insulin antibody to the insulin receptor leads to hyperglycemia, which may interfere with the insulin signaling pathway (Jackson *et al.*, 2012 and Ryan *et al.*, 2010).

Anti-insulin antibodies are biomolecules produced by the immune system. Their levels are determined routinely in clinical practice to provide important information on the humoral (immunoglobulins) immune status of patients. Studies in Spain have shown that increased

concentrations of some circulating anti-insulin antibodies may be nonspecific signs of the development of diabetic complications (Rodriguez-Segade *et al.,* 1991; Rodriguez-Segade *et al.,* 1996). The determination of anti-insulin antibody levels in serum of subjects with diabetic retinopathy from the study using Enzyme-linked immunosorbent assay (ELISA) is important for assessing the extent to which these levels have changed especially immunoglobulin E (IgE) and immunoglobulin G (IgG). Enzyme-linked immunosorbent assay is an important diagnostic tool in this research because of its role in detection of antigens and antibodies present in a sample with high sensitivity and high specificity (Moore, 2020; Sakamoto *et al.,* 2017). ELISA also offers more accuracy, does not require radioisotopes or counter, is efficient, reliable and cost- effective compared to other techniques such as radioimmunoassay (RIA). In contrast, ELISA demonstrates the following disadvantages; labor-intensive and expensive to prepare antibody because it is a sophisticated technique. There is also the high possibility of false positive or negative results due to insufficient blocking of the surface of microtiter plate (Sakamoto *et al*., 2017). Taking together, the advantages of ELISA method outweighs the disadvantages, makingit a more preferred biotechnical tool for measurement of antibody levels in serum.

Quantification of IgG and IgE levels in serum of DR subjects could lead to further important information about immune dysfunction in these subjects. This could inform policy makers in the formulation of new management policies on diabetes mellitus and associated complications. It may also help determing the cause of the disease and the possible preventive measures that will help combat this disease burden. The formulation of new management policies on diabetes mellitus and associated complications will facilitate substantial reduction of preventable and treatable blindness and economic loss to people and families with diabetes.

### 1.4 Hypothesis

There is no relationship between anti-insulin antibody levels among diabetic and non-diabetic subjects in Ghana.

### 1.5 Aim

The aim of the study was to evaluate the relationship between anti-insulin antibodies and subjects with diabetic retinopathy.

### 1.6 Specific objectives

The specific objectives of the study were:

- To determine whether individuals without diabetes, individuals with diabetes without retinopathy, and individuals with diabetic retinopathy differ with regard to levels of anti-insulin IgE and IgG antibodies.
- To investigate associations between participants‘ features of each study group and levels of anti-insulin IgE and IgG antibodies

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 DIABETES MELLITUS

Diabetes Mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins (Baynes, 2015). Diabetes mellitus is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago (Sazid *et al*., 2017). In the year 1936, the difference between type 1 and type 2 DM was distinctly made. Type2 diabetes mellitus was described as a component of metabolic syndrome in 1988 (Awad *et al.,* 2019 and Deepthi *et al.,* 2019). The origin and etiology of DM can vary but always include defects in either insulin secretion or response or in both (Nora, 2017). Mostly patients with diabetes mellitus have either type 1 diabetes or type 2. Type 1 may be immune-mediated or idiopathic. Type 2 DM also known as non-insulin dependent, is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency (Baynes,2015**) .** Type 2 DM results from interaction between genetic, environmental and behavioral risk factors.

The improvement in outcomes for individual patients with diabetes has not resulted in similar improvements from the public health perspective (Chen *et al*., 2014 and Pouya *et al*., 2019). This is because; worldwide prevalence of diabetes has continued to increase dramatically. Globally, as of 2019, an estimated 463 million people had DM, with type 2 making up about 90% of the cases (Pouya *et al.,* 2019). The number of people with type 2 DM is increasing in every country with 80% of people with DM living in low- and middle-income countries (Chen *et al*., 2014).

Increase in prevalence in both rural and urban setting, and affecting both gender proportionally. This means that prevalence of diabetes in both males and females living in both rural and urban communities worldwide is increasing at alarming rate (Pouya *et al*., 2019). According to the International Diabetes Federation Africa Region in 2019, prevalence of diabetes was 3.9% with 60% of adults living with diabetes unaware of the condition.

Although type 2 DM is widely diagnosed in adults, its frequency has markedly increased in the pediatric age group over the past two decades (American Diabetes Association (ADA), 2000). The prevalence of type 2 DM in the pediatric population is higher among girls than boys, just asit is higher among women than men (Craig *et al.,* 2014).This variation is due to the fact that the hormones oestrogen, Human placental lactogen present in females in large amount (oestrogen) and testosterone present in males play important roles in regulation of insulin function. Type 2 DM develops only if inadequate beta-cell function is associated with other risk factors (Soltesz *etal.,* 2007).

**2.2 INSULIN**

Insulin is a hormone secreted by the β-cells in the pancreas (Shashikala *et al*., 2014, Nora *et al*., 2014, Ryan *et al*., 2010 and ADA, 2014). Insulin is Monomeric biologically active molecule which is made up of two long amino acid chains or polypeptide chains (Ananya, 2019). The chains are chain A with 21 amino acids and chain B consists of 30 amino acids. There are two disulfide bridges covalently bond the chains. Chain A contains an internal disulfide bridge(Ananya, 2019). Insulin reduces blood glucose concentration, increases cell porosity to monosaccharides, amino acids and fatty acids (Ananya, 2019; Qaid, 2016; Haggstrom *et al.,* 2016). It increases glycolysis, the pentose phosphate cycle, and glycogen production in liver, inhibits glycogenolysis,

accelerates protein synthesis, cholesterol and triglyceride synthesis, stimulates production of very low density lipoprotein cholesterol, and hinders both hepatic gluconeogenesis and ketogenesis (Haggstrom *et al*., 2016; Qaid, 2016).

Glycemic control is one of the main functions of insulin (Qaid, 2016). This is caused by a lot of factors in non-subjects and in diabetic subjects. One of the factors contributing to impede insulin function is the presence of insulin antibodies (anti- insulin antibodies) (Qaid, 2016).

**2.3 ANTI- INSULIN ANTIBODIES**

The anti-insulin antibodies (AIAs) present in circulation in insulin-treated subjects has been recognized as early as 1950s (Kurtz *et al.,* 1980; Ryan *et al*., 2010). The prevalence and concentrations as determined by titration of AIAs have markedly declined since the use of highly purified animal and human insulin preparations, but the production of these antibodies could not be stopped completely (Sahin *et al*., 2010). Receiving animal insulin in the past triggers AIA binding levels. Subjects treated with human insulin AIAs can also produce against the insulin analogues even though it was demonstrated to be less immunogenic as compared to the human insulin (Sahin *et al.,* 2010 and Copenh, 1976). The findings of severe hypoglycaemia, elevated plasma immune reactive insulin (IRI) concentration and anti-insulin auto antibodies, in theabsence of prior exposure to exogenous insulin, were first recognized by co-workers in 1970 (Casesnoves *et al.,*1998). Some evidence suggests that the formation of anti-insulin antibodies may be linked to the major histocompatibility complex locus (Casesnoves *et al.,* 1998).

Treatment of diabetic subjects with common available insulin preparations in most cases results in production of immunoglobulins (Ig) called anti-insulin-antibodies (Copenh.1976). During the last ten to fifteen (10-15) years it has been shown, that these immunoglobulins, especially IgE, may be responsible for allergical manifestations. Moreover it has been demonstrated, that

immunoglobulins, especially IgG, may be responsible for insulin resistance (Copenh, 1976). Immunoglobulin E (IgE) after mast cells activation by hyperglycemia whose degranulation may contribute to a vicious cycle, resulting in blood retinal barrier breakdown (BRB) and leakage, endothelial dysfunction, acellular capillaries, extravasation, neovascularization and lipoprotein modification (Tapan *et al.,* 2015, Alessandro *et al.,* 2016 and Olga *et al.,* 2016). Antibodies can be agonists to the insulin receptor and cause hypoglycemia.

Research conducted by Shashikala and Naidu in 2017 discovered that insulin-auto antibodies could also be one of the causes for decreased insulin efficacy and accelerated frequency of complications in type 2 DM. All the investigations carried out by the researchers, supported the assumption that, anti-insulin-antibodies may shorten the remission period, probably due to neutralizing effect upon the endogenous insulin supply and also, anti-insulin-antibody complexesmight deteriorate late diabetic vascular complications such as retinopathy (Ryan *et al.,* 2010, WHO, 2019 and Lauren *et al.,* 2014).

## 2.4 INSULIN PATHOPHYSIOLOGY IN DIABETES MELLITUS

Whenever there are abnormal high levels of blood glucose (hyperglycemia), the brain recognizes it and sends a message through nerve impulses to pancreas and other organs to decrease its effect (Nora, 2017). Defects in insulin are a cause of diabetes mellitus insulin-dependent type1 (*Shashikala et al.,* 2014). Insulin-dependent diabetes mellitus is a several factorial disorder of glucose homeostasis that is characterized by vulnerability to ketoacidosis in the absence of insulin treatment (Shashikala *et al.,* 2014). Clinical features are polydipsia, polyphagia and polyuria which result from hyperglycemia-induced osmotic diuresis and secondary thirst (Shashikala *et al.,* 2014). These clinical features result in long-term complications that affect the eyes, kidneys, nerves, and blood vessels (Shashikala *et al*., 2014). Defects in insulin action are

the cause of maturity-onset diabetes of the young MODY10 (Shashikala *et al*., 2014, WHO, 2019).

Maturity-onset diabetes of the young (MODY10) is a form of diabetes that is characterized by an autosomal dominant mode of inheritance, onset in childhood or early adulthood is usually before twenty five (25) years of age (Shashikala *et al*., 2014). Clinical symptoms of type 1 DM include high levels of blood glucose concentrations, ketoacidosis and lean body mass and usuallyzero to thirty five years (0-35) years of age (Shashikala *et al.*, 2014). Whereas type 2 DM occurin people who are more than thirty (35) years of age, usually obese, glucose levels being moderately raised and ketosis is very rare and microangiopathies are common complications that emanates from peripheral insulin resistance, impaired insulin secretion and promote glucose formation ( Shashikala *et al.*, 20014).

In ten to forty percent (30-40%) of type-1 diabetes mellitus and type-2 diabetes mellitus (type 2 DM) subjects are anti-insulin antibody (AIA) positive and have microangiopathies of differing areas (Shashikala *et al*,. 2011). The circulating AIA and other antibodies associated withdiabetes reflect the severity of diabetic complications (Shashikala *et al*., 2014). The incidence of autoimmune disease has inclined over the last three decades (Shashikala *et al.,* 2011). Diabetic related antibodies could be one of the causes for increased frequency of complications in type 2 DM. Nephropathy and retinopathy are highly associated with type 2 DM subjects with higher levels of glutamic acid decarboxylase (GAD) than type 2 DM subjects with lower levels GAD. In absolute terms, the plasma insulin concentration in both fasting and meal stimulated usually is increased, although ―relative‖ to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis (Ryan *et al.*, 2010).

Keeping in mind the intimate relationship between the secretion of insulin and the sensitivity of hormone action in the complicated control of glucose homeostasis, it is practically impossible to separate the contribution of each to the etiopathogenesis of type 2 DM (Nora, 2017). Insulin resistance and hyperinsulinemia eventually lead to impaired glucose tolerance (Shashikala *et al.,* 2017 and Sekikawa *et al.,* 1993). Research carried by Naidu and Shashikala showed that insulin treated type 2 DM subjects with retinopathy and neuropathy have higher levels of glutamic acid decarboxylase antibody and lower levels of glutamic acid decarboxylase antibody than in non-treated type 2 DM subjects with retinopathy and neuropathy.

## 2.5 AUTOIMMUNE ANTIBODIES

Autoimmune antibodies against β-cells are produced, randomly and in response to foreignprotein or substance within the body (Shashikala *et al*., 2011). Initially, one β-cell produces one specific kind of antibody (Baynes, 2015). The β-cell proliferate‘s or is killed off through a process called clonal deletion (Haggstrom, 2016; Shashikala *et al*., 2017; Baynes, 2015 and ADA, 2014**).** Insulin autoantibodies (IA2) are antibodies that attack the natural insulin production (Shashikala, *et al.,* 2014, 2016 and 2017). These are one of the primary antibody markers for type one diabetes mellitus; others are glutamic acid decarboxylase (GAD) and islet cell antibodies (ICA). Glutamic acid decarboxylase is considered to be one of the strongest candidate auto-antigen involved in triggering β-cell-specific autoimmunity whereas islet cell antibodies are markers that appear when insulin producing beta cells are damaged. For instance, Anti-insulin antibodies in circulation may alter physiological function of insulin by increasing itselimination (half-life) which can lead to diabetes complications such as retinopathy or hypoglycemia.

In type 1 and type 2 diabetes mellitus subjects thirty to forty percent (30-40%) are anti-insulin antibody (AIA) positive and have microangiopathies of differing areas (Shashikala *et al.,* 2011).

About eighty five percent (85%) of patients have circulating islet cell antibodies, and the majority also have detectable anti-insulin antibodies before receiving insulin therapy (Ryan *et al.,* 2010). Most islet cell antibodies are directed against glutamic acid decarboxylase (GAD) within pancreatic β-cells. (Alhomsi *et al.,* 1992, DMICC, 2014 and ADA, 2014).

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with type 1 DM (Laura *et al*., 2004). In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagons in type 1 DM patients (AlHomsi *et al.,* 1992). Normally, hyperglycemia leads to reduced glucagons secretion, however, in patients with type 1 DM, glucagons secretion is not suppressed by hyperglycemia (Nora, *2017*). Although insulin deficiency is the primary defect in type 1 DM, there is also a defect in the administration of insulin.

Deficiency in insulin leads to uncontrolled breakdown of lipids and increased levels of free fatty acids concentrations in the plasma, which impedes glucose metabolism in peripheral tissues such as skeletal muscle (Baynes, 2015). This impairs glucose metabolism and insulin deficiency also reduces the expression of a number of genes essential for target tissues to respond normally to insulin such as glucokinase in liver and the glucose transporter 4 (GLUT 4) class of glucose transporters in adipose tissue explained that the main metabolic derangements, which result from insulin defect or inadequate in type 1 DM are impaired glucose, lipid and protein metabolism (Kerner1 *et al.,* 2014, DMICC, 2014 and Ryan *et al.,* 2010). The resultant inappropriately

increased glucagon levels exacerbate the metabolic defects due to insulin deficiency (ADA. 2014). Although insulin deficiency is the primary defect in type 1 DM, there is also a defect in the administration of insulin or insulin therapy.

## 2.6 ACUTE COMPLICATIONS OF DIABETES MELLITUS

The three main metabolic complications of diabetes are diabetic ketoacidosis (DKA)hyperosmolar non-ketotic coma, and hypoglycaemia (Nora, 2017). Diabetic ketoacidosis occurs when the diabetic system produces high levels of blood acids called ketones as a result of the body inability to produce enough insulin. Without enough insulin, the body begins to break downlipids to produce free fatty acids as fuel. This process produces accumulation of acids in theblood called ketones, if not treated leads to diabetic ketoacidosis according to Mayo Foundation for Medical Education and Research , 2019 (MFMER, 2019)

Hyperosmolar non-ketotic coma also known as hyperosmolar hyperglycaemic state (HHS), is one of the acute complications of diabetes. It occurs as a result of very high blood glucose levels. This condition can affect both types of diabetes, yet it usually occurs amongst people with type 2 diabetes. Especially when the blood glucose levels above 33mmol/L for extended periods of time presents a risk of hyperosmolar non-ketotic coma according to Global Diabetes C ommunity, 2019 (GDC, 2019). The risk of hyperosmolar non-ketotic coma is seen in subjects with type 2 DM who have some concomitant illness that leads to reduce fluid intake. Infection is the most common preceding illness, but many other conditions, such as stroke or myocardial infarction, can cause this state.

Hypoglycaemia is a condition that occurs due to a very low level of blood glucose. Hypoglycaemia is important when blood sugar levels are at 3.9mmol/L or below and may show the following signs and symptoms such as fatigue, pale skin, shakiness, sweating, hunger,

irritability, anxiety and an irregular heart rhythm (MFMER, 2019). These acute metabolic complications of diabetes were considered to be the cause of death in three point five percent (3.5%), three point four percent (3.4%) and two point three percent (2.3%) of patients respectively, in a hospital admissions in Sher-i-Kashmir Institute of Medical Sciences , Srinagar, Kashir, India (Zargar *et al.,* 2009). Diabetic keto acidosis (DKA) is the commonest cause of death in children, teenagers and young adults with diabetes; it causes up to a third of all deaths in people with diabetes younger than 24 years (White, 2000). The major contributing factors to such high mortality are the chronic lack of availability of insulin, delays in seeking medical assistance by newly diagnosed type 1 patients presenting with ketoacidosis, misdiagnosis of diabetes, and poor health care in general and diabetic care in particular (Rwiza *et al.,* 1986). Hyperosmolar non-ketotic coma is usually a complication of type 2 diabetes and is less common and accounts for about 10 percent of all hyperglycaemic emergencies in developing countries (Samuel, 2014). Infection is the leading precipitating factor for both diabetic ketoacidosis and hyperosmolar non-ketotic coma, followed by non-compliance with a medical regimen (Zouvanis *et al.,* 1997).

## 2.7 CHRONIC DIABETES COMPLICATIONS

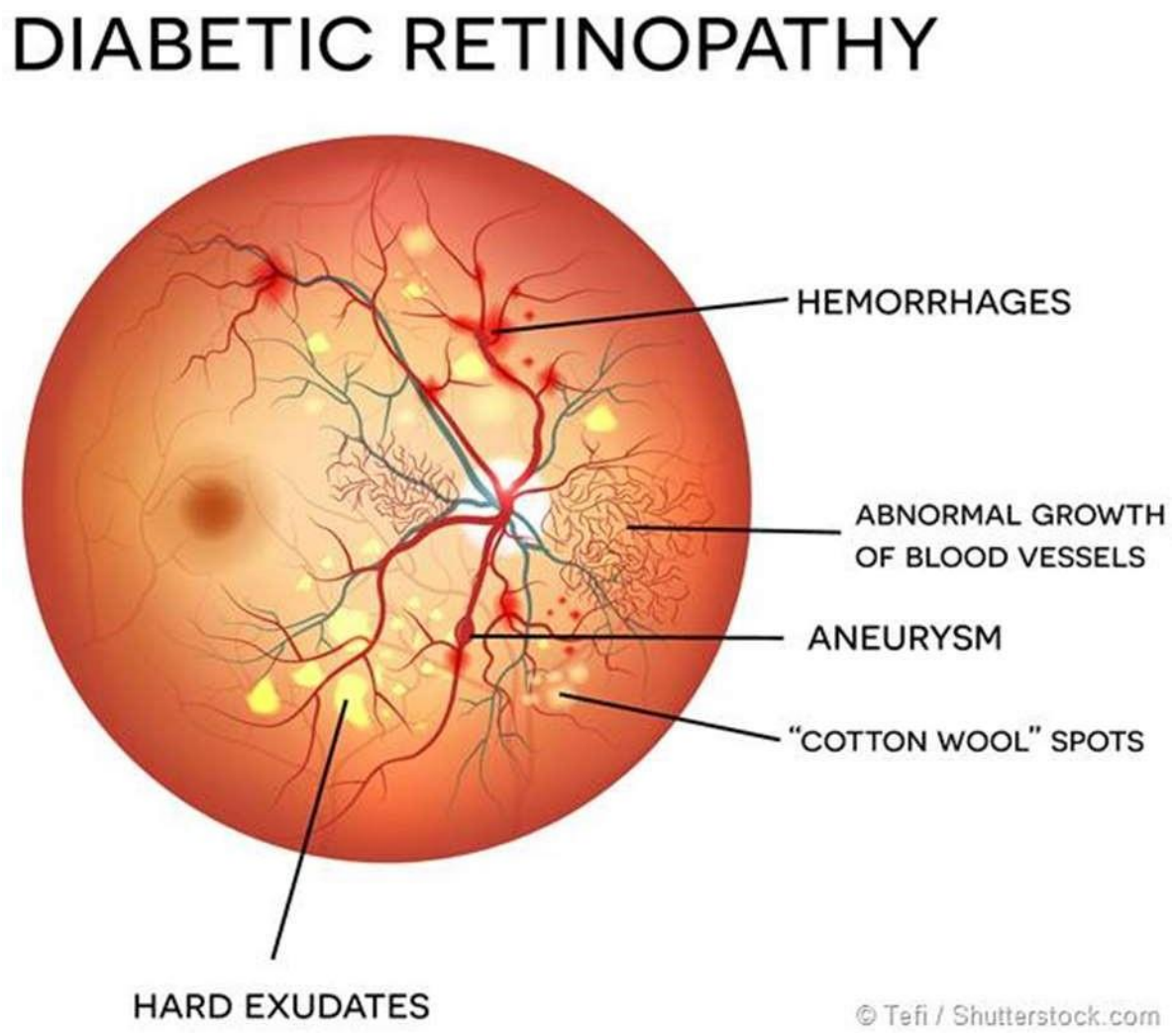
Diabetes is a chronic metabolic disease resulting from the body‘s inadequate formation or use of insulin, a hormone responsible for regulating glucose levels in the blood and body`s tissues (Lauren *et al*., 2014 and Yu *et al.,* 2013). Primarily, the chronic complications have been classified as micro vascular (that is, diabetic nephropathy, neuropathy, and retinopathy) and macrovascular (that is, coronary artery disease, peripheral arterial disease, and stroke) ( Baynes, 2015). Poorly managed diabetes results in a number of complications over time, damaging nearly every organ system, including the retinal tissue. In addition to increased risk for

glaucoma and cataracts, the most dangerous ocular implication of diabetes is diabetic retinopathy, an aggressive disorder clinically associated with a variety of retinal microvascular damages (Jackson *et al.,* 2012 and Fong *et al*., 2004).

## 2.8 DIABETIC RETINOPATHY

Diabetic retinopathy is the main ocular complication associated with diabetes, and is the leading cause of blindness in the working-age population of developed world (Lauren *et al.,* 2014). Although long-established clinical diagnosed based on abnormalities of the retinal microvasculature, diabetic retinopathy is now globally recognized as a neurovascular disease. While all subjects with diabetes are at higher risk for eye disease including diabetic retinopathy, proactive measures, and timely intervention can avoid or delay subsequent vision loss (Ryan *et al.,* 2014). Diabetic retinopathy has been typically classified as non-proliferative diabetic retinopathy (NPDR) less severe, characterised by microaneurysms, intraretinal hemorrhaging, and other microvascular aberrations, and severe proliferative diabetic retinopathy (PDR) , characterized by the onset of neovascularization and vitreal hemorrhaging ( Bandello *et al*., 2010and Wilkinson *et al.,* 2003). Diabetic macular edema (DME), another stage of diabetic retinopathy involving macular thickening due to fluid accumulation caused by blood retina barrier breakdown as a result of inflammatory mediators associated with diabetes. It is accountable for a great proportion of diabetes-related vision loss (Bandello *et al.,* 2010).

Diabetic retinopathy has many economic implications for healthcare systems and the overall population, as well as a variety of personal consequences on patient quality of life (Rein *et al*., 2006 Filho *et al*., 2011 and Lauren *et al.*, 2014). However, if addressed early and proactively, the incidence of total vision loss from diabetic retinopathy can be significantly declined.




Figure; 2.8.1 (Tefi *et al*., 2020) /https//www.shutterstock.com/image illustration/diabetic

Systemic management policy of diabetes by controlling glycemia, blood pressure, and serum anti-insulin antibodies levels are the most important method of preventing diabetic retinopathy onset and progression (Lauren *et al.*, 2014). Once detected, surgical and medical interventions including photocoagulation, vitrectomy, and intravitral drug injection can help avoid vision loss.

However, the need for advanced detection methods and therapies that will allow earlier diagnosis and treatment remains a problem. This investigation will help for the prevention and intervention for diabetic retinopathy, and examines ongoing developments in the search for new endpoints and therapies as they apply to preventing vision loss associated with diabetes.

## 2.9 PREVENTIVE INTERVENTION

The prevalence of diabetes globally, continuously increases, incidence of vision-threatening diabetic retinopathy is estimated to nearly triple in the next forty (40) years (Lauren *et al*., 2014). For subjects diagnosed with diabetes mellitus, the most essential management method of avoiding visual complications, such as retinopathy, is to manage (control) the diabetes at a systemic level (Zhu CH *et al.,* 2013). Tight regulation of glycemia through intensive insulin treatment significantly decreases the risk for retinopathy prevalence and progression. In addition to its influence on glycemic and circulating insulin levels, systemic insulin treatment has been demonstrated to have a local effect on ocular tissue as well, including restoration of retinal insulin receptor signaling cascade and rod photoreceptor function, (Filho *et al*., 2011 and Lauren *et al.*, 2014).

The standard of care for subjects with high-risk PDR remains pan retinal (scatter) photocoagulation surgery, administered in effort to stop angiogenesis and avoid hemorrhaging (Mazhar *et al.,* 2011 and Ryan *et al*., 2010) . Scatter laser treatment is also another clinical method to reduce progression of retinopathy and subsequent vision loss in select subjects with non-high-risk PDR and even severe NPDR; however, side effects such as loss of peripheral and night vision often deem the treatment at this level less favorable. When DME is clinically diagnosed, focal photocoagulation surgery is the best option for treatment, preferably before pan-retinal treatment if both are necessary (Lauren *et al*., 2014). While laser treatment restores no

already vision lost, the damage it inflicts is in preference over that which would occur ifretinopathy continued to progress. In advanced cases where laser treatment is not an option or recommended, vitrectomy is an option to reduce hemorrhaging, as well as to correct retinal detachment and scarring. Vitreous surgery has proved to be the recommended therapy for both severe PDR and DME and drastic improvement of quality of life has been investigated in both cases (Bandello *et al*., 2010 and Wilkinson *et al.*, 2003).

# CHAPTER THREE

# METHODOLOGY

## 3.1 Study design

The study was a case-control one. Information relating to the demographic characteristics and health status of study participants were documented using a standard questionnaire.

## 3.2 Study site

Recruitment of study participants were carried out at the National Diabetes Research Center and the eye unit of the Korle-Bu Teaching Hospital (KBTH). Sample analysis was carried out at the Chemical Pathology Laboratory, University of Ghana Medical School, College of Health Sciences, University of Ghana.

## 3.3 Study population

The population studied included twenty five (25) non-diabetic subjects, twenty five (25) subjects with diabetes and fifty (40) subjects with diabetic retinopathy.

## 3.4 Inclusion criteria

All diabetic individuals aged 25 years and above who consented to inclusion were enrolled. The individuals with diabetic retinopathy were diagnosed by an ophthalmologist specialist at Korle- bu Teaching Hospital eye unit. Control subjects for the study were recruited from NDMRC. Their fasting blood samples were taken and screened using glucose oxidase method to be sure they are non-diabetic. The diabetes group included diagnosed diabetic patients without any diabetic complications on management at the National Diabetes Management and Research Centre (NDMRC) and diabetic subjects with retinopathy and attending the eye clinic in KBTH.

## 3.5 Exclusion criteria

Individuals with familial hyperlipidaemia and opaque ocular media were excluded**.** Patients who

were immunosuppressed such as those with immunoglobulin deficiency syndrome, HIV,and Hepatitis B were excluded. Also those who tested for the urine nitrite test or bacterial and parasitic infections were excluded from the study (the above has proven to affect immunoglobulin levels in subjects).

### 3.6 Sampling method

Convenience sampling technique, a non-probability method was used. Thus, successive participants who met all the inclusion and none of the exclusion criteria during the period of study were recruited.

### 3.7 Procedure

Patients visiting the physician at the National Diabetic Management and Research Centre (NDMRC) and referred to the eye unit were recruited into the study**.** Staff of KBTH were screened for controls and was confirmed based on results of fasting blood glucose (FBG) < 5.6 mmol/L (ADA, 2010). Patients were interviewed using a structured questionnaire. Visual acuity of subjects were assessed using Snellen‘s chart. Each patient‘s blood pressure was measured as he/she was in sitting position, after 5–10 minutes of rest using an automatic cuff blood pressure machine. The pupils were dilated using tropicamide, phenylephrine, or cyclopentolate eye drop every 5 minutes for 15 to 20 minutes after instilling 0.5% tetracaine hydrochloride, after which stereoscopic binocular examination of the fundus was carried out using a slit lamp and a 90 D loupe. Fundus findings were counter-checked by a consultant ophthalmologist**.**

### 3.8 Sample size

The minimum sample size was determined using Cochran`s (1977) formula as shown below:

The minimum sample size was determined using the formula:

$$n = \frac{z^2 \times p(1-p)}{}$$

$m^2$

Where $n$ = minimum sample size

z = confidence level at 95% (standard value of 1.96)

m = margin of error at 5% (standard value of 0.05)

$p$ = the reported prevalence (P) range for diabetic retinopathy (DR) in Africa = 7.0%to 62.4% (Burgess *et al.*, 2013).

Hence in total, 90 individuals were recruited for the study.

### 3.9 Data collection and tools

Subjects who consented and met the inclusion criteria were recruited into the study. Data were collected between November and December, 2019. Anthropometric measurements and blood samples were collected at the National Diabetes Centre and Korle-Bu Teaching Hospital Eye Unit. The questionnaire (Appendix-ш) was structured in English language. All questionnaire were printed comprising of twenty- three questions structure to solicit the necessary information from patients and were administered by the primary investigator.

### 3.10 Anthropometric measurement

For all the participants body weight and height were measured using a standard physician_s scale and a mounted meter rule, to the nearest 1.0 kg and 0.005m respectively. These measurements were taken when the subjects were without footwear and wearing light clothing. The body mass indices (BMIs) of the participants were calculated as weight/height (kg/m2).The participants' blood pressure were measured in sitting position, after 5-10 minutes of rest using anautomatic cuff blood pressure machine and stethoscope.

### 3.11 Sample collection and preparation

Five milliliters (5mls**)** of venous blood sample were taken from each participant by a phlebotomist.

Three (3) mls of blood was allowed to clot and the serum separated by centrifugation at 3000 rpm for 10 minutes at room temperature. Contamination by hemolysis or lipemia was avoided. The sera was stored at -20 $^{o}$C up to six months until required for biochemical analysis. The remaining 2 ml of patients'venous blood was put into fluoride tubes and centrifuged at 3000 rpm for 10 minutes at room temperature for measurement of plasma blood glucose.

**3.12 Measurements**

**3.12.1 Fasting plasma glucose (FPG)**

Fasting plasma glucose (FPG) was determined by glucose oxidase method. The normal value of this test is below 5.6 mmol/L (ADA, 2010). Glucose oxidase (GOX) convert's glucose to gluconic acid. Hydrogen peroxide formed in this reaction in the presence of peroxidase (POD) oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. This dye has absorbance maximum at 505nm (500-550nm). The intensity of the colour complex is directly proportional to the concentration of glucose in sample. The mechanism of the reaction equation is shown below

$$\text{(a)}\ \beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{\text{(GOX)}} \text{D-glucono-}\delta\text{-lactone} + H_2O_2$$

$$\text{(b)}\ 2H_2O_2 + \text{p-hydroxybenzoic acid} + \text{4-aminoantipyrine} \xrightarrow{\text{(POD)}} \text{quinoneimine dye} + 4H_2O$$

**3.12.2 IgG and IgE Measurements by ELISA**

Enzyme linked immunosorbent assay (ELISA) was used for measurement of serum anti-insulin antibodies (IgE and IgG). ELISA is a test that uses antibodies and color change to identify a

substance. The principle of the test (ELISA) is that, a mixture of highly purified preparation of recombinant human insulin is bound to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unbound serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patients antibodies forming a conjugate/ antibody/ antigen complex. Washing of microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450nm. The amount of color is directly proportional to the concentration of IgG anti-bodies present in the original sample.

**3.13 Data processing**

The data gathered in the current study were analyzed with the help of Statistical Products and Services Solutions (SPSS), version 25. Descriptive statistics (means, standard deviations, and proportions) were used to summarize the socio-demographic and clinical features of the study participants. Furthermore, using an alpha level of 0.05, one-way between-groups analysis of variance (ANOVA) was used to compare the three study groups regarding their levels of anti-insulin IgE and IgG antibodies. In addition, within each group, the Pearson‘s product-moment correlation was used to determine associations between participants‘ sociodemographic and clinical features that were continuous variables and the levels of anti-insulin IgE and IgG antibodies.

Regarding associations between participants‘ sociodemographic and clinical features and the levels of anti-insulin IgE and IgG antibodies, point biserial correlations were conducted. These associations were presented using the Pearson product moment correlation coefficient. Moreover, the coefficient of determination ($r^2$), which explains how much variance two associated variables

share, was also computed for each association. Preliminary analyses were also performed to ensure no violations in the assumptions of homogeneity of variances, normality, linearity, and homoscedacity.

### 3.14 Ethical consideration

Ethical approval (ID: CHS-Et/M.3-P1.9/20017-2018) was obtained from the ethical and protocol review committee (EPRC), CHS, University of Ghana. Approval was also obtained from Korle-Bu Teaching Hospital's Scientific and Technical Committee (KBTH-STC) and Korle Bu Teaching Hospital Institutional Review Board (KBTH-IRB) (ID: KBTH-STC00040/20019). Rational for the study was explained to the Patients. Informed consent was then obtained(Appendix I).

### 3.15 Dissemination of results

The results will be disseminated through the Department of Chemical Pathology UG, peer-reviewed publication and conferences.

# CHAPTER FOUR

# RESULTS

## 4.1 Sociodemographic and biochemical features of the study participants

In total, ninety (90) individuals, comprising of twenty-five (25) individuals without diabetes, twenty-five (25) individuals with diabetes without retinopathy, and forty (40) individuals with diabetes retinopathy were recruited in the current study. With the exception of the control group, which had a higher proportion of its participants being males (60%, $n$ = 15), most of the participants were females in both the retinopathy (65%, $n$ = 26) and diabetes (76%, $n$ = 19) groups. Details of the demographic features of the study participants are presented in Table 4.1.

**Table 4.1: Socio-demographic characteristics of the study participants**

| | Retinopathy Group | | Diabetes Group | | Control Group | |
|---|---|---|---|---|---|---|
| **Feature** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| **Gender** | | | | | | |
| Female | 26 | 65 | 19 | 76 | 10 | 40 |
| Male | 14 | 35 | 6 | 24 | 15 | 60 |
| **Education level** | | | | | | |
| None | 8 | 20 | 3 | 12 | 2 | 8 |
| Basic | 13 | 32.5 | 7 | 28 | 7 | 28 |
| Secondary | 12 | 30 | 11 | 44 | 11 | 44 |
| Tertiary | 7 | 17.5 | 4 | 16 | 5 | 20 |
| **Occupation** | | | | | | |
| Retired | 14 | 35 | 12 | 48 | 3 | 12 |
| Self employed | 22 | 55 | 13 | 52 | 17 | 68 |
| Civil servant | 1 | 2.5 | 0 | 0 | 0 | 0 |
| Teacher | 2 | 5 | 0 | 0 | 0 | 0 |
| Nurse | 1 | 2.5 | 0 | 0 | 2 | 8 |
| Student | 0 | 0 | 0 | 0 | 3 | 12 |

*Table4.1: Socio-demographic characteristics of the study participants. This shows sociodemographic characteristics of female, male, none, basic, secondary, tertiary, retired, self-employed, civil servant, teacher, nurse and student. Data presented in frequency (No.) and percentages (%)*

None of the participants of the control group were on any medication, whereas the members of both the retinopathy and diabetes groups were largely on a variety of medications, details of which are summarized in Table 4.2 below.

**Table 4.2: Medications being taken by the study participants**

| Medication | Retinopathy Group | | Diabetes Group | |
|---|---|---|---|---|
| | **No.** | **%** | **No.** | **%** |
| Tropicamide | 40 | 100 | 1 | 4 |
| Phenylephrine | 39 | 97.5 | 1 | 4 |
| Metformin | 38 | 95 | 23 | 92 |
| Cyclopentolate | 37 | 92.5 | 0 | 0 |
| Nifedipine | 13 | 32.5 | 10 | 40 |
| Amaryl | 2 | 5 | 22 | 88 |
| Lisinopril | 6 | 15 | 9 | 36 |
| Dionil | 6 | 15 | 0 | 0 |
| Insulin | 5 | 12.5 | 1 | 4 |
| Losartan | 2 | 5 | 7 | 28 |
| Atorvastatin | 2 | 5 | 9 | 36 |
| Amlodipine | 5 | 12.5 | 8 | 32 |
| Aspirin | 2 | 5 | 1 | 4 |

*Table4.2: Medications being taken by the study participants. This shows a variety of medication administered to diabetic subjects and retinopathy subjects. These medications aspirin, amlodipine, losartan, dionil, lisinopril,amaryl, nifedipine, cyclopentolate, metformin, phenylephrine and tropicamide.Data was presented in frequency (No.) and percentages (%)*

Among the study participants, the respective mean ages of the retinopathy, diabetes, and control groups were 58.45 ± 9.48 years, 63.56 ± 11.02 years, and 43.16 ± 16.30 years. Furthermore, the anti-insulin IgE (46.49 ± 29.41) and IgG (47.90 ± 29.41) antibodies were highest in the diabetes group. Higher levels of IgE and IgG concentrations were observed to be higher in diabetic

retinopathy subjects than those of the diabetic without retinopathy group ($p< 0.05$).Table 4.3 below summarizes these and other clinical features of the study participants.

**Table 4.3: Biochemical features of the study participants**

| VARIABLES | Controls (n=25) | DM without RD (n=25) | DM with Retinopathy (n=40) |
|---|---|---|---|
| Age (years) | 43.16 ± 16.30 | 63.56 ± 11.02* | 58.45 ± 9.48# |
| BMI ($Kg/m^2$) | 26.64 ± 6.92 | 31.40 ± 8.60* | 27.98 ± 5.13# |
| SBP (mmHg) | 134.2 ± 23.26 | 146.64 ± 21.35* | 154.5 ± 29.72# |
| DBP (mmHg) | 86.08 ± 14.92 | 77.64 ± 14.09 | 91.43 ± 22.70# |
| FBG (mmol/L) | 4.88 ± 0.45 | 8.24 ± 2.78* | 8.69 ± 2.52 |
| IgE | 184.3 ± 147.2 | 96.7 ± 136.1 | 176.6 ± 189.8# |
| IgG | 184.0 ± 154.5 | 95.8 ± 131.9 | 159.4 ± 179.1# |
| Period of DM | N/A | 15.25±235 | N/A |
| Period of DR | N/A | N/A | 5.91±5.01 |

*Table 4.3: Biochemical features of the study participants. This shows biochemical features, age and period with the diabetes and diabetes with retinopathy of the study participants. Data presented as mean and standard deviation. SD = Standard deviation; BMI = Body mass index; BP = Blood pressure, NA=Not applicable, Ig is immunoglobulin.*p< 0.001 when compared with control, #p<0.05 when compared with diabetics without retinopathy*

## 4.2 A comparison of the study groups regarding their levels of anti-insulin IgEand IgG antibodies

The one-way between-group analysis of variance conducted (see Table 4.4 below) demonstrated that the three study groups under investigation differed significantly from each other regarding their levels of anti-insulin IgE ($F = 4.10$, $p = 0.020$) and IgG ($F = 6.03$, $p = 0.004$) antibodies.

**Table 4.4: A comparison of the study groups regarding their levels of anti-insulin IgE and IgG antibodies**

| | | Sum of squares | *df* | Mean square | *F* | *p* value |
|---|---|---|---|---|---|---|
| **IgE** | **Between groups** | 3789.78 | 2 | 1894.89 | 4.10 | 0.020 |
| | **Within groups** | 40193.26 | 87 | 461.99 | | |
| | **Total** | 43983.03 | 89 | | | |
| **IgG** | **Between groups** | 6213.91 | 2 | 3106.96 | 6.03 | 0.004 |
| | **Within groups** | 44835.64 | 87 | 515.35 | | |
| | **Total** | 51049.56 | 89 | | | |

**Table 4.4** shows the association between IgE and IgG of the study groups regarding their levels anti-insulin antibodies. *Df* = Degree of freedom p-value < 0.05 significant.

As observed in Table 4.5, the multiple comparisons conducted revealed that the significant differences observed among the study groups were as follows: the diabetes group had significantly higher levels of IgE ($p = 0.018$) and IgG ($p = 0.003$) than did the control group, but did not differ significantly from the retinopathy group for each of IgE ($p = 0.58$) and IgG ($p = 0.53$) levels respectively. Also, the retinopathy group did not differ significantly from the control group with regard to IgE levels ($p = 0.14$), but had significantly higher levels of IgG ($p = 0.04$) than did the control group.

**Table 4.5: Multiple comparisons of the study groups regarding their levels of anti-insulin IgE and IgG antibodies**

| | Categories | | MD (I-J) | *p*-value | Standard Error | 95% *CI* |
|---|---|---|---|---|---|---|
| **IgE** | **Control Group** | **Diabetes Group** | -17.14* | 0.018 | 6.08 | -31.93 – -2.35 |
| | | **Retinopathy Group** | -10.87 | 0.139 | 5.48 | -24.11 – 2.37 |
| | **Diabetes Group** | **Retinopathy Group** | 6.27 | 0.579 | 5.48 | -6.97 – 19.52 |
| **IgG** | **Control Group** | **Diabetes Group** | -21.70* | 0.003 | 6.42 | -37.32 – -6.07 |
| | | **Retinopathy Group** | -14.70* | 0.036 | 5.79 | -28.68 – -0.71 |
| | **Diabetes Group** | **Retinopathy Group** | 7.00 | 0.534 | 5.79 | -6.98 – 20.99 |

*Table 4.5: shows association of the study population regarding their levels of IgE and IgG anti-insulin antibodies*
**Significant at 0.05 alpha level; MD = Mean difference; CI = Confidence interval*

### 4.3 Associations between participants' features and anti-insulin antibodies

The Pearson product-moment and point biserial correlations conducted to determine associations between participants' features and anti-insulin IgE antibodies (see Table 4.6) revealed that in the retinopathy group, it was only being on nifedipine medication that had a significant negative correlation with the levels of anti-insulin IgE antibodies ($r = -0.32$, $p = 0.04$). However, none of the participants' features within the diabetes and control groups had any significant correlations with the levels of anti-insulin IgE antibodies ($p > 0.05$).

**Table 4.6: Association between participants' biochemical features and anti-insulin IgE antibodies**

| | Retinopathy Group | | Diabetes Group | | Control Group | |
|---|---|---|---|---|---|---|
| **Participants' features** | **R** | ***P*** | **R** | ***P*** | **R** | ***P*** |
| Age | -0.04 | 0.83 | 0.01 | 0.97 | 0.30 | 0.15 |
| Gender | 0.22 | 0.18 | 0.21 | 0.31 | 0.07 | 0.74 |
| BMI | -0.13 | 0.43 | -0.12 | 0.58 | 0.13 | 0.55 |
| Educational level | -0.11 | 0.51 | 0.13 | 0.53 | -0.37 | 0.07 |
| Systolic BP | -0.01 | 0.97 | -0.09 | 0.67 | 0.24 | 0.24 |
| Diastolic BP | 0.06 | 0.74 | -0.16 | 0.45 | 0.05 | 0.83 |
| Period with diabetes / retinopathy | 0.12 | 0.46 | 0.01 | 0.96 | N/A | N/A |
| Occupation | 0.05 | 0.78 | 0.01 | 0.98 | -0.37 | 0.07 |
| Fasting blood glucose | 0.10 | 0.52 | -0.30 | 0.15 | -0.27 | 0.19 |
| Metformin | -0.16 | 0.33 | -0.20 | 0.92 | N/A | N/A |
| Lisinopril | 0.13 | 0.44 | 0.06 | 0.77 | N/A | N/A |
| Amaryl | -0.21 | 0.18 | 0.23 | 0.28 | N/A | N/A |
| Nifedipine | -0.32* | 0.04 | 0.15 | 0.47 | N/A | N/A |
| Losartan | -0.22 | 0.17 | 0.13 | 0.52 | N/A | N/A |
| Atorvastatin | 0.03 | 0.84 | 0.01 | 0.96 | N/A | N/A |
| Phenylephrine | -0.28 | 0.08 | 0.24 | 0.24 | N/A | N/A |
| Amlodipine | -0.14 | 0.37 | -0.07 | 0.73 | N/A | N/A |
| Aspirin | 0.11 | 0.49 | -0.21 | 0.30 | N/A | N/A |

Table 4.6: Shows association between participants' features and anti-insulin IgE antibodies *Significant at 0.05 alpha level; N/A = Not applicable

The Pearson product-moment and point biserial correlations conducted to determine associations between participants' features and anti-insulin IgG antibodies (see Table 4.7) revealed that in the retinopathy group, it was only being on metformin medication that had a significant medium

negative correlation with the levels of anti-insulin IgE antibodies (r = 0.32, $p$ = 0.04). However, none of the participants‘ features within the diabetes and control groups had any significant correlations with the levels of anti-insulin IgG antibodies.

**Table 4.7: Association between participants' features and anti-insulin IgG antibodies**

| | **Retinopathy Group** | | **Diabetes Group** | | **Control Group** | |
|---|---|---|---|---|---|---|
| **Participants' features** | **R** | ***p*** | **R** | ***P*** | **R** | ***P*** |
| Age | -0.18 | 0.26 | -0.02 | 0.91 | 0.07 | 0.73 |
| Gender | 0.22 | 0.18 | 0.22 | 0.28 | 0.13 | 0.53 |
| BMI | -0.21 | 0.19 | -0.14 | 0.51 | 0.03 | 0.88 |
| Educational level | 0.003 | 0.98 | 0.16 | 0.45 | -0.05 | 0.81 |
| Systolic BP | -0.01 | 0.96 | -0.08 | 0.72 | 0.06 | 0.76 |
| Diastolic BP | 0.06 | 0.73 | -0.14 | 0.49 | 0.01 | 0.98 |
| Period with diabetes / retinopathy | 0.11 | 0.51 | 0.01 | 0.96 | N/A | N/A |
| Occupation | 0.17 | 0.30 | 0.01 | 0.98 | -0.26 | 0.21 |
| Fasting blood glucose | 0.04 | 0.79 | -0.30 | 0.15 | -0.22 | 0.29 |
| Metformin | 0.32* | 0.04 | -0.02 | 0.94 | N/A | N/A |
| Lisinopril | 0.07 | 0.69 | 0.04 | 0.86 | N/A | N/A |
| Amaryl | -0.04 | 0.83 | 0.20 | 0.34 | N/A | N/A |
| Nifedipine | -0.04 | 0.79 | 0.15 | 0.47 | N/A | N/A |
| Losartan | -0.77 | 0.64 | 0.16 | 0.46 | N/A | N/A |
| Atorvastatin | -0.10 | 0.55 | 0.02 | 0.93 | N/A | N/A |
| Aspirin | -0.20 | 0.22 | -0.21 | 0.31 | N/A | N/A |

*Significant at 0.05 alpha level; N/A = Not applicable

# CHAPTER FIVE

# DISCUSSION

Anti-insulin anti-antibody, also called insulin antibody, belongs to a class of proteins called immunoglobulin (Ig). Igs are made by specialized white blood cells which identify, opsonize, agglutinate, neutralize and releases inflammatory organic molecules to destroy materials foreign (pathogen) to the immune system (Behl *et al*., 2015; Pini *et al*., 2016; Zhang *et al*., 2016 and Ghose *et al*., 2020).

Diabetic retinopathy (DR) is the ocular manifestation of end-organ damage in diabetes mellitus as a result of progressive vascular injury due to chronic hyperglycemia (Behl *et al*., 2015; Pini *et al*., 2016; Zhang *et al*., 2016 and Shad *et al*., 2017). Although there are other risk factors of DR, the major risk factors are hyperglycemia and increased duration of diabetes (Ryan *et al.*, 2010). These risk factors result in retinal metabolic changes and microvascular damage. This wasthe first study to investigate the levels of anti-insulin IgE and IgG antibodies in patients with diabetic retinopathy, Diabetic and non -diabetic subjects.

A major focus of this study was to determine whether individuals without diabetes, individuals with diabetes without retinopathy, and individuals with diabetic retinopathy differ significantly with regard to levels of anti-insulin IgE and IgG antibodies. Another major focus of this study was to investigate associations between participants‘ features in each study group with levels of anti-insulin IgE and IgG antibodies.

As observed, participants‘ occupation, age, gender, fasting blood glucose levels, and being on either of tropicamide, methyldopa, or phenylephrine had significant associations with either anti-insulin IgE or IgG antibody levels. This study seems to be the first to report such findings.

Serum immunoglobulin G and immunoglobulin E were measured using Enzyme-linked immunosorbent assay (ELISA). High levels of IgE and IgG concentrations were observed in diabetic retinopathy subjects than those of non-diabetic and diabetic subjects (Table 4.3). The results however did not reveal any statistical difference when the three study groups were compared statistically with regards to their anti-insulin antibodies – whether IgE or IgG.

Higher levels of IgE and IgG concentrations were observed to be higher in diabetic retinopathy subjects than those of the diabetic without retinopathy group. The higher levels of IgG concentrations in the DR group agree primarily with the existing information that IgG is the most abundant immunoglobulin (Botchey, 2014). This observation could also be a result of chronic metabolic dysfunction associated with hyperglycemia and insulin resistance according to Mayo Foundation for Medical Education and Research, 2020 (MFME, 2020). Hyperglycemia could influence up-regulation of inflammatory mediators. Additionally, vascular permeability could increase from loss of pericytes, neovascularilization and accelerate endothelial proliferation in retina capillaries resulting in breakdown of blood retina barrier (BRB) allowing fluids to accumulate in the deep retinal layers, leading to damages in photoreceptors and other neural tissues and causing macula edema, leading to visual loss in diabetes (Zhang *et al*., 2016). High levels of IgE concentration that was also observed in diabetic retinopathy subjects and diabetic subjects could be as a result of allergic reaction. Again, observed higher IgE concentrations among subjects with DR could be implicated in the development of microvascular permeabilityin patients with diabetes and diabetes complications.

Immunoglobulin E, due to its higher affinity for mast cells and basophils activation, could release inflammatory mediators such as histamine, leukotrienes and prostaglandins (Scott *et al*., 2012). These inflammatory mediators could promote vascular permeability, endothelial cell loss,

leukostasis, aggravation of retinal inflammation, up regulation of oxidative stress and cellular apoptosis in retina and increased retina neovascularization. These clinical features are the cardinal pathophysiologic features of diabetic retinopathy (Scott *et al*., 2012; Behl *et al.,* 2015; Zhang *et al*., 2016; Pine *et al*., 2016).

Non-diabetic and diabetic subjects showed no difference with regards to levels of anti-insulin IgG and IgE antibodies concentrations. This observation could be due to the fact that persons with diabetes and DR in this study were on prescribed medications and such management practice could have been responsible for such observation made.

Body mass index (BMI) in this study was found to be significantly higher in the diabetic and diabetic retinopathy subjects than non-diabetic subjects. This observation confirmed findings by researchers (Knowler *et al*., 2002; Wang *et al*., 2005). Body mass index was identified as a pre-disposing factor in the development of diabetes mellitus (Inter Act Consortium, 2012).

In this study, the investigator did not distinguish type 2 from type 1 with regards to the levels of anti-insulin IgE and IgG antibodies which may account at least in part for the inconsistent results obtained. This view was supported by another researcher (Hoddinott *et al*., 1982).

Diabetic and non-diabetic subjects Systolic and diastolic blood pressures demonstrated significant different (Table 4.3). Aggravating glycaemic status tended to be associated with increases in body mass index as well as systolic and diastolic blood pressures according to Amoah *et al*. (2002).

It was therefore expected that the diabetic subjects in the study like diabetic retinopathy subjects would have presented higher diastolic and systolic pressures compared to the non-diabeticsubjects. The diabetic and diabetic retinopathy subjects in this study were on anti-diabeticmedications. Those who had developed hypertension were on anti-hypertensive medication.

These variety of medications as management practices for diabetes could be responsible for the systolic and diastolic pressures which were significantly different. Although, the diabetic retinopathy subjects were on medications, their systolic and diastolic blood pressures were significantly ($p< 0.05$) higher compared with the diabetic without complication and non-diabetic groups indicating a possible non adherence to the taking of their anti-hypertensive drugs. This study seems to be the first to report such findings

# CHAPTER SIX

# CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS

## 6.1 Conclusions

It is concluded that among the study participants sampled, neither diabetes nor diabetic retinopathy influenced the levels of anti-insulin IgE and IgG antibodies. Furthermore, the factors that had significant associations with anti-insulin IgE and IgG antibodies were: fasting blood glucose, occupation, age, gender, and being on tropicamide, methyldopa, and Phenylephrine medications.

## 6.2 Limitations

The assay used for determining the levels of the antibodies did not differentiate between anti-insulin antibodies and autoantibodies. This study did not distinguish type 2 from type 1 with regards to the levels of anti-insulin IgE and IgG antibodies which may have potentially affected study outcomes.

## 6.3 Recommendations

This study could be replicated using larger sample sizes, with an assay that distinguishes between anti-insulin antibodies and autoantibodies.

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## APPENDIX I

## RESEARCH PARTICIPATION INFORMATION SHEET

### The level of Anti-insulin Antibody in Subjects with Diabetic Retinopathy in Ghana

Diabetes mellitus is a chronic condition that can lead to complications over a period. The complications include coronary heart disease, cerebrovascular disease, neuropathy, retinopathy and nephropathy.

However, anti-insulin antibodies are antibodies that attack insulin, usually external insulin. Administration of exogenous insulin within a short amount of time, the presence of endogenous insulin will cause IA2 not to be a meaningful diagnostic test for type one diabetes (T1DM). Anti-insulin antibodies in circulation may alter physiological function of insulin by increasing the elimination (half-life) of insulin which can lead to diabetes complications such as retinopathy or hypogycemia.

Increasing prevalence of diabetes mellitus in the past few decades with complications because of poor glycaemic control is severe in low and middle income countries (WHO, 2016).

In Africa, it is estimated that prevalence of diabetic retinopathy is ranged between 7% to 62.4% in Sahara Africa (Burgess *et al*., 2013).

In Ghana, data on diabetes and diabetic retinopathy is scanty. Recent studies showed 6.3% crude prevalence of diabetes mellitus. The increasing in visual complications and blindness burdendue to diabetic retinopathy is not known to the best of my knowledge in Ghana and this study sought to establish the nature and magnitude of diabetic retinopathy and levels of anti- insulin antibody among diabetics.

Diabetes mellitus complications have a serious impact on those affected and their families, hence the need for early detection and prompt and adequate management.

Anti-insulin antibodies could be one of the causes for increased frequency of diabetes complications especially retinopathy in Ghana and in the much of the world.

This study is intended to fill that gap. Findings may contribute to the formulation of new management policies on diabetes and associated complications such as retinopathy.

This study will take a small amount of blood (4ml) from you by inserting a needle in your forearm. The risk involved in this blood collection procedure is negligible and it will cause only minimal pain and bruising. This sample will be just once and will be used for analyses fasting blood glucose and anti-insulin antibody levels in subjects with diabetic retinopathy in Ghana. This will be followed by a detailed fundus examination by a senior ophthalmologist to assess for presence of diabetic retinopathy.

It will be appreciated if you will agree to take part in this study. Your participation in the study is voluntary, and you can leave the study at any time without any disadvantage concerning your medical care at this Hospital. All information gathered will be treated with strict confidentiality.

If you have further questions on the study you can contact;

**Bismark Mohammed Nluki** (0249742640), Department of Chemical Pathology, or **Dr. Amissah Arthur** (0276864343), Medical Doctor Eye Unit KBTH and **Dr. Seth Amanquah** (0244293987), Chemical Pathology Department, Medical School University of Ghana.

**APPENDIX II**

**RESEARCH PARTICIPATION CONSENT FORM**

I have been invited to take part in this study. The purpose of this study has been made clear to me and I have been provided the opportunity to ask questions. I have also been given adequate time to rethink my decision to participate in the study. I have been made to understand that my participation is voluntary and that I can withdraw anytime without given reasons. I understand this study will not influence the regular care and treatment I receive from my health care providers. I know that this study has been approved by the Ethic and Protocol Review Committee of the school of Biomedical and Allied Sciences, University of Ghana. I also know that the information obtained from this research will be confidential and also for scientific purposes only. I agree to this, provided my privacy is guaranteed. I hereby consent to participate in this study.

**I ……**

**of ……. give my consent for my sample to be used for the research project stated above which has been explained to me.**

**By……**

**Patient's signature…….....Date……**

**Doctor's signature……Date……..**

Doctor's signature……Date……..

**APPENDIX III**

**QUESTIONNAIRE**

GENERAL INFORMATION STUDY No.

1. AGE (in years). ----------
2. SEX: a) Male ☐ b) Female ☐

Name.....IP/OP NO----------

3. Weight----------

4. Height----------

5. Occupation..... .

6. E-mail.....

7. Educational background; None ☐ Primary ☐ Secondary ☐ Tertiary ☐

8. Have you heard of Diabetes Mellitus? a) Yes ☐ b) No ☐

9. Is it a hereditary disease? ----------

10. Can diabetes be controlled with the following?

a) Diet----------

b) O.H.A----------

c) Insulin----------

11. When was diagnosis made? ----------

12. How many visits do you make per year to the physician? ----------

13. Do you know the disease can affect the eye? a) Yes ☐ b) No ☐

14. If yes? Source of information

a) Health facility ☐ b) Nurse ☐ c) Dialectologist ☐ d) General practitioner ☐

e) Community nurse ☐ f) Nutritionist ☐

g) Physician from diabetic clinic

**O R**

h) Mass media ☐ j) Press release ☐ k) Internet ☐ l) Radio Station ☐

m) Radio talks ☐ n) TV advertisements o) Newspaper articles

p) Mobile clinics ☐ q) SMS ☐

Others----------

15. Have you seen the eye doctor? Yes ☐ No ☐

16. What took you?----------

a) Visual complains (self-referral) OR Referral by the physician ----------

b) Others (specify) ----------

17. Is diabetic retinopathy one of the complications of diabetes?

a) Yes ☐ b) No ☐

18. Is laser treatment an option for Proliferative diabetic retinopathy?

a) Yes ☐ b) No ☐

19. Any previous eye examination for the diabetes

a) Yes ☐ b) No ☐

20. How far do you stay? ----------

21. Cost of travel----------

22. Did you come with an escort? ----------

23. Concomitant illness. ☐

a) Hypertension ☐

b) Nephropathy ☐

c) Glaucoma ☐

Others ----------

**LABORATORY:**

Fasting blood Sugar (mmol/L) ----------

B/P (mmHg) - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

**Drug (g/mg)**----------