KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

HAEMATO-BIOCHEMICAL PROFILE OF DIABETES MELLITUS PATIENTS IN THE DUNKWA METROPOLIS

By

GOCKAH-ADAPOE ERNEST

BSC. (HONS) MEDICAL LABORATORY TECHNOLOGY

A THESIS SUBMITTED IN
FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY IN
CHEMICAL PATHOLOGY

In the

Department of Molecular Medicine,
School of Medical Sciences

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,
KUMASI

MAY, 2016

DECLARATION

The experimental work described in this thesis was carried out at the Dunkwa Municipal Hospital Laboratory (Central Region), SMS Biochemical Laboratory (KNUST), and Rayben Diagnostics Kumasi. I hereby declare that this work has not been submitted for any other degree here or elsewhere. This thesis presents results of original research undertaken by me personally. Information taken from other works has been specially and duly acknowledged.

Ernest Gockah-Adapoe	……	……..
MPhil Candidate	Signature	Date
Dr. W.K.B.A. Owiredu	…….	…….
Academic Supervisor	Signature	Date
Prof. Benjamin Eghan	…….	…….
Co- Supervisor	Signature	Date
Prof. Francis Agyemang Yeboah	……	……..
Head of Department	Signature	Date

DEDICATION

I dedicate this work to the Almighty God without whom this work would not simply have been possible. A change in topic at the eleventh hour, ordering of reagents that seemed never to arrive and a broken leg were humanly very good reasons for this research not to see the light of day but these were just a way of God telling us that with him everything is possible.

ACKNOWLEDGEMENT

I wish to express my foremost thanks to the Almighty God and Father for sustaining me throughout this course. My sincere thanks and gratitude to Dr. W.K.B.A. Owiredu for his selflessness, guidance and enthusiasm in steering me through this course. A lot of thanks goes to Prof. Francis Agyemang-Yeboah for believing in me when we had to change the topic due to emerging evidence in the said field and for his continuous interest and support throughout the period of preparation of this work. I am equally indebted to Dr. E. F. Laing for his direction and forbearance in the course of preparation for this thesis.

To all the Lecturers and Staff of the department of Molecular Medicine, School of Medical Sciences (SMS)-KNUST for their diverse support in this research, I say thank you.

My special thanks goes to Dr. Abraham Matey the Medical Superintendent of the Dunkwa Municipal Hospital for granting me permission to undertake this research at the facility and also to the Laboratory staff who assisted me all through the early and late hours of preparing participants, administration of questionnaires and collection of some of the samples. I am truly grateful.

To Dr. Anthony Afum-Adjei Awuah and Enoch Odame Anto I am immensely grateful for the time we spent analyzing and making meaning to the large data generated.

Last but not the least I wish to acknowledge my mother, Ms. Monica Mabel Gockah for her love, encouragement, support and personal interest in my professional development. I am also thankful to my family and friends especially Frimpomaa Agyemang for her continuous support and prayers. I thank you all and pray that the Lord bless you in all your endeavors.

ABSTRACT

Background: Anaemia is a known factor of complication in diabetes. Diabetic patients, compared to non-diabetics have been shown to be susceptible to a severe form of anaemia as Glomerular Filtration Rate (GFR) decreases. In spite of these, anaemia is unrecognized and often untreated in Diabetic patients in Ghana. Prevalence of anaemia; the haematobiochemical profile of diabetics and the association between anthropometric and renal function of diabetics under clinical management were studied at the Dunkwa Municipal

Hospital, Dunkwa-On-Offin, Upper Denkyira East, Central Region, Ghana.

Methods: A cross-sectional base line study for diabetics was conducted from January 2015 to March 2015 on diabetes mellitus (DM) patients attending the Dunkwa Municipal Hospital. A systematic random sampling technique was used to recruit the 103 participants who had an age range between 20 and 80 years.

Out of 6ml of venous blood collected from the participants; 4ml was dispensed into yellow capped gel separator test tubes for onward separation into Eppendoff tube for the biochemical assays and 2ml was dispensed into EDTA anticoagulant test tubes for haematological assays. The data were double entered and analysis performed using Microsoft excel and Graph Pad Prism version 5.0 with statistical significance set at $p<0.05$. Tests of normality for all groups were done using the D`Agostino and Pearson omnibus normality test. Additionally all graphs shown in this thesis were drawn using this software. The Mann-Whitney U test was used for comparison of two groups while the Kruskal Wallis test was used when comparing more than two groups. Test correlations were performed using the Spearman correlation test.

Results: A total of 103 DM patients were recruited for the study with 73(70.9%) being anaemic. 5.5% had renal insufficiency (<60 GFR ml/min/1.73m^2) while 94.5% had GFR >60 ml/min/1.73m^2 using the CKD-EPI criteria. Using the MDRD criteria, 4.1% of the patients had renal insufficiency (<60 GFR ml/min/1.73m^2) while 95.9% had GFR >60 ml/min/1.73m^2. There was a significant correlation between serum ferritin and anaemia and serum iron and anaemia (r=0.565; p<0.0001) and (r=0.333; p=0.0038) respectively.

Conclusion: Anaemia is a common complication of diabetes in the Dunkwa metropolis of the Central Region of Ghana, and it occurs early even in the absence of renal impairment which therefore necessitates early screening for anaemia and further studies to elucidate the possible aetiology.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADA: American Diabetes Association

Akt: Protein Ser/Thy Kinase B

ATP: Adenosine Triphosphate

BMI: Body Mass Index

Ca^{2+}: Calcium ion

CAT- 1: Carnitine Acyl Transferase

CHRPE: Committee on Human Research Publication and Ethics

CI: Confidence Interval

CKD: Chronic Kidney

Disease Cl^-: Chlorine ion cm :

Centimetre

DBP : Diastolic Blood Pressure

DCCT: Diabetic Control and Complications Trial

DKA: Diabetic Ketoacidosis

DM: Diabetes Mellitus

DN: Diabetic Nephropathy EDTA:

Ethylenediaminetetra acetic Acid eGFR:

estimated Glomerular Filtration Rate

EP: Erthrocyte Protoporphyrin

ESRD: End Stage Renal Disease

FBS: Fasting Blood Sugar

FFAs: Free Fatty Acids

fL: femtolitres g/dl:

Gram per Decilitre

GDM: Gestational Diabetes Mellitus

GFR: Glomerular Filtration Rate

GI: Gastrointestinal

GLUT: Glucose Transporter

GSK-3: Glycogen Synthase Kinase

Hb: Haemoglobin

HbA1c: Glycated Haemoglobin

HCT: Haematocrit

HHS: Hyperosmolar Hyperglycaemic State

HTN: Hypertension

IDDM: Insulin-dependent Diabetes Mellitus

IFG: Impaired Fasting Glycaemia

IGT: Impaired Glucose tolerance

IR: Insulin Receptor

K^+: Potassium ion

Kg/m^2 : Kilogram per Meter square

LDL – C: Low Density Lipoprotein Cholesterol

LVH: Left Ventricular Hypertrophy

MCH: Mean Cell Haemoglobin Count

MCV: Mean Corpuscular Volume

MDRD: Modification of Diet for Renal Disease Mg^{2+}: Magnesium ion mmHg : Millimeter mercury mmol/L: Millimole per Litre Na^+: Sodium ion

NaCl: Sodium Chloride

OGTT: Oral Glucose Tolerance Test

PDK: PI3- K – Dependent Kinase (PDK)-1

PEPCK: Phosphoenolpyruvic Carboxykinase

Pg: Picogram

PI3-K: Phosphatidylinositol 3-Kinase

PIP3: Phosphatidylinositol 3, 4, 5 – Phosphate

PO_4: Phosphate ion RDW: Red

Cell Distribution Width sTfR:

Serum Transferrin Receptor

T1DM: Type 1 Diabetes

T2DM: Type 2 Diabetes

TIBC: Total Iron Binding Capacity

UIBC: Unsaturated Iron Binding Capacity

WC: Waste Circumference

WHO: World Health Organization

WHR: Waist to Hip Ratio

WtHR: Weight to Height Ratio

β – Cell: Beta cells

μmol/L : Micromole per Litre

Chapter 1 INTRODUCTION

1.1 BACKGROUND

About 382 million people have been diagnosed with diabetes worldwide (WHO, 2013). About 90% of the cases are known to be type 2 diabetes, representing about 8.3% of the adult population (Mathers and Loncar, 2006). According to the WHO, diabetic related deaths were estimated at 1.5 million in 2012, thus the 8th leading cause of death with over 80% these deaths occurring in low and middle - income countries (Mathers and Loncar, 2006; WHO, 2013).

Diabetes is a major health problem, also affecting approximately 20.8million people in the United States (Cowie *et al.,* 2009). Diabetes mellitus could result from defects in insulin secretion, action or both and is characterized by chronic hyperglycaemia with disturbances in fat, carbohydrate and protein metabolism (WHO, 1999). Diabetes mellitus is a major global life - threatening disease with profound public health consequences. Untreated, diabetes could lead to many complications such as diabetic ketoacidosis, ketotic hyperosmolar coma, kidney failure, cardiovascular disease, cerebrovascular accidents, foot ulcers and retinopathy (Menz and Lord, 1999; Kitabchu *et al.,* 2009).

Diabetes has been significantly implicated in end - stage renal disease and the commonly associated anaemia (Herzog *et al.,* 2007). Furthermore, anaemia may be more common in diabetes (Weiner *et al.,* 2005) and is seen earlier in diabetic patients than in patients with renal function disorders of other etiology (Bonakdaran *et al.,* 2011). Investigation of anaemia is recommended when the Hb concentration falls below 12g/dl in women and

13g/dl in men. Anaemia is a common concern in patients with type 1 or type 2 diabetes mellitus and may occur in about 25% of the patients (Thomas *et al.,* 2003; Kanapuru *et al.,* 2007).

Contributing factors to anaemia in diabetic nephropathy (DN) include damage to the renal interstitium, systemic inflammation and autonomic neuropathy (Bonakdaran *et al.,* 2011). Furthermore, anaemia in diabetic patients is linked with a more rapid decline in the glomerular filtration rate (GFR) than in patients with kidney diseases of other type or etiology (Rossing *et al.,* 2006). Prior to measurable changes in GFR, renal dysfunction may be observed, as seen in many pathophysiological changes of DN.

Whether anaemia plays a direct role in the pathogenesis of DN or that of diabetic renal disease is yet to be proven. However, diabetic patients may suffer the effects of anaemia since many have marked cardiovascular disease and organ damage secondary to hypoxia. Thus haemoglobin levels may also be implicated in increased risk of cardiovascular events, hospitalization, and mortality (Levin *et al.,* 2002; Ezekowitz *et al.,* 2003; Keane *et al.,* 2003).

Anaemia can lead to falsely diminished levels of glycated haemoglobin (HbA1c), which may lead to under-management of hyperglycemia which in turn contributes to the progression of both micro and macro vascular diabetic complications (Kilpatrick *et al.,* 2004). The assertion that effective management of anaemia has marked positive outcomes in patients with failing renal function, has no scientific evidence except for improvement in the quality of life (Cody *et al.,* 2001; Thomas *et al.,* 2004b). Renal impairment is not marked in most patients with DN and as a result a majority of them are managed by their general physician or endocrinologist. Meanwhile significant pathology would have been

evident in such patients with DN before attaining the criteria for referral to a nephrologist (GFR - 30ml/min) (Reddan *et al.,* 2003).

1.2 PROBLEM STATEMENT

Prevalence of diabetes is on the increase globally as a result of population growth, aging, urbanization and the increasing prevalence of obesity and physical inactivity. Estimates of current and future diabetes prevalence have all shown increase. Evidence in medical research indicates that anaemia in diabetic patients is associated with a rapid decline in glomerular filtration rate (GFR) than in patients with kidney diseases of other cause (Rossing *et al.,* 2006). Therefore, this study is intended to assess unrecognized anaemia in patients with diabetes which would provide adequate knowledge in the management of diabetes mellitus in Ghana.

1.3 JUSTIFICATION

Several studies have been conducted in anaemia and diabetes in other countries. However limited studies have been conducted in Ghana and especially in the area of unrecognized anaemia in diabetes since Ghana has a different culture, geographical location and genetic makeup. This research seeks to find the prevalence of unrecognized anaemia in diabetes. Knowledge from this study will inform and enhance better management of patients with diabetes. All data derived from this research will increase knowledge and enhance early identification of anaemia in diabetics with the sole aim of improving diagnosis, treatment and ultimately prevention.

1.4 AIM OF THE STUDY

The study is aimed at assessing unrecognized anaemia in patients with diabetes visiting the Dunkwa Municipal Hospital to inform and enhance better management of patients.

1.5 SPECIFIC OBJECTIVES

1. To determine the prevalence of unrecognized anaemia in diabetic patients visiting the Dunkwa Municipal Hospital.
2. To determine the haemato-biochemical profile of diabetics under management at the Dunkwa Municipal Hospital.
3. To determine the correlation between anaemia and anthropometric indices as well as renal function test in the study subjects.

Chapter 2 LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus could result from defects in insulin secretion, action or both and is characterized by chronic hyperglycaemia with disturbances in carbohydrate, fat and protein metabolism (WHO, 1999). Insulin is a peptide hormone that is produced by the beta cells (β - cells) located in the islets of Langerhans of the pancreas. Insulin is used by liver, muscle

and adipose cells to retrieve glucose from the bloodstream for energy production (Alberti and Zimmet, 1998). When insulin is not present in sufficient levels or is unable to be used by the cells properly, glucose builds up in the bloodstream resulting in hyperglycaemia (Kitabchu *et al.*, 2009).

Diabetes mellitus is a major global life - threatening disease with profound public health consequences. Untreated, diabetes could lead to many complications such as diabetic ketoacidosis, ketotic hyperosmolar coma, kidney failure, cardiovascular disease, cerebrovascular accidents, foot ulcers and retinopathy (Menz and Lord, 1999; Kitabchu *et al.*, 2009). Acute complications include diabetic ketoacidosis and non ketotic hyperosmolar coma (Greene, 1986; Murphy, 1998; Pinhas-Hamiel and Zeitler, 2007). The prevention of diabetes involves a healthy diet, healthy lifestyle physical exercise and maintenance of a normal body weight. Insulin injections may be employed in the management of Type 1 diabetes whiles Type 2 diabetes may be managed with medications with or without insulin (Picot *et al.*, 2009).

2.2 CLASSIFICATION OF DIABETES MELLITUS

The two main types of diabetes mellitus (DM) are Types 1 and 2, though there are other rare forms of it. Type 1 diabetes mellitus (T1DM) is characterized by insulin deficiency resulting from pancreatic beta cell destruction. The aetiology of T1DM is either immune mediated, related to either damage to pancreatic tissue or idiopathic. However, the most prevalent form of DM, type 2 diabetes mellitus (T2DM) accounting for over 90% of all diabetes mellitus cases, presents as multiple metabolic abnormalities with relative insulin deficiency and prominent insulin resistance (WHO, 1999). Other categories of diabetes

mellitus are gestational diabetes mellitus (GDM) and impaired glucose tolerance (IGT) (Kuzuya and

Matsuda, 1997).

2.2.1 Type 1 Diabetes Mellitus

T1DM was previously known to clinicians as insulin - dependent diabetes mellitus (IDDM), because it results from autoimmune destruction of the cell responsible for insulin production

(β – cells of the pancreas). Insulin injections are thus required for glucose control and survival

(Rother, 2007). The destruction of the β - cells may eventually lead to a total loss of insulin production, which renders insulin dependent cells unable to properly metabolize glucose resulting in hyperglycaemia. This excess glucose is then passed out of the body in urine (Atkinson and Maclaren, 1994). Although the exact cause of type 1 diabetes mellitus remains unknown, it is believed that it is the result of the combination of autoimmune, environmental, and genetic factors (Atkinson and Maclaren, 1994). Traditionally T1DM was termed as juvenile diabetes because majority of it occurred often in children. It has however been shown to be prevalent amongst adults too (Mayer-Davis *et al.*, 2009).

T1DM has been associated with impaired counter-regulatory response to hypoglycemia and gastroparesis, irregular and unpredictable hyperglycemia, ketosis and serious hypoglycemia (Mayer - Davis *et al.*, 2009).

2.2.2 Type 2 Diabetes Mellitus

T2DM is usually characterized by defects in insulin secretion along with insulin resistance in cells (Orchard *et al.*, 2006). In these patients, the ability to produce insulin does not completely disappear, but they instead become increasingly insulin deficient and/or resistant which also results in hyperglycaemia. The disease presentation may range from insulin resistance with some loss of insulin production, to predominant loss of insulin secretion with some or no insulin resistance (Orchard *et al.*, 2006). It is typically not diagnosed until the development of complications. T2DM is due primarily to lifestyle and genetic factors (Risérus *et al.*, 2009). It is typically found to be associated with obesity, poor diet, stress, physical inactivity and urbanization (Risérus *et al.*, 2009).

2.2.3 Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is likened to T2DM in several respects as it involves defective insulin secretion and response. GDM is prevalent in about 2 - 10% of all pregnancies and may improve or disappear after delivery (Buchanan and Xiang, 2005). However, GDM persists in approximately 5 - 10% of women as DM of the second type postpartum (Beers *et al.*, 2006; Lapolla *et al.*, 2009).

Though, treatment outcomes for gestational diabetes is very positive, careful medical supervision throughout the pregnancy is strongly advised. Clinically advised dietary changes, blood glucose monitoring and in some cases insulin may be required. Gestational diabetes, if left untreated, can damage the health of the foetus or mother. Clinical evidence has shown skeletal muscle malformations, congenital cardiac and central nervous system

anomalies and macrosomia (high birth weight) as some of the risks to the baby (Turok *et al.*, 2003; Beers *et al.*, 2006).

2.3 CLINICAL STAGES OF DIABETES MELLITUS

The subtypes of diabetes mellitus are the insulin dependent and non-insulin dependent type (Kuzuya and Matsuda, 1997). Insulin is required for metabolic control in the insulin dependent subtype. Insulin may be secreted endogenously but has been shown to be largely insufficient. Exogenous insulin is therefore required to achieve normal blood glucose levels. The insulin independent subtype can be satisfactorily controlled by other drugs and dietary modifications other than insulin (WHO, 1999; ADA, 2003).

Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) are intermediate metabolic states between normal glucose levels and DM (Harris, 1989; Charles *et al.*, 1991; Nathan *et al.*, 2007). IFG refers to fasting glucose concentrations above the normal reference range but below diabetic thresholds. Whiles the IGT refers to fasting 2-hour post glucose concentrations above the normal reference range but below diabetic thresholds (Engelgau *et al.*, 1997). IGT and DM are often seen coexisting in some individuals with IFG based on the oral glucose tolerance test (OGTT) (Engelgau *et al.*, 1997; DCCT, 2004).

2.4 PATHOPHYSIOLOGY OF DIABETES MELLITUS

Deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of DM as it is the principal hormone involved in regulation of the uptake of glucose from the blood into the body's cells (Bao *et al.*, 1996). Sources of glucose for the body includes intestinal absorption of food, glycogen breakdown and gluconeogenesis (DeFronzo,

1997). Insulin is responsible for the hormonal management of glucose levels in the body. Its main mechanisms include inhibition of the breakdown of glycogen and gluconeogenesis, stimulating the storage of glucose into muscle cells and adipocyte in the form of glycogen (Bao *et al.*, 1996).

Insulin is released into the blood in response to blood glucose levels, typically after eating (Bao *et al.*, 1996), and used by the body's cells to absorb glucose from the blood to be used as fuel, converted into other needed molecules, or stored as glycogen. Lower glucose levels in the blood decrease the release of insulin and increase glycogenolysis. Glycogenolysis is mainly controlled by glucagon, which acts antagonizes insulin (DeFronzo, 1997). In the case low levels of insulin, insulin insensitivity or insulin resistance, there would be defective absorption and storage of glucose by the body. The clinical effects are poor protein synthesis, persistent hyperglycaemia among others (DeFronzo, 1997).

The kidneys reach the reabsorption threshold when blood glucose levels remain high over time and glucose will be excreted in the urine (glycosuria) (DeFronzo, 1997). Osmotic pressure of urine thus increases thereby inhibiting the reabsorption of water by the kidneys resulting in increased urine production (polyuria) and fluid loss. Furthermore, dehydration and increased thirst (polydipsia) will result from water held in the body's cellular stores being used to replenish the diminished blood volume (Bao *et al.*, 1996).

Although the central figure is hyperglycaemia, the effect of diabetes mellitus is not limited to carbohydrate metabolism. A significant role in the disease progression is played by lipid and protein metabolism. Abnormalities in glucose metabolism results in unregulated biochemical processes that glycosylate haemoglobin and other bodily proteins and lipids

(Bao *et al.*, 1996). The effects of the dysmetabolism in carbohydrates, lipids, and proteins include chronic organ failure and damage (Bao *et al.*, 1996).

2.5 METABOLIC EFFECTS OF INSULIN AND DIABETES MELLITUS

Insulin is synthesized by the beta cells (β - cells) of the pancreas. It is produced as proinsulin, a precursor, which is broken down to form insulin and C-peptide which are equivalent amounts into the portal circulation (Bao *et al.*, 1996). The secretion of insulin is mainly controlled by plasma glucose concentration. The mature insulin molecule comprises of the A and B polypeptide chains which are linked together by two inter - chain disulphide bridges with an intra - chain disulphide bridge present in the A chain (Frier *et al.*, 1981).

The principal function of insulin is the control of glucose uptake and utilization by peripheral tissues via the glucose transporter. Insulin also inhibits hepatic gluconeogenesis and glycogenolysis, and its activity is counteracted by glucagon, growth hormone, thyroxine, epinephrine (adrenaline) and cortisol (Frier *et al.*, 1981). Blood levels of insulin in T1DM are very low, whereas the opposite happens in T2DM (Frier *et al.*, 1981).

Research has established that a lot of factors essential for normal growth and development are dependent on insulin signaling at the respective target tissue. It has also been established that insulin resistance and insulin secretory dysfunction are involved in the pathogenesis of T2DM (Ferrannini, 1998; Weyer *et al.*, 2001).

After secretion, circulating insulin interacts with the insulin receptor (IR) which is a transmembrane tyrosine kinase at the target tissue. The receptor is expressed as a tetramer in an α- 2 - β - 2 configuration and joined by disulphide cross - bridges (Ebina *et al.*, 1985).

The receptor is rapidly reconfigured after binding with insulin resulting in an autophosphorylation of specific intracellular tyrosine residues of the β - subunits through a transphosphorylation mechanism further igniting the receptor's tyrosine kinase activity (Lee *et al.,* 1997).

The catalytic site of the tyrosine kinase is occluded in the inactive state by the activation - loop precluding access of ATP and various substances. ATP and other substrates reach the catalytic site secondary to conformational changes caused by the auto-phosphorylation of the tyrosine residues at positions 1158, 1162, and 1163 in the activation - loop (Hubbard *et al.,* 1994; Hubbard, 1997). Docking sites for downstream effectors are formed from the phosphorylated tyrosine residues (Feinstein *et al.,* 1993).

Primarily, mediation of the metabolic response to insulin is via the phosphatidylinositol 3 - kinase (PI3 - K) pathway. Following the association of the p85/p110 complex of PI3 - K with the insulin receptor substrate molecules, the PI3-K activity results in the production of phosphatidylinositol 3, 4, 5 - phosphate (PIP3) which binds to the PH domains of PI3 - K - dependent kinase (PDK) - 1 and Akt (protein Ser/Thr kinase B) (Czech and Corvera, 1999). PDK–1 is activated as a result, and in turn phosphorylates and activates Akt associated with the regulation of the translocation of glucose transporter (GLUT) 4, which is insulin - sensitive glucose transporter expressed by muscle and lipid cells (Czech and Corvera, 1999).

Stimulation of glycogenesis is also another key metabolic effect of insulin. Activation of glycogen synthase in response to insulin is mediated by glycogen synthase kinase - 3 (GSK - 3). The activation of Akt by insulin in turn activates GSK - 3 by phosphorylation thereby limiting its ability to inhibit glycogen synthase activity (Cross *et al.,* 1995). Insulin -

mediated activation of Akt reverses processes that inhibit protein synthesis (Welsh and Proud, 1993).

2.6 METABOLIC COMPLICATIONS OF DIABETES MELLITUS

2.6.1 Carbohydrate Metabolism

In the case of absolute or relative insulin deficiency, development of hyperglycaemia is consequent of increased gluconeogenesis, accelerated glycogenolysis or impaired glucose utilization by the peripheral tissues (Vaag *et al.,* 1992). Gluconeogenic precursors have been implicated in increased hepatic glucose production. This is complicated further by high levels of stress hormones in diabetic ketoacidosis (DKA) and hyperosmolar hyperglycaemic state (HSS) (Felig and Wahren, 1971; Foster and McGarry, 1983; Siperstein, 1992).

The major pathophysiological event responsible for hyperglycemia in diabetic patients is hyper production of glucose by the liver and kidney, with gluconeogenesis playing a major role than glycogenolysis (Schade and Eaton, 1980; Siperstein, 1992; Exton, 1987; Hue, 1987).

Levels of glucagon are in diabetics are higher than in non-diabetics. A high glucagon - to - insulin ratio inhibits production of fructose - 2, 6 – bisphosphate, an important metabolic regulator. This in turn stimulates the activity of fructose - 1, 6 - bisphosphatase (an enzyme that catalyzes the hydrolysis of fructose - 1, 6 - bisphosphate to fructose - 6 - phosphate) and inhibits the rate-limiting enzyme in the glycolytic pathway (phosphofructokinase) (Pilkis *et al.,* 1990). Gluconeogenesis is promoted further by the stimulation of

phosphoenolpyruvic carboxykinase (PEPCK) by the high glucagon - to - insulin ratio in the presence of increased cortisol in DKA (Pilkis *et al.*, 1990; Granner and Pilkis 1990.

Hydrolysis of glucose - 6 - phosphate to glucose is catalyzed by hepatic glucose - 6 – phosphatase and stimulated by increased catabolic hormones and decreased insulin levels. Major substrates for gluconeogenesis include alanine, glutamine, lactate and glycerol. These are provided by excess proteolysis and decreased protein synthesis which occurs as a result of increased catabolic hormones and decreased insulin (Felig and Wahren, 1971; Wasserman and Vranic, 1986).

Hyperglycaemia leads to glycosuria in DKA and HHS causing an osmotic diuresis, polyuria, dehydration, thirst and polydipsia, resulting in hypovolemia and decreased GFR, which increases the severity of hyperglycaemia. Some amounts of gluconeogenesis occur via the kidneys (Mayer-Davis *et al.*, 2009).

2.6.2 Lipid and Ketone Metabolism

Phosphorylation and activation of hormone - sensitive lipase in adipose tissue could occur as a result of insulin deficiency and increased concentrations of counter regulatory hormones, particularly epinephrine. This further results in a surge in ketone production (Jensen *et al.*, 1989; McGarry, 1979; Nurjhan *et al.*, 1992). The hyperactivity of tissue lipase initiates a breakdown of triglyceride into glycerol and free fatty acids (FFAs). Glycerol is used in the liver as a substrate for gluconeogenesis, whereas the massive release of FFAs express damage in the liver, where they serve as precursors of the ketoacids in DKA (McGarry, 1979; DeFronzo *et al.*, 1994).

The FFAs being stimulated by glucagon, are oxidized into ketone bodies in the liver. High glucagon levels in DKA block the metabolic conversion of pyruvate to acetyl – CoA thereby reducing the hepatic levels of malonyl – CoA (McGarry, 1979; Nurjhan *et al.*, 1992). Malonyl - CoA inhibits carnitine acyl transferase I (CAT - I), the rate - limiting enzyme for transesterification of fatty acyl - CoA to fatty acyl - carnitine, thereby regulating oxidation of fatty acids to ketone bodies.

Movement of FFA into the mitochondria for oxidation requires Carnitine acyl transferase I.

The increased fatty acyl - CoA and CAT - I activity in DKA, lead to increased ketogenesis (McGarry *et al.*, 1989; Nosadini *et al.*, 1989; Balasse and Fery, 1989; Zammit, 1994).

The role of glucagon in ketogenesis has been well established by research (Beylot *et al.*, 1991; Carlson *et al.*, 1993). Ketogenesis is mildly increased as a result of increasing plasma glucagon in insulin - deficient subjects (Beylot *et al.*, 1991). The role of cortisol in ketogenesis is however predictable in contrast to ambiguous actions of physiological or near - physiological concentrations of glucagon (Goldstein *et al.*, 1994).

Growth hormone has been shown to bring about a significant increase circulating levels of ketone bodies and FFAs (Press *et al.*, 1984; Moeller *et al.*, 1992).

Lipolysis in adipocytes is enhanced by epinephrine (adrenergic stimulation) thereby increasing concentrations of FFAs in plasma especially in the presence of insulin deficiency. In addition, epinephrine facilitates hepatic ketogenesis directly (Avagaro *et al.*, 1993).

Norepinephine plays a similar role but at concentrations similar to those observed in the synaptic cleft (Keller *et al.*, 1984).

2.6.3 Diabetic Ketoacidosis and Hyperosmolar Hyperglycaemic State

Considering type 1 and 2 diabetes, DKA and HHS are the two most serious complications present. They have respective mortality rates of about <5% and 15% (Basu *et al.*, 1992). Increasing age, coma and hypotension correlates significantly with poor prognosis (Malone *et al.*, 1992).

Diabetic ketoacidosis is complicated considering that it consists of hyperglycaemia, ketonaemia and acidaemia. It has been established that the degree of hyperglycaemia in DKA is quite variable and may not be a prominent cofactor of the severity of DKA. This however is not the case in HHS, where hyperglycaemia and hyperosmolality is more severe. While HHS may present with varying degrees of clinical ketosis, it often presents without coma. A significant correlation has been established between serum osmolality and mental status in both DKA and HHS (Ennis *et al.*, 1994).

DKA and HHS are major effects of defective carbohydrate regulation that can occur in diabetes mellitus (Polonsky *et al.*, 1994; Umpierrez *et al.*, 1997). Each condition can present in distinctive forms though there is a considerable overlap in metabolic symptoms (Ennis *et al.*, 1994).

Insulin deficiency in DKA can be total or insufficient relative to an excess of counter regulatory hormones. On the other hand, there is a reserve amount of insulin secretion that's sufficient enough in regulating ketosis but not hyperglycaemia. These results in marked

dehydration and defects in renal function leading to reduced excretion of glucose (Ennis *et al.*, 1994).

Infections such as pneumonia and those affecting the urinary tract have been implicated in the development of DKA or HHS accounting for about 30 - 50% of all cases (Ellemann *et al.*, 1984). Other implicates include pulmonary embolism, pancreatitis, alcohol abuse, drugs that alter carbohydrate metabolism among others (Petzold *et al.*, 1971; Ellemann *et al.*, 1984).

Recurrent ketoacidosis is an identifiable clinical problem which could occur as a result of poor compliance and some psychological factors leading to defaults in insulin therapy. This has been shown to be a major problem amongst African - Americans living in urban areas and medically indigent patients (Musey *et al.*, 1995; Morris *et al.*, 1997; Umpierrez *et al.*, 1997).

2.6.4 Water and Electrolyte Metabolism

Osmotic diuresis in DKA and HHS is largely due to hyperglycaemia. In DKA, ketoanions excreted via urine is significantly lesser than half of that of glucose on molar basis (DeFronzo *et al.*, 1975). In addition, certain electrolytes and minerals (PO_4, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and Na^+) are lost in marked amounts as a result of osmotic diuresis. Reabsorption of salt and water in the proximal and distal nephron and phosphate reabsorption in the proximal tubule are all stimulated by insulin. Thus hyperglycaemia, hyperketonemia (in DKA) and a deficiency in insulin, would result in severe disturbances in water and electrolytes balance in DKA and HHS (DeFronzo *et al.*, 1975; 1976).

Impairment of NaCl in the proximal tubule and loop of Henle occurs as a result of the osmotic effects of glycosuria. The strong ketoacids formed during DKA are fully dissociated at physiological pH. Thus, ketonuria ultimately leads to excretion of positively charged cations (Na^+, K^+, and $NH4^+$). Plasma bicarbonate buffering the hydrogen ions results in metabolic acidosis whiles retention of ketoanions leads to an increase in the plasma anion gap (DeFronzo *et al.*, 1976).

The extent of dehydration in HHS is usually greater than in DKA. This could be due to the more gradual onset and longer duration of metabolic decompensation in patients with HHS despite the absence of severe ketonuria (Wachtel *et al.*, 1987) and partially to the fact that these patients often present with an impaired fluid intake. Known co-factors include diuretic use, fever, diarrhoea, nausea and vomiting. This together with the higher average age of incidence of HHS and the presence of other associated diseases could explain the higher mortality of HHS (Wachtel *et al.*, 1987).

Increased plasma tonicity occurring as a result of hyperglycaemia and water loss leads to a loss of intracellular water and potassium. The presence of acidosis and intracellular protein breakdown due to insulin deficiency further aggravates the potassium shifts (Castellino *et al.*, 1987).

Increasing volume depletion leads to decreased glomerular filtration rate and greater glucose and ketoanion retention in plasma. Patients with a better dietary history during diabetic ketoacidosis present with greater degrees of hyperchloremic metabolic acidosis. Other findings include better kidney function, greater ketonuria, lower ketonaemia and anion gap (Adrogue *et al.*, 1982).

Patients with a poor dietary history however present with marked volume depletion, hyperosmolality, renal function impairment and a higher plasma anion gap due to plasma retention of glucose and ketoanions. These patients could also present with greater alteration of sensoria, commonly found in HHS than in DKA (Wachtel *et al.,* 1987). The inability to take fluid (often in elderly patients) plus other pathogenic mechanisms lead to greater hyperosmolality.

The urinary loss of ketoanions in turn results in loss of potential bicarbonate. In insulin treated DKA, hydrogen ions are consumed, as ketoanion metabolism is facilitated leading to regeneration of bicarbonate, correction of metabolic acidosis, and decrease in plasma anion gap recovered within few days (Halperin and Cheema-Dhadli, 1989).

2.7 DIAGNOSIS OF DIABETES MELLITUS

Clinical diagnosis of DM is often supported by symptoms such as polyuria with glycosuria, recurrent infections, polydipsia, unexplained weight loss, drowsiness and coma (Umpierrez *et al.,* 1997). Diagnosis of diabetes mellitus in non - pregnant individuals may be warranted by a single random blood glucose measurement above the diagnostic value, venous plasma ≥11.1 mmol/L and venous whole blood ≥10.0 mmol/L. Fasting glucose greater than the reference range (venous plasma ≥7.0 mmol/L, venous whole blood ≥6.1mmol/L) and/or 2 - hour post glucose load (venous plasma ≥11.1 mmol/L, venous whole blood ≥10.0 mmol/L) may confirm the diagnosis of DM in these individuals (Alberti and Zimmet, 1998).

Random blood glucose results lie within the indeterminate range (venous plasma ≥5.5 and

<11.1 mmol/L, venous whole blood ≥4.4 and <10.0 mmol/L) as well as intermediate fasting blood glucose levels may require further investigation by OGTT (Alberti and Zimmet, 1998).

Glycated haemoglobin (HbA1c), which is an alternative to blood glucose estimation, reflects the average glycaemia over the preceding 2 - 3 months (McCance *et al.*, 1994). Research has described HbA1c as an index of metabolic control for diabetic patients in clinical settings (Goldstein, 1984), and participants in epidemiological studies as well as a risk factor for developing micro and macrovascular complications (Moss *et al.*, 1994; Krolewski *et al.*, 1995).

Gestational diabetes mellitus (GDM) is diagnosed by a standard OGTT which is performed after an overnight fast (12 - 14 hours). Plasma glucose is measured after the fasting and the patient is given 100g of anhydrous glucose dissolved in 250 - 300ml of water. Plasma glucose is then measured at hourly intervals for 3hours after the glucose intake. Pregnant women whose results meet the WHO criteria for diabetes mellitus or IGT are classified as having Gestational Diabetes Mellitus (Alberti and Zimmet, 1998).

2.8 EPIDEMIOLOGY OF DIABETES MELLITUS

About 382 million people have been diagnosed with diabetes worldwide (WHO, 2013). Out of these patients, type 2 diabetes account for about 90% of the cases representing 8.3% of the adult population (Mathers and Loncar, 2006). Diabetes resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death (WHO, 2013). Over 80% of these deaths occurred in low and middle - income countries (Mathers and Loncar2006).

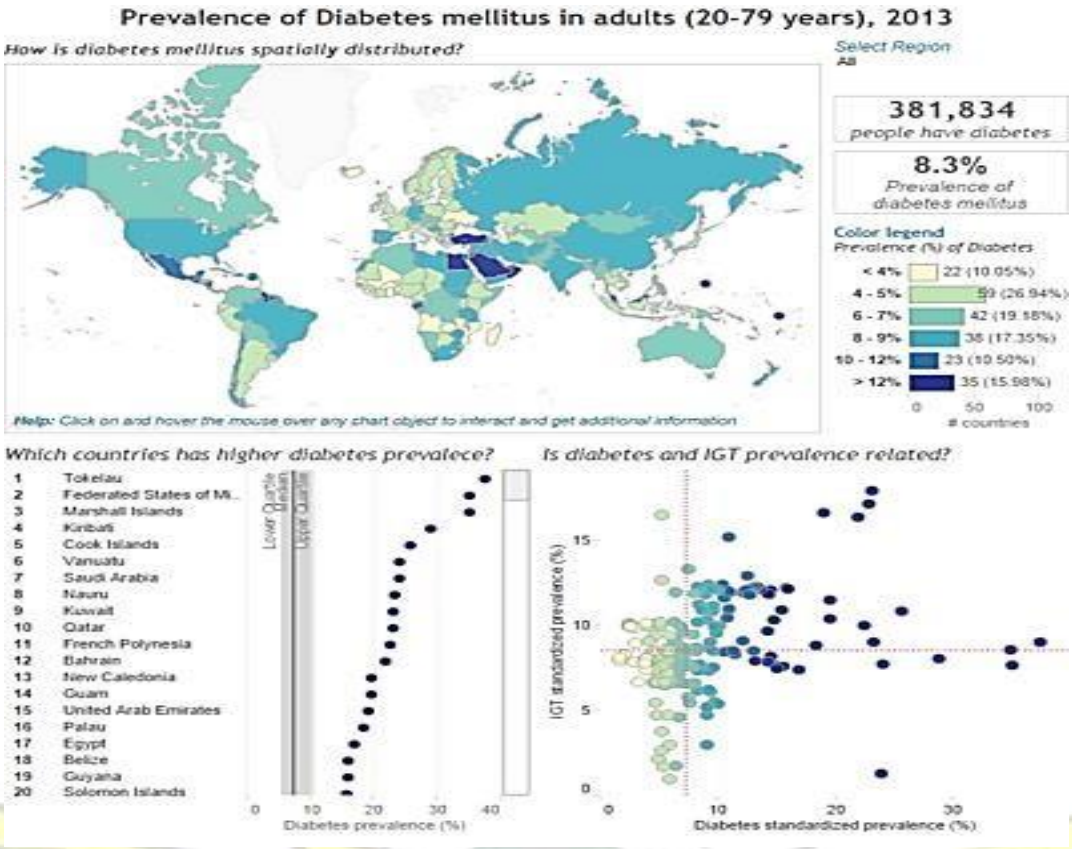


Figure 1 global estimates of diabetes for 2013 and projections for 2035, (Source: International Diabetes Federation Global Atlas 2013).

In 2010, 12.1 million were found to be suffering from diabetes in Africa (about 4% of the world total). This is expected to increase to 23.9 million by 2030, with the biggest increase in those aged 45-64 years (International Diabetes Federation, 2009). The number of affected patients in Africa is projected to double (at 24 million) in 20 years (Mbanya *et al.*, 2010: Hall *et al.*, 2011). Health systems in Africa are fraught with so many challenges such that adequate healthcare does not reach a considerable portion of the population thereby aggravating these incidence rates (Mbanya *et al.*, 2006).

Current estimates in Africa state that as of 2010, 35,700 children have type 1 diabetes, with

5,800 new cases diagnosed each year (International Diabetes Federation, 2009). Compared to developed countries, the mean age of presentation in Africa is later and in most countries there is a female preponderance (Kalk *et al.,* 1993).

2.9 DIABETES MELLITUS IN GHANA

In Ghana, data on the overall prevalence of DM are unavailable. However, prevalence studies have been carried out in most parts of the country. Rates in the Greater Accra Region were found to be 6.3% with age adjusted prevalence for DM, IFG and IGT being 6.4, 6.0 and 10.7% respectively (Amoah *et al.,* 2002a). It was also established by Amoah *et al.* (2002a) that prevalence amongst males (7.7%) were higher than in females (5.5%), and worsening of glycaemic status correlates with increase in age, body mass index, systolic and diastolic blood pressures.

Efficiency of fasting glucose and oral glucose tolerance test (OGTT) criteria for diagnosis has also been investigated (Amoah, 2002). Based on internationally accepted criteria, prevalence of undiagnosed diabetes mellitus by fasting (3.2%) and OGTT (3.1%) criteria were similar (Amoah, 2002), with both yielding cumulative prevalence of 4.5%. Prevalence of IGT (15.8%) was higher than that of IFG (10.7%).

A study conducted by Eghan *et al.* (2007) in Kumasi revealed that the prevalence of microalbuminuria in patients with diabetes as 43% and the significant predictors of microalbuminuria as duration of diabetes and serum concentration of creatinine. Prevalence autoimmune type 1 diabetes was found to be 7.5% in Kumasi with that of autoimmune diabetes in the total diabetic population as 19.2% (Agyei - Frempong *et al.,* 2008). Another study conducted by Brenyah *et al.* (2013) in Kumasi revealed that the frequency of

proteinuria in Ghanaians with type 2 diabetes mellitus as 13.8% and the significant predictors of proteinuria as age, hypertension and duration of the disease.

Severe beta - cell dysfunction and moderate reduction in insulin sensitivity contributed to the pathogenesis of type 2 DM in Ghana (Amoah *et al.,* 2002b). Dosoo *et al.* (2001) found significantly reduced total antioxidant levels in type 2 DM patients, suggesting existence of lower antioxidant defence in uncontrolled type 2 DM and hence use of antioxidant therapy in such patients.

Ocimum canum extracts are commonly used by diabetics to control blood sugar. Research in diabetic mice has shown that Aqueous extracts of *Ocimum canum* decrease levels of fasting blood glucose, serum total cholesterol, low density lipoprotein cholesterol (LDL - C), free radicals and body weight, while serum high density lipoprotein cholesterol (HDL - C) increased (Nyarko *et al.,* 1997; 2002).

Quality food, availability, cost-effectiveness and training of health care personnel in DM management and education are crucial factors in enhancing diabetic care (Amoah *et al.,* 1998; 2000; De - Graft, 2004).

2.10 ANAEMIA IN DIABETES MELLITUS

The WHO recommend anaemia investigation when the haemoglobin concentration is < 12g/dl in women and <13g/dl in men. Anaemia is a common concern in patients with type 1 or type 2 diabetes mellitus, and can occur in about 25% of the patients (Thomas *et al.,* 2003; Kanapuru *et al.,* 2007). The evaluation of anaemia in diabetic patients involves a routine history and physical examination. Routine laboratory tests employed include full

blood count, a reticulocyte count, serum iron, ferritin and total iron binding capacity estimations as well as a faecal test for occult blood (Macdougall *et al.*, 2007).

Untreated anaemia is known to contribute to functional morbidity, including impaired cognitive function, poor sleep, anorexia and depression in patients with DM. Physical activity, which is an important predictor of adverse outcomes and reduced quality of life in diabetes, is affected directly by the haemoglobin concentration, contributing to tiredness, fatigue, and reduced physical independence (Keane and Lyle, 2003). Also, anaemia may directly influence the development and progression of diabetic complications (Stevens *et al.*, 2003).

In the general population, a decrease in the mean haemoglobin concentration is associated independently with an increase in cardiovascular disease, stroke, and premature mortality as well as more complicated ischemic heart disease (Zeidman *et al.*, 2004). The major causes of anaemia in DM with chronic kidney disease (CKD) patients are iron and erythropoietin deficiencies and defective erythropoietin response. Also, in DM patients with CKD, iron deficiency anaemia could result from gastrointestinal bleeding, dietary deficiency and low intestinal absorption (Fishbane *et al.*, 2009).

Erythropoietin deficiency and hyporesponsiveness (probably caused by reduced renal mass, hormonal depletion, systemic inflammation and microvascular damage in the bone marrow) contributes to anaemia in diabetic patients with CKD (Erslev and Besarab, 1997; Thomas, 2007). Patients with hyporesponsiveness regularly require high doses of erythropoietin in order to raise the blood level of haemoglobin in the absence of iron deficiency (Erslev and Besarab, 1997; Thomas, 2007).

2.10.1 Anaemia and the Diabetic Kidney

More than 5% of newly diagnosed patients with T2DM may already have diabetic kidney disease, and a further 30 - 40% will develop diabetic nephropathy mostly within 10 years of diagnosis (Cooper *et al.,* 2005). In patients with diabetes worldwide, nephropathy constitutes one of the major risks for premature mortality. Anaemia is a major risk factor for the progression to end - stage renal disease (ESRD) in patients with CKD, with or without diabetes (Yokoyama *et al.,* 1997; Keane and Lyle, 2003; Ueda *et al.,* 2003)

Anaemia may not be directly involved in microvascular injury in the kidney or result in ESRD. It however, may modulate the pathways that lead to progressive renal damage in diabetes. Mitogenic and fibrogenic effects on the kidney as a result of hypoxia is associated with expression of multiple growth factors, hormones, vasoactive reagents, and enzymes (Fine *et al.,* 2000). Haemoglobin levels also correlate closely with oxidative stress because erythrocytes represent a major antioxidant component of the blood (Deicher and Horl, 2003).

It is conceivable that in the setting of diabetes, the combination of tissue hypoxia, oxidative stress, and reduced renoprotection associated with erythropoietin deficiency may act to enhance renal damage in the diabetic kidney (Deicher and Horl, 2003). In some patients with diabetes and anaemia, the renal capacity to produce erythropoietin is not simply abolished because the erythropoietin response to hypoxia may be preserved even though erythropoietin levels are inappropriately low for their degree of anaemia (Bosman *et al.,* 2002). This finding, together with the fact that erythropoietin levels remain in the normal range in most patients with diabetes and anaemia suggests that, erythropoietin synthesis and release pathways are not simply lost in the diabetic kidney (Bosman *et al.,* 2002).

The mechanisms leading to uncoupling of erythropoietin synthesis and haemopoiesis in diabetes remain to be established (Bardet *et al.,* 1991). However, it may be that continued hypoxic stress in the diabetic kidney has an important role in reducing its sensitivity to changes in haemoglobin levels and other haemopoietic stressors (Zou and Cowley, 2003).

Increased energy demands in the diabetic kidney, associated with salt retention, also may serve to induce a functional hypoxia in the tubulointerstitium. Considering diabetic nephropathy, erythropoietin levels correlate with fractional sodium reabsorption (Donnelly, 2001). Low haemoglobin levels and increased sodium reabsorption in the diabetic's nephron produce contradicting stimuli for erythropoietin production, potentially resulting in normal erythropoietin levels as a counterbalance to salt retention, but at the expense of anaemia (Thomas *et al.,* 2004ab). At a local level, inflammatory cytokines and the accumulation of oxidized nucleic acids, endogenous polyamines, cobalt, and tryptophan metabolites may inhibit the production of erythropoietin in the diabetic kidney (Thomas *et al.,* 2004b).

Autonomic dysfunction may play a role in erythropoietin deficiency in patients with diabetes. Polyneuropathy has been strongly correlated with anaemia associated with diabetes. Splanchnic denervation for example leads to a blunted production of erythropoietin in response to hypoxia (Finne and Skoglund, 1970; Winkler *et al.,* 1999; Bosman *et al.,* 2002).

There have been several prospective clinical studies involving diabetic patients with advanced nephropathy in which correction of anaemia with exogenous erythropoietin partly was able to attenuate the deterioration of renal function (Kuriyama *et al.,* 1997; Jungers *et al.,* 2001). However, this effect was overall less prominent in diabetic compared with

nondiabetic patients, possibly reflecting the key adjunctive role of other nonrenal factors in the anaemia associated with diabetes (Jungers *et al.*, 2001).

2.10.2 Anaemia and the Diabetic Heart

Cardiac dysfunction is common in patients with diabetes, in whom it is an independent risk factor for premature mortality. The prognosis of heart failure is significantly worse in patients with diabetes than in nondiabetic patients. A study had showed that structural and functional cardiac abnormalities are present in more than 70% of adults with T2DM (Srivastava *et al.,* 2006). Moreover, the kind of patients who have cardiac failure are those with increased age, hypertension, and renal impairment, who are also the same individuals at greatest risk for anaemia, as part of the cardio - renal - anaemia syndrome (Srivastava *et al.,* 2006).

Consequently, it is conceivable that unrecognized anaemia may contribute to the increased burden of cardiac dysfunction and the disproportionate increase in left ventricular mass observed in patients with diabetes (Srivastava *et al.,* 2006). Also, about 94% of diabetic patients with anaemia had evidence of some cardiac abnormality on echocardiography (Srivastava *et al.,* 2006). Anaemia has also been linked with onset of heart failure (Sandgren *et al.,* 2005), and a marker for poor outcomes in patients with established cardiac dysfunction, even after adjusting for conventional risk factors (Brucks *et al.,* 2004).

Chronic anaemia results in volume overload, increased cardiac output, increased heart rate, and ultimately, progressive left ventricular hypertrophy (LVH) and diastolic dysfunction. A modest decrease in haemoglobin concentration (10.5 g/dL) may independently be associated with a 33% increase in LVH (Levin *et al.,* 1999). However, a partial regression

in LVH may be possible after the correction of the anaemia in CKD (Portoles *et al.*, 1997; Hayashi *et al.*,

2000).

The correction of anaemia in individuals with heart failure is currently the matter of large and ongoing studies. Some studies have shown an improvement in cardiac function with regression of left ventricular mass after erythropoietin treatment (Hayashi *et al.*, 2000; Silverberg *et al.*, 2001). In addition, there is evidence that the number of hospitalizations may be reduced by correction of anaemia in diabetes. The correction of anaemia may also result in improved exercise tolerance and patient well - being as well as maintaining patients in a community setting (Ezekowitz *et al.*, 2003; Silverberg *et al.*, 2003).

2.11 MANAGEMENT OF DIABETES MELLITUS

2.11.1 General Management

Management of glucose levels is necessary to prevent diabetic associated complications. Ideally, diabetes management should be provided by a well - coordinated team of physicians, dieticians, pharmacists, laboratory scientists, nurses and mental health professionals (ADA, 2012). The age, physical activity level, diet, social situation and culture of the patients as well as the presence or absence of any complication must all be taken into consideration when designing specific treatment plans for diabetic patients. All treatment regimens, however, should have the same goal which is the maintenance of normal blood glucose level (ADA, 2012).

There are two standard ways of monitoring glucose control: patient self - monitoring of blood glucose and glycated hemoglobin (HbA1c). The timing and number of self -

monitoring of blood glucose depends on the particular needs and goals for the patient (ADA, 2012). Patients using multiple insulin injections are recommended to monitor at least three times per day, but for patients at higher risk for hypoglycemia more tests may be needed. It is very important that patients using this method of control be educated on how to properly use their glucose meter, know when to test, and how to adjust their diet, exercise, insulin intake or other treatment options accordingly (ADA, 2012).

Glycated haemoglobin (HbA1c) estimation is another way of assessing glucose levels in the blood. Haemoglobin is the major protein in the red blood cells and its main function is to transport oxygen in the blood stream (Nathan *et al.,* 2005). When glucose is present in the blood stream, it causes a glycation of the haemoglobin molecules. The levels of glycated haemoglobin can then be used as a measure of the average glucose levels (Nathan *et al.,* 2005).

Since red blood cells, and thus their haemoglobin molecules, are present in the blood stream for up to 90 days, this measurement can present a snap shot of the average glucose levels of a person for the last 3 months (Nathan *et al.,* 2005). Results from the Diabetes Control and Complications Trial (DCCT) have showed that patients who had HbA1c <7% have much better outcomes, and therefore most physicians use this as a general prognostic marker for their patients (ADA, 2012).

However, in children the acceptable HbA1c levels are slightly higher to reduce the risk of hypoglycaemia (Nathan *et al.,* 2005). It is recommended that the HbA1c tests be performed quarterly in patients who are not meeting glycaemic levels or whose therapy has changed. But in patients who are meeting their glycaemic levels and are under consistent therapy, may need only two tests per year (Nathan *et al.,* 2005).

There are several limitations to the glycated haemoglobin test. Patients with conditions that affect their red blood cells (haemolysis or those with haemoglobin variants) or those with high blood loss or anaemia may have inaccurate HbA1c levels. Also, as this test provides a picture of average control, it does not accurately reflect glycaemic variability within a patient. Therefore, a combination of both glycated haemoglobin and glucose monitoring methods may be the best option (ADA, 2012).

2.11.2 Specific Therapy Approaches

For those diagnosed with T1DM, insulin therapy is essential for survival and to reach normal glycaemic levels. There are currently several types of insulin available, each with a different activity. When insulin is normally secreted by the pancreas, hexamers are formed by the binding of six individual insulin molecules. These hexamers must be broken down into dimers and monomers before they can enter the bloodstream. Scientists have found ways to manipulate this characteristic as well as combining additional products to control the action time of injected insulin (ADA, 2012).

Rapid acting insulin (Lispro, Humalog, NovoRapid/NovoLog, Apidra) was developed in such a way that hexamer formation is greatly reduced, resulting in an absorption within 5 - 10 minutes, with peak action at 1 hour. Short - acting insulins (Actrapid, Humulin Regular S, Insuman Rapid) have the same molecular structure as those secreted from the pancreas and begin to work 20 - 30 minutes after injection with a peak at 1.5 - 2 hours and can last for up to 5 hours (ADA, 2012).

In people without diabetes, there is always a low level of insulin in the blood to cover the glucose that is released from the liver between meals. This is known as basal or background insulin. Ideally, an insulin regimen should mimic the profile of a non - diabetic person's natural response to prevent hyperglycemia and avoid hypoglycemia. Therefore basal insulin injections are also given to diabetic patients (ADA, 2012).

The Diabetes Control and Complications Trial showed that intensive insulin therapy (three or more insulin injections per day or continuous subcutaneous insulin infusion) in patients with T1DM improved blood glucose levels and decreased complication rates. However, it was also found that the intensive treatment increased the rate of severe hypoglycemia. It is therefore recommended that patients use multiple injections a day of basal and prandial insulin, choose doses of insulin based on carbohydrate intake at meals and predicted physical activity, and use newer insulin analogs (Nathan *et al.*, 2005). It is also important that patients with T1DM also be screened for additional autoimmune diseases such as thyroid dysfunctions, vitamin B12 dysfunctions and celiac disease if they have any symptoms (Nathan *et al.*, 2005).

Patients with type 2 diabetes mellitus usually require oral medication therapy to aid with their diabetes control. The three established types of these drugs; those that increase insulin production, those that improve insulin sensitivity, and those that delay carbohydrate absorption from the blood. There are several different drugs in each of these categories and the physician uses the patient's symptoms and contraindications to decide which of these drugs most appropriate (ADA, 2012).

Chapter 3 MATERIALS AND METHODS

3.1 STUDY POPULATION

A total of 103 clinically confirmed diabetic patients who have started chemotherapy were recruited from the diabetic clinic at the Dunkwa Municipal Hospital for the study.

3.2 STUDY AREA

Dunkwa-On-Offin or simply Dunkwa, is the capital town of the Upper Denkyira East Municipal District in the Central Region of Ghana and has a total population of about 33, 379 people and a landmass elevated 117m above sea level.

3.3 STUDY DESIGN

We conducted a cross-sectional base line study for diabetic participants with diabetic nephropathy visiting the diabetic clinic at the Dunkwa Municipal Hospital. The 103 participants recruited had an age range between 20 years and 80 years and were recruited participants between January 2015 and March 2015.

3.4 INCLUSION CRITERIA

- All patients with diabetes visiting the diabetic clinic at the Dunkwa Municipal Hospital

3.5 EXCLUSION CRITERIA

- Pregnant women
- Individuals with chronic infections e.g. HIV, Hepatitis B and Pulmonary Tuberculosis.

3.6 STATISTICAL ANALYSIS

The data were double entered and analysis performed using Microsoft excel and Graph Pad Prism version 5.0 (Graph Pad software San Diego Califonia, USA) with statistical significance set at $p<0.05$. Tests of normality for all groups were done using the D`Agostino and Pearson omnibus normality test. Additionally all graphs shown in this thesis were drawn using this software. The Mann-Whitney U test was used to compare of two groups while the Kruskal Wallis test was used when comparing more than two groups. Test correlations were performed using the Spearman correlation test.

3.7 DATA HANDLING

All data and information obtained from patients have been anonymized and cannot be linked to the patient in any way. Participants' names have not been used in my thesis, any publication or reports from this study. Only the principal investigator and co-investigators have access to the data. All samples collected in this study have been given code numbers. Only theses code numbers were used in processing and analysis of any results obtained from these samples. Also all data and information obtained from patients will not appear anywhere with names on it and cannot be linked to the participant in anyway.

3.8 ETHICAL APPROVAL

Ethical approval for this study was obtained from the Committee on Human Research, Publication and Ethics (CHRPE) at the School of Medical Sciences (SMS) at the Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi, Ghana. Approval was also given by the Dunkwa Municipal Hospital. A copy of the approval letters can be

found in the Appendix. Additionally, informed voluntary consent was sought from participants before questionnaires were administered.

3.9 ANTHROPOMETRIC MEASUREMENTS

Anthropometric measurements were taken which included the height in meters, weight in kilograms, waist circumference in centimeters, hip circumference in centimeters, arm circumference in centimeters, the Body Mass Index [Weight(kg)/Height^2 (m^2)] and the waist to hip ratio. A SECA (Japan) Weighing scale was used to measure weight, whiles a Shahe

Height Meter (Beijing, China) was used to measure height and Butterfly measuring tape (Beijing, China) was used to measure the waist and hip circumference. After patients have rested for 5 minutes blood pressure was measured twice by means of a fully automated medical Lloydspharmacy blood pressure device (Coventry, U.K) with patient in a sitting position and the averages used for analysis.

3.10 SAMPLE COLLECTION

About 6mls of venous blood sample was collected from participant by anticubital venepuncture after overnight fast (12-16hours) by a qualified biomedical scientists. A fasting blood sugar reading was immediately estimated using whole blood on an Accutrend glucometer (Indianapolis, USA). About 4mls of this blood was separated into yellow capped gel separator test tubes (Vacuette serum clot activator) for biochemical assays. These samples were centrifuged at 5000rpm for 5min and alliquoted into Eppendorf safe-lock micro centrifuge (volume 1.5ml) tubes for storage at -20˚C.

The remaining 2mls of whole blood was separated into EDTA (Ethylenediaminetetra acetic Acid) violet capped test tubes for hematological assays all under sterile conditions. Two blood film smears for blood film comments for each participant were immediately prepared and the Full Blood Count results were estimated using a calibrated automated Sysmex Hematology Analyzer within 2 hours of sample collection.

3.11 LABORATORY ASSAY

3.11.1 Preparing Samples.

Samples centrifuged at 5000rpm for 5min and aliquoted into Eppendorf safe-lock micro centrifuge (volume 1.5ml) tubes for storage at -20˚C (Figure 2).

Figure 2. The yellow capped tubes are BD Vacutainer SS II Advance REF 367955 tubes with gel separator for which whole blood is separated and transferred to the transparent Eppendorf tubes on the left.

3.11.2 Thin blood films preparation

To a clean grease free slide 2ul of whole well mixed blood is used in preparing a thin film which is allowed to air dry and fixed immediately with absolute Methanol for onward staining with 1 in 10 Giemsa (Rowmanosky stain) and washed with buffered distilled water, air-dried and examined under oil immersion at X100 objective lens.

Figure 3. Thin blood film preparations, Municipal Hospital Dunkwa

3.11.3 Automated assay of Serum Ferritin

The samples for the assay of Ferritin was thawed and the auto analyzer Siemens Immulite 1000 System (Erlagen, Germany) was calibrated with control standards. Upon passing the calibration the serum samples are loaded and the quantitative assay is started.

Method: Solid Phase Enzyme Labelled Chemiluminescent Immunometric Assay.

Principle: The solid phase (bead) was coated with monoclonal murine anti-ferritin antibody. The liquid phase consist of alkaline phosphatase (bovine calf intestine) conjugated to the polyclonal goat anti-ferritin antibody. Both Sample and bead were incubated together for 30 minutes during which ferritin formed an antibody sandwich complex with the monoclonal murine anti-ferritin antibody and the enzyme conjugated polyclonal goat anti-ferritin antibody on the bead and in the reagent respectively.

Centrifugal washes helped remove unbound sample and enzyme conjugate. Finally, the chemiluminescent substrate is added to the bead containing test unit and the signal is generated in proportion to the bound enzyme.

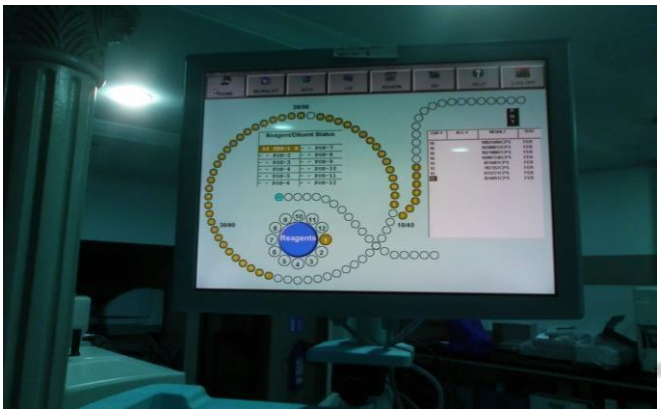


Figure 4. Serum Ferritin being assayed on a Siemens Immulite 1000 Auto analyzer-**Rayben Diagnostics Ghana**

3.11.4 Automated Assay of Serum Transferrin

Samples for the assay were thawed to room temperature from -20˚C while the Siemens Dimension Xpand Plus is calibrated with standard controls. Upon passing the calibration, the samples are loaded according to the batching codes and assayed quantitatively.

Method: Quantitative Turbidimetric Method

Principle: This method is based on the precipitation of transferrin by its polyclonal antibody.

Transferrin from serum or plasma reacts with its polyclonal antibody to form immune complexes. Addition of polyethylene glycol accelerates formation of these complexes. The resulting turbidity is measured by bichromatic end-point measurements at 340 and 700nm.

The increase in turbidity is proportional to the concentration of transferrin in the sample and the results are reported in grams per liter (g/L).

Figure 5 Transferrin Being Assayed on Siemens Dimension Xpand Plus Auto analyzer-

Rayben Diagnostics Ghana

3.11.5 Automated Assay of Serum Iron

Samples for the assay are thawed to room temperature from -20˚C while the Siemens Dimension Xpand Plus is calibrated with standard controls. Upon passing the calibration, the samples are loaded according to the batching codes and assayed.

Method: Colorimetric with precipitation method

Principle: Under acidic conditions, iron (Fe3+) bound to the protein transferrin is released. In the presence of the reducing agent ascorbic acid. (Fe^{3}+) is reduced to (Fe^{2}+) which forms a blue complex with 5, 5' (3-(2-pyridyl)-1, 2, 4-triazine-5, 6-diyl)-bis-2-furansulfonic acid disodium salt (Ferene).

The absorbance of the complex, measured using a bichromatic (600nm, 700nm) endpoint technique is directly proportional to the concentration of iron in the serum.

3.11.6 GLUCOSE

Glucose is enzymatically oxidized by glucose oxidase (GOD) to gluconic acid and hydrogen peroxide (H_2O_2). In the presence of peroxidase (POD), H_2O_2 produces the

oxidative coupling of phenol with 4-aminophenazone (AP) with maximum absorbance at 505 nm according to the following scheme:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\text{AP} + \text{phenol} \xrightarrow{\text{POD}} 4H_2O + \text{4-aminophenazone}$$

3.11.7 UREA

Urease specifically decomposes urea producing CO_2 and ammonia. The latter reacts with phenol and hypochlorite in alkaline medium yielding indophenol blue, which is calorimetrically measured at 540nm. This is known as Bethelot`s reaction (modified)

Salicylate was used to react with the ammonium ions to form a green complex, which is measured at 600nm

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

3.11.8 CREATININE

Serum or plasma is mixed with alkaline picrate reagent, which forms a yellow-red complex with creatinine. The absorbance of the complex is read at 490nm. A second absorbance reading after acidifying the solution corrects for the non-creatinine chromogens in the sample. This is known as the Jaffe reaction.

3.11.9 ION SELECTIVE ELECTRODE

This method uses a different principle for the determination of electrolytes. Most chemical analyzers are fitted with ISEs. Which usually contains Na^+ with glass membranes and K^+ electrodes with liquid ion-exchange membranes. High selectivity is one of its advantages.

The principle of potentiometry is employed and can be simply be stated as determination of change in electrometric forces(EMF) in the potential-measuring circuit between a measurement electrode(the ISE) and a reference electrode, as the selected ion(i.e. Na^+, K^+, Cl^- etc.) interacts with the membrane of the ISE. Low and high calibrator standard solutions containing the ion of the interest are run and the potential of the standard is determined and subsequently, the factor of change in EMF/Log concentration is stored in the microprocessor memory for the calculation of the unknown concentration when its EMF is measured. The concentration of the unknown is the presented in a digital output.

Chapter 4 RESULTS

4.1 Sociodemographic characteristics

Table 1 shows the sociodemographic characteristics of study participant categorized by gender. The mean age of participants was 57.71 years (SD 1.85). Higher number (68) of the participants were married. There was more female (61.8%) married participants than male (38.2) participants. Majority (79.6%) of the participants were employed. Higher proportion of unemployed participants were females (85.7%) than males (14.3%). A higher number of the participants had completed secondary education and most of them were females (64.9%) than males (35.1%). Most (58) of the participants had more than 5 years duration of diabetes and higher proportion was females (69.0%) than male (31.0%). More males (66.7%) compared to female (33.3%) participants took alcoholic beverages. More females (72.5%) compared to male did regular physical exercise (27.5%).

1 Sociodemographic, duration of diabetes and lifestyle characteristics of the studied

Table

participants stratified by gender

Variables	Total N=103	Male (n=30)	Female (n=73)
Age (Mean ± SD)	57.71 ± 1.85	57.74 ± 2.54	57.67 ± 1.15
Marital status Single	1	-	1(100.0)
Married	68	26(38.2)	42(61.8)
Divorced	15	2(13.3)	13(86.7)
Widowed	18	1(5.6)	17(94.4)
cohabiting	1	1(100.0)	-
Occupation Unemployed	21	3(14.3)	18(85.7)
informal	67	16(23.9)	51(76.1)
Formal	15	11(73.3)	4(26.7)
Level of education No education	27	3(11.1)	24(88.9)
Primary	33	8(24.2)	25(75.8)
Secondary	37	13(35.1)	24(64.9)
Tertiary	6	6(100.0)	-
Duration of diabetes (years)			
<5	45	12(26.7)	33(73.3)
5 and more	58	18(31.0)	40(69.0)
Alcohol intake			
Yes	9	6(66.7)	3(33.3)
Exercise Yes	80	22(27.5)	58(72.5)

4.2 Hematobiochemical profile categorised by diabetes only and both diabetes and hypertension

Table 2 summarises the hematobiochemical profile stratified by diabetic (DM) only and both diabetic and hypertension (DM + HTN). Participants with both DM + HTN were significantly older (60.29 ± 1.26years) compared to those with only DM (53.93 ± 1.79 years) ($p=0.0035$). Participants with both DM + HTN had a significantly increased BMI, WHR and eGFR compared to those with only DM ($p<0.05$). Hematobiochemical profile was not statistically significantly different between the two studied groups ($p>0.05$).

2 Hematobiochemical profile stratified by diabetic (DM) and both diabetic and

Table
hypertension (DM + HTN)

Parameters	Total N=103	DM Only (N=43)	DM + HTN (N=60)	p-value
Age (years)	57.11 ± 1.79	53.93 ± 1.79	60.29 ± 1.26	**0.0035**
WBC ($x10^3/\mu L$)	5.44 ± 0.21	5.51 ± 0.21	5.37 ± 0.25	0.6717
RBC ($x10^6/\mu L$)	4.39 ± 0.09	4.37 ± 0.09	4.41 ± 0.07	0.7285
Hb (g/dL)	11.51 ± 0.23	11.59 ± 0.23	11.44 ± 0.16	0.5793
HCT (%)	36.29 ± 0.76	36.24 ± 0.76	36.34 ± 0.56	0.9080
MCV (fL)	82.95 ± 1.06	83.44 ± 1.06	82.41 ± 0.88	0.4552
MCH (pg)	26.40 ± 0.32	26.60 ± 0.32	26.20 ± 0.28	0.3482
MCHC (g/dL)	31.81 ± 0.12	31.95 ± 0.12	31.66 ± 0.15	0.1636
RDW-CV (%)	10.49 ± 0.11	10.49 ± 0.11	10.49 ± 0.11	0.1579
RDW-SD (fL)	25.22 ± 0.36	25.24 ± 0.36	25.19 ± 0.26	0.9163
PLT ($x10^3/\mu L$)	189.4 ± 9.08	181.2 ± 9.08	197.6 ± 9.43	0.2284
PDW (fL)	14.58 ± 0.26	14.71 ± 0.26	14.44 ± 0.23	0.4438
FBS (mmol/L)	11.14 ± 0.89	11.56 ± 0.89	10.72 ± 0.87	0.5089
Urea (mmol/L)	3.73 ± 0.19	3.72 ± 0.19	3.73 ± 0.19	0.9627
Creatinine (µmol/L)	76.13 ± 4.13	78.07 ± 4.13	74.19 ± 3.11	0.4457
Transferrin (%)	2.17 ± 0.06	2.18 ± 0.07	2.16 ± 0.05	0.8340
Log_{10} Ferritin (ng/mL)	2.06 ± 16.54	2.03 ± 0.05	2.09 ± 0.03	0.3996
TIBC/UIBC	2.71 ± 0.07	2.72 ± 0.08	2.70 ± 0.06	0.8778
Serum iron levels (µmol/L)	12.89 ± 0.72	12.98 ± 0.72	12.80 ± 0.54	0.8760
BMI (kg/m^2)	24.15 ± 0.76	22.77 ± 0.76	25.53 ± 0.63	**0.0059**
WC (cm)	52.41 ± 3.62	51.93 ± 3.62	52.88 ± 3.12	0.8429
HC (cm)	57.36 ± 4.18	57.41 ± 4.18	57.31 ± 3.54	0.9856
WHR	0.92 ± 0.01	0.91 ± 0.01	0.93 ± 0.01	**0.0476**
CKD-EPI (GFR)	104.31 ± 2.11	96.52 ± 2.11	112.1 ± 3.72	**0.0418**
MDRD (GFR)	109.45 ± 3.02	107.2 ± 3.02	111.7 ± 6.82	0.1739

4.3 Hematobiochemical profile categorised by Metformin and non-metformin use

Mean MCH, MCHC, PLT and creatinine were significantly reduced while RDW-CV, and GFR (both criteria) were significantly elevated among metformin-treated diabetics compared to nonmetformin uses ($p<0.05$). Mean Hb, HCT, MCV, Ferritin were reduced among metformintreated diabetics but was not statistically difference compared to non-metformin uses ($p>0.05$) **(Table 3).**

Table 3 Hematobiochemical profile stratified by Metformin and non-metformin use

Parameters	Total N=103	Metformin use (N=76)	Non-metformin use (N=27)	p-value
Age (years)	58.04 ± 1.31	56.46 ± 1.31	59.62 ± 1.97	0.1457
WBC ($x10^3$/µL)	5.42 ± 0.19	5.41 ± 0.19	5.43 ± 0.31	0.9503
RBC ($x10^6$/ µL)	4.35 ± 0.06	4.43 ± 0.06	4.26 ± 0.10	0.1784
Hb (g/dL)	11.52 ± 0.15	11.48 ± 0.15	11.56 ± 0.30	0.8159
HCT (%)	36.32 ± 0.50	36.26 ± 0.50	36.37 ± 1.09	0.9178
MCV (fL)	83.56 ± 0.84	82.49 ± 0.84	84.62 ± 1.13	0.1795
MCH (pg)	26.63 ± 0.25	26.13 ± 0.25	27.12 ± 0.34	**0.0398**
MCHC (g/dL)	31.89 ± 0.12	31.65 ± 0.12	32.12 ± 0.13	**0.0397**
RDW-CV (%)	10.52 ± 0.09	10.74 ± 0.09	10.30 ± 0.12	**0.0125**
RDW-SD (fL)	25.20 ± 0.26	25.24 ± 0.26	25.15 ± 0.37	0.8456
PLT ($x10^3$/µL)	197.5 ± 7.81	182.7 ± 7.81	212.3 ± 11.78	**0.0491**
PDW (fL)	16.17 ± 0.32	16.74 ± 0.32	15.59 ± 0.53	0.0721
FBS (mmol/L)	10.80 ± 0.79	11.47 ± 0.79	10.13 ± 0.83	0.3684
Urea (mmol/L)	3.80 ± 0.15	3.63 ± 0.15	3.98 ± 0.28	0.2515
Creatinine (µmol/L)	78.71 ± 2.67	72.73 ± 2.67	84.69 ± 5.43	**0.0328**
Transferrin (%)	2.19 ± 0.05	2.13 ± 0.05	2.25 ± 0.08	0.1930
Log_{10} Ferritin (ng/mL)	2.08 ± 0.05	2.07 ± 0.03	2.08 ± 0.08	0.8231
TIBC/UIBC	2.74 ± 0.05	2.66 ± 0.06	2.81 ± 0.10	0.1909
Serum iron levels (µmol/L)	12.65 ± 0.05	13.17 ± 0.53	12.12 ± 0.83	0.3032
BMI (kg/m^2)	24.27 ± 0.60	24.54 ± 0.60	24.00 ± 0.97	0.6443
WC (cm)	49.83 ± 2.91	53.92 ± 2.91	45.73 ± 3.27	0.1243
HC (cm)	54.49 ± 3.305	58.89 ± 3.305	50.08 ± 4.05	0.1503
WHR	0.92 ± 0.01	0.92 ± 0.01	0.92 ± 0.01	0.9046
SBP (mmHg)	142.1 ± 3.05	144.1 ± 3.05	140.1 ± 3.22	0.4655
DBP (mmHg)	84.50 ± 1.69	84.65 ± 1.69	84.35 ± 1.99	0.9221
CKD-EPI (GFR)	93.96.0 ± 2.55	101.0 ± 2.55	86.92 ± 3.94	**0.0047**
MDRD (GFR)	100.45 ± 3.78	109.5 ± 3.78	91.40 ± 4.44	**0.0094**

4.4 Hematobiochemical profile stratified by anaemic and non-anaemic diabetic participants

Mean FBS, Urea and creatinine were significantly elevated while median iron, mean ferritin, and BMI were significantly reduced among diabetic associated with anaemia compared to nonanaemic counterparts ($p<0.05$). GFR (both CKD-EPI & MDRD) was reduced in anaemic compared to non-anaemic participants ($p>0.05$) **(Table 4).**

Table 4 Hematobiochemical profile stratified by anaemic and non-anaemic diabetic participants

Parameters	Total N=103	Hb status		p-value
		Anaemia (N=73)	Non-anaemia (N=30)	
Age (years)	56.95 ± 1.28	58.29 ± 1.28	55.60 ± 2.04	0.262
WBC ($x10^3$/µL)	5.57 ± 0.18	5.30 ± 0.18	5.84 ± 0.36	0.1421
RBC ($x10^6$/ µL)	4.53 ± 0.06	4.22 ± 0.06	4.84 ± 0.06	**< 0.0001**
Hb (g/dL)	11.69 ± 0.25	10.39 ± 0.25	12.99 ± 0.13	**< 0.0001**
HCT (%)	37.73 ± 0.46	34.48 ± 0.46	40.97 ± 0.42	**< 0.0001**
MCV (fL)	83.62 ± 0.82	82.00 ± 0.82	85.23 ± 1.09	**0.0297**
MCH (pg)	26.69 ± 0.24	26.00 ± 0.24	27.17 ± 0.37	**0.0095**
MCHC (g/dL)	31.73 ± 0.12	31.80 ± 0.12	31.66 ± 0.13	0.5217
RDW-CV (%)	10.61 ± 0.09	10.65 ± 0.09	10.56 ± 0.13	0.5871
RDW-SD (fL)	25.23 ± 0.28	25.15 ± 0.28	25.31 ± 0.20	0.7249
PLT ($x10^3$/µL)	189.50 ± 8.39	190.8 ± 8.39	188.2 ± 8.75	0.8594
PDW (fL)	16.58 ± 0.34	16.26 ± 0.34	16.90 ± 0.47	0.2952
FBS (mmol/L)	10.35 ± 0.78	11.97 ± 0.78	8.729 ± 0.82	**0.0178**
Urea (mmol/L)	3.61 ± 0.17	3.96 ± 0.17	3.26 ± 0.19	**0.0204**
Creatinine (µmol/L)	75.06 ± 3.21	77.77 ± 3.21	72.35 ± 3.19	**0.0361**
BUN/UREA	22.71 ± 0.93	23.95 ± 0.93	21.47 ± 1.09	0.128
Transferrin (%)	2.17 ± 0.05	2.17 ± 0.93	2.17 ± 0.07	0.9194
Log_{10} Ferritin (ng/mL)	2.09 ± 0.05	2.03 ± 0.04	2.14 ± 0.05	0.1117
TIBC/UIBC	2.72 ± 0.05	2.71 ± 0.06	2.72 ± 0.09	0.9233
Serum iron levels (µmol/L)	13.61 ± 0.06	11.85 ± 0.44	15.37 ± 0.09	**0.0002**
BMI (kg/m^2)	24.89 ± 0.58	23.53 ± 0.58	26.24 ± 0.89	**0.0127**
WC (cm)	51.94 ± 2.82	52.95 ± 2.82	50.93 ± 4.17	0.6969
HC (cm)	56.45 ± 3.27	58.29 ± 3.27	54.60 ± 4.55	0.5324
WHR	0.93 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	0.0952
SBP (mmHg)	142.8 ± 2.85	143.9 ± 2.85	141.7 ± 4.35	0.6822
DBP (mmHg)	84.08 ± 1.55	84.93 ± 1.55	83.23 ± 2.59	0.5645

CKD-EPI (GFR)	98.81± 2.69	95.52 ± 2.69	102.1 ± 3.72	0.1775
MDRD (GFR)	106.95 ± 3.22	102.2 ± 3.22	111.7 ± 6.82	0.1574

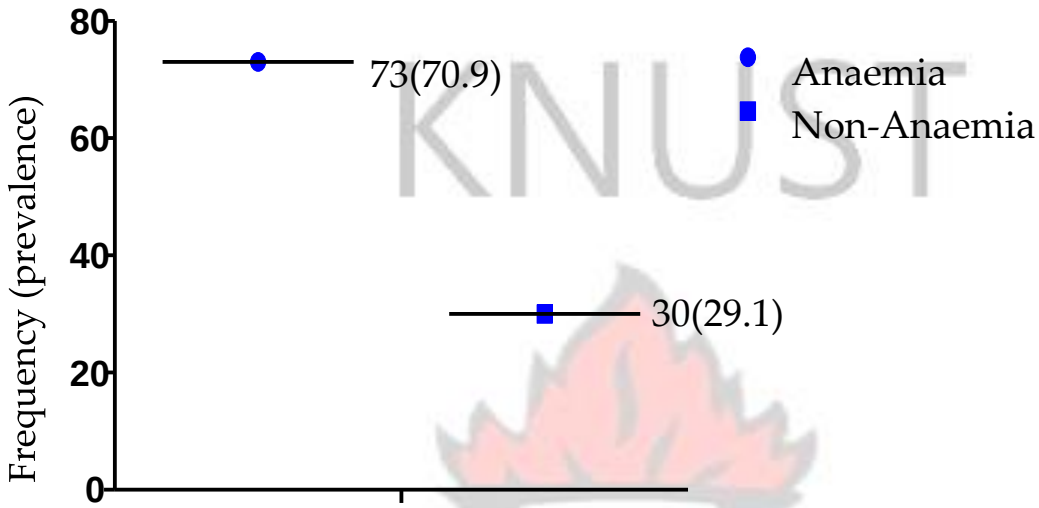


Figure 6 Prevalence of anaemic and non-anaemic diabetic studied participants

As shown in **Figure 6** the prevalence the prevalence of unrecognized anaemia was 70.9%.

Table

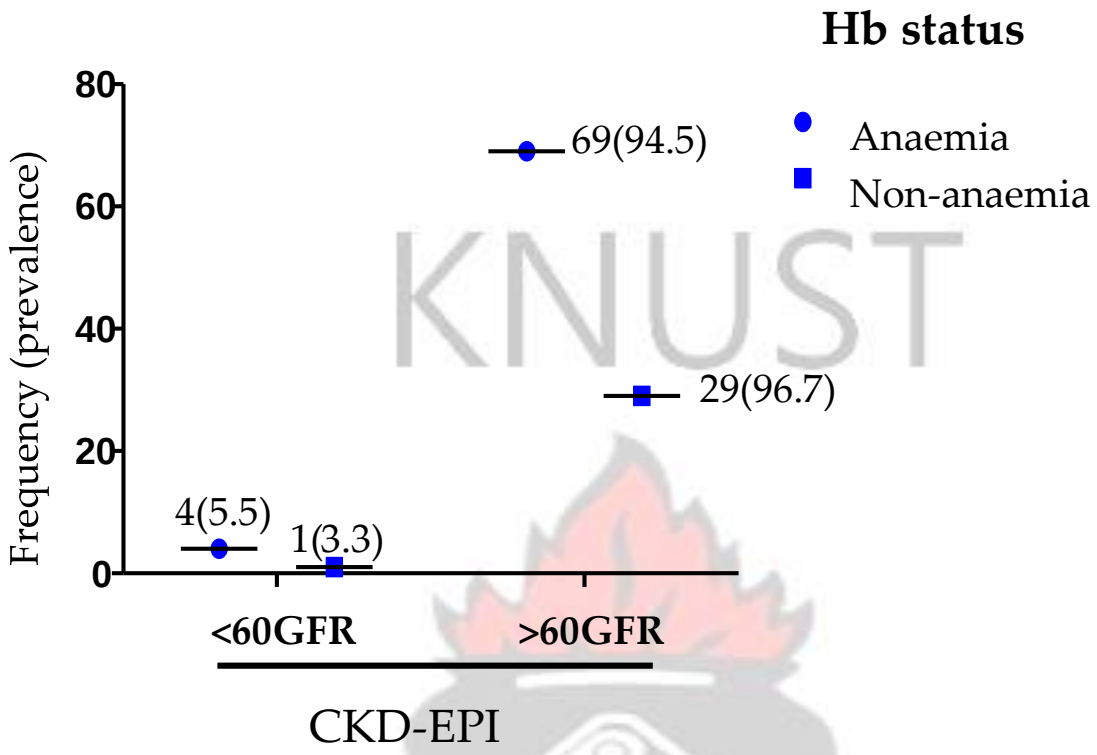


Figure 7 Prevalence of anemic and non-anemic diabetic participants stratified by renal insufficiency using CKD-EPI

As shown in **Figure 7**, Out of a total of 73 anaemic participants, 5.5% (4/73) had renal insufficiency (<60 GFR ml/min/1.73m^2) while 94.5% (69/73) had GFR >60 ml/min/1.73m^2 using the CKD-EPI criteria. One (1) person (3.3%) out of a total of 30 participants who were non-anaemic had renal insufficiency.

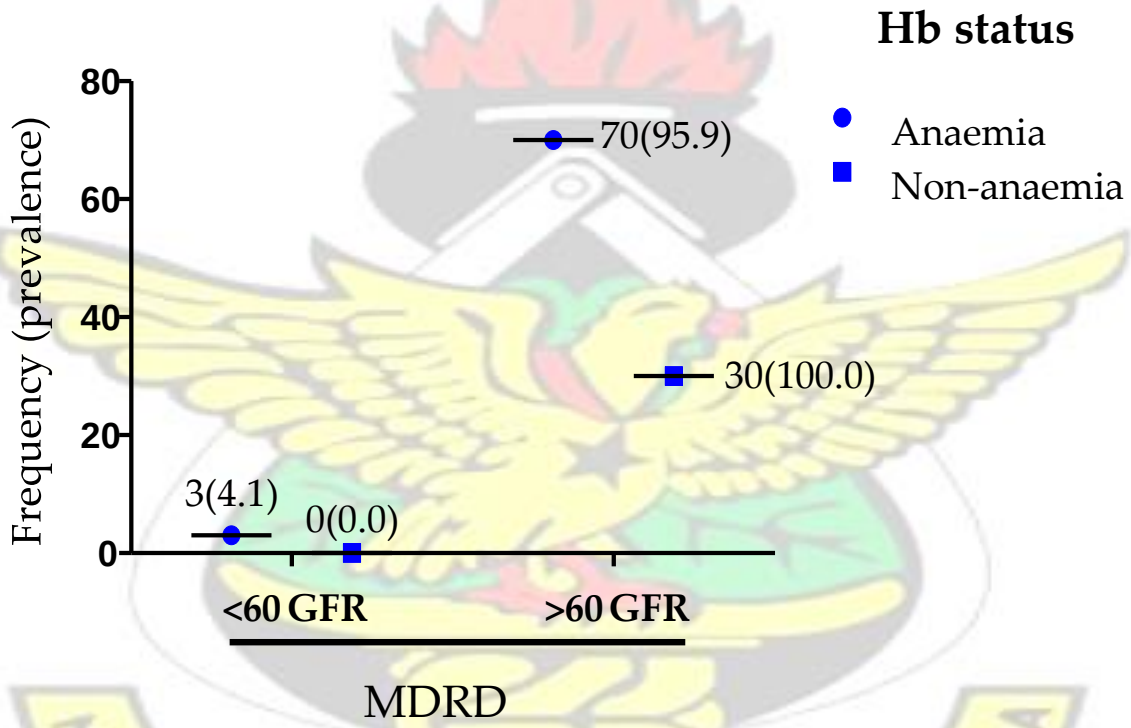


Figure 8 Prevalence of anemic and non-anemic diabetic participants stratified by renal insufficiency using Modification of diet for renal disease (MDRD).

As shown in **Figure 8**, Out of a total cohort of 73 anaemic participants, 4.1% (3/73) had renal insufficiency (<60 GFR ml/min/1.73m^2) while 95.9% (70/73) had GFR >60 ml/min/1.73m^2 using the MDRD criteria.

4.5 Association of gender, film comment and eGFR staging with haemoglobin status

A higher proportion of females (74.0%) were anaemic compared to male (63.3%) participants. Anaemia was more of microcytic (87.9%) (<80fL MCV) than normocytic (62.9%) (80-100fL MCV). Blood film comments showed 100.0% predominately microcytic cells with 95.2% mild to moderate hypochromasia, anisocytosis, poilkilocytosis and target cells. The proportion of anaemia was higher among participant with stage 4 CKD (100.0%) followed by those with stage 2 (79.3%), stage 3 (75.0%), and stage 1 CKD (66.7%) using CKD-EPI criteria. Conversely, proportion of anaemia was higher among participant with both stage 4 CKD and stage 3 CKD (100.0%) followed by stage 2 (77.7%) and stage 1 (67.1%) **(Table 5).**

Table 5 Association of gender, film comment and GFR staging with haemoglobin status

Variables	Total	Hb status		p-value
		Anaemia	Non-anaemia	
Gender				0.2802
Male	30(29.1)	19(63.3)	11(36.7)	
Female	73(70.9)	54(74.0)	19(26.0)	
MCV status				0.0091
Microcytic (<80fl)	33(32.0)	29(87.9)	4(12.1)	
Normocytic (80-100)	70(67.9)	44(62.9)	26(37.1)	
Film comment				
Red				
Mild to moderate hypochromasia, anisocytosis, poilkilocytosis, target cells	21(20.4)	20(95.2)	1(5.0)	0.0121
Predominantly microcytic cells	6(5.8)	6(100.0)	-	
Normochromic red cells	76(73.8)	47(61.8)	29(38.2)	
White cell and platelet				
Normal White cell and platelet count	101(98.1)	73(72.3)	28 (27.7)	0.0259
reactive lymphocytes	2(1.9)	-	2(100.0)	
Glomerulus filtration rate (GFR) mL/min/1.73 m2				
CKD-EPI				0.6702
>90	69(66.9)	46(66.7)	23(33.3)	
60-89	29(28.2)	23(79.3)	6 (20.7)	
30-59	4(3.9)	3(75.0)	1 (25.0)	
15-29	1(0.9)	1(100.0)	-	
<15	-	-	-	
MDRD				0.5859

>90	73 (70.9)	49 (67.1)	24 (32.9)
60-89	27(26.2)	21 (77.7)	6 (22.2)
30-59	2(1.9)	2 (100.0)	-
15-29	1(0.9)	1 (100.0)	-
<15	-	-	-

4.6 Association of gender and eGFR staging

Table 6 shows an association between gender and eGFR. Stage 4 CKD was mostly associated with females (100.0%) than males (0.0%) irrespective of the GFR criteria used. Meanwhile, the higher proportion of males compared to females were more like to have stage 3 CKD using both EPI criteria (75.0% vs 25.0%) and MDRD (100.0% vs 0.0%). There no significant association between gender and GFR ($p>0.05$).

Table 6 Association of gender and eGFR staging

Estimated Glomerulus filtration rate (GFR) mL/min/1.73 m2	Total N=103	Males N=30	Females N=73	
CKD-EPI criteria				0.0983
>90	69(66.9)	18(26.0)	49(71.0)	
60-89	29(28.2)	7(24.1)	22(75.9)	
30-59	4(3.9)	3(75.0)	1 (25.0)	
15-29	1(0.9)	-	1(100.0)	
<15	-	-	-	
MDRD criteria				0.0642
>90	73 (70.9)	22 (30.1)	51 (69.9)	
60-89	27(26.2)	6 (22.2)	21(77.8)	
30-59	2(1.9)	2 (100.0)	-	
15-29	1(0.9)	-	1 (100.0)	
<15	-	-	-	

4.7 Association between of haemoglobin status, anaemia and film comments with DM and metformin use

A higher proportion anaemia was common among participants with both DM+HTN (42.7%) compared to those with only DM (28.2%) (p=0.2697) and those with metformin treatment (52.4%) compared to those without metformin treatment (18.4%) (p=0.7862). Participants with both DM+HTN compared to those with only DM were mostly associated with microcytic anaemia (<80fL MCV) (22.3% vs 9.7%), microcytic red cell (3.9% vs 1.9% on film comment) and mild to moderate hypochromasia, anisocytosis, poilkilocytosis and target cells (12.6% vs 7.8%) (p>0.05). Similarly, participants with metformin treatment compared to those without metformin treatment were mostly associated with microcytic anaemia though not statistically significant (<80fL MCV) (27.2% vs 4.9%), microcytic red cell (4.9% vs 0.9% on film comment) and mild to moderate hypochromasia, anisocytosis, poilkilocytosis and target cells (16.5% vs 3.9%) (p>0.05) **(Table 7)**

Table 7 Association of haemoglobin status, anaemia and film comments with DM and metformin use

Variables	Total N=103	DM Only N=43	DM + HTN N=60	p-value	Metformin used N=76	Non-metformin used N=27	p-value
Hemoglobin status				0.2697			0.7862
Anaemia	73	29(28.2)	44(42.7)		54(52.4)	19(18.4)	
Non-anaemia	30	14(14.6)	16(15.5)		22(21.4)	8(7.8)	
MCV status				0.0703			0.1315
Microcytic (<80fL)	33	10(9.7)	23(22.3)		28(27.2)	5(4.9)	
Normocytic (80-100fL)	70	34(33.0)	36(34.0)		48(46.6)	22(21.4)	
Film comment Red blood cells				0.8807			0.7156
Mild to moderate hypochromasia, anisocytosis, poilkilocytosis, taget cells	21	8(7.8)	13(12.6)		17(16.5)	4(3.9)	
Predominantly microcytic cells	6	2(1.9)	4(3.9)		5(4.9)	1(0.9)	
Normochromic red cells	76	33 (32.0)	43(41.7)		54(52.4)	22(21.4)	
White cell and platelet count				0.8111			0.4003
Normal White cell and platelet count	101	42(40.8)	59(57.3)		74(71.8)	27(26.2)	
reactive lymphocytes	2	1(0.9)	1(0.9)		2(1.9)	0	

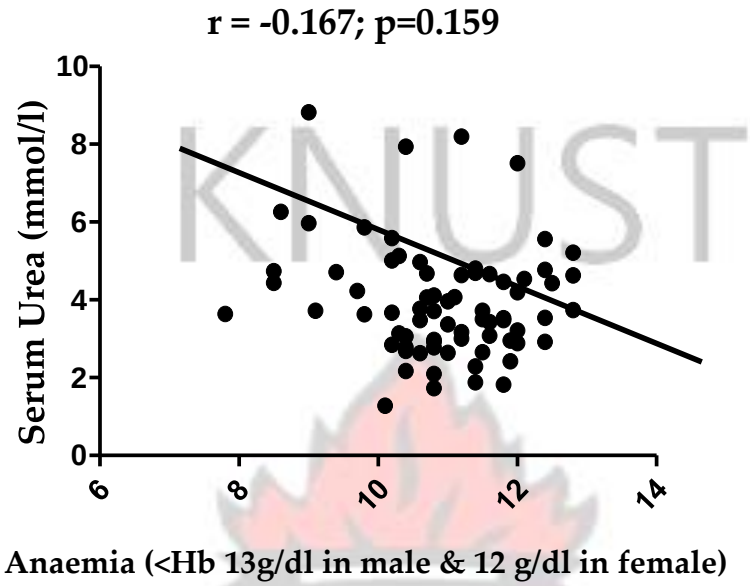


Figure 9 Scatterplot of Pearson correlation between serum urea and anaemia

As shown in **Figure 9**, there was non-statistically significant negative correlation between serum urea and anemia (r=-0.167; p=0.159).

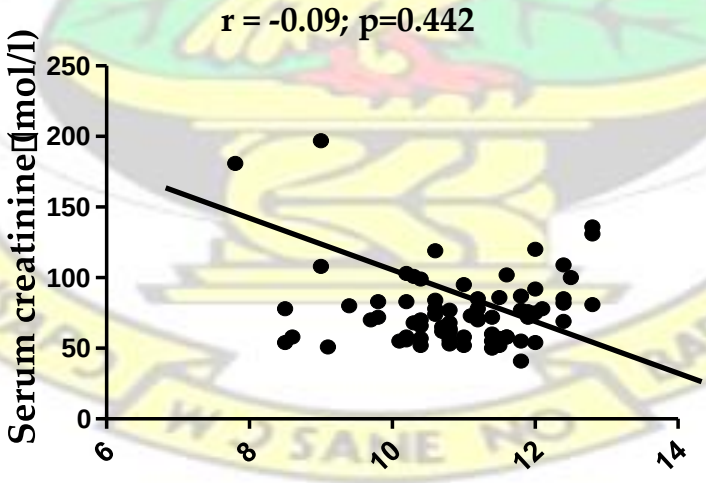


Figure 10 Scatterplot of Pearson correlation between serum creatinine and anaemia

As shown in **Figure 10**, there was non-statistically significant negative correlation between serum creatinine and anemia (r=-0.090; p=0.442).

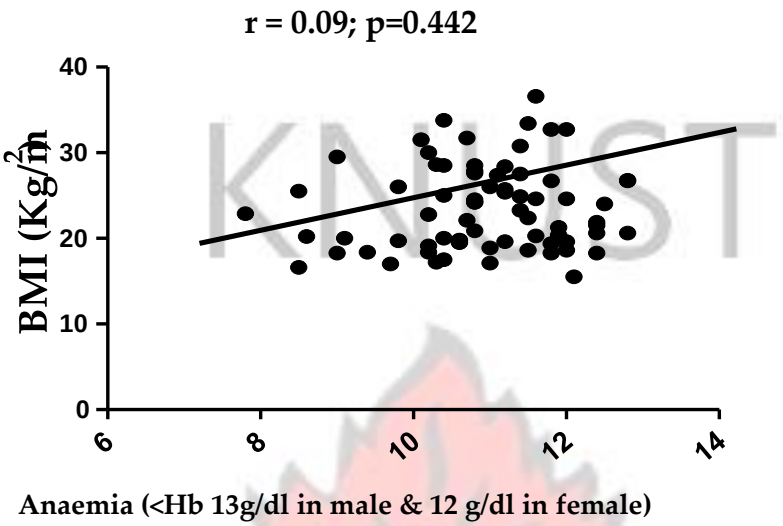


Figure 11 Scatterplot of Pearson correlation between BMI and anaemia

As shown in **Figure 11**, there was non-statistically significant positive correlation between BMI and anemia (r=0.09; p=0.442).

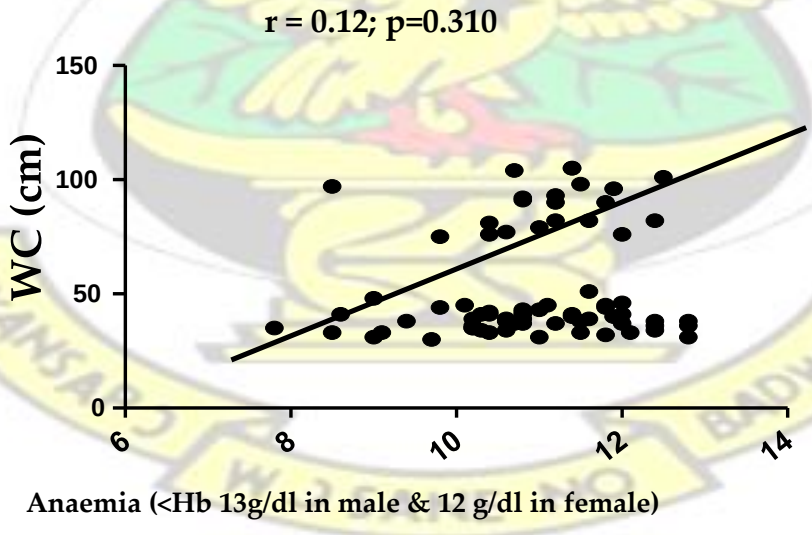


Figure 12 Scatterplot of Pearson correlation between WC and anaemia

As shown in **Figure 12**, there was non-statistically significant positive correlation between WC and anemia (r=0.12; p=0.310).

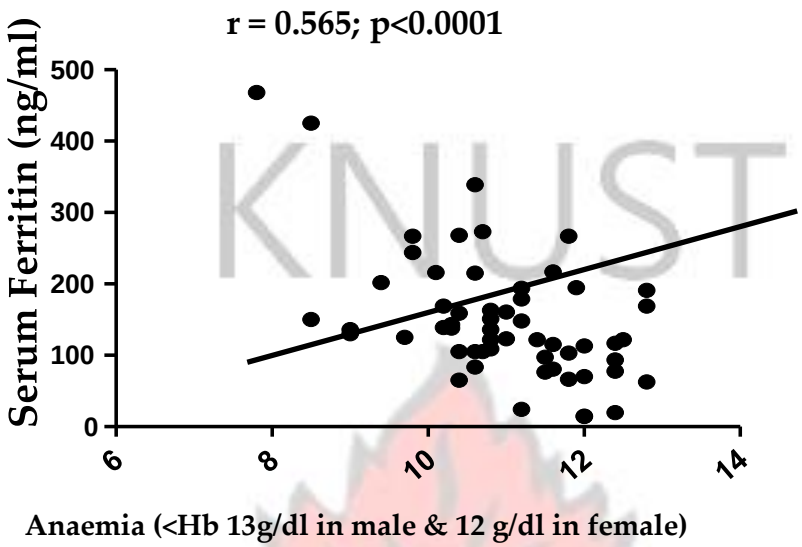


Figure 13 Scatterplot of Pearson correlation between serum Ferritin and anaemia

As shown in **Figure 13**, there was statistically significant positive correlation between serum ferritin and anaemia (r=0.565; p<0.0001).

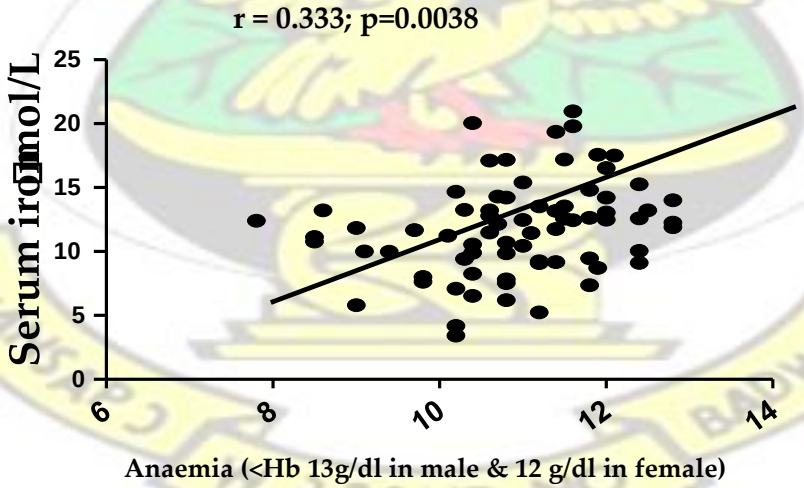


Figure 14 Scatterplot of Pearson correlation between serum iron and anaemia

As shown in **Figure 14** there was statistically significant positive correlation between serum iron and anaemia (r=0.333; p=0.0038).

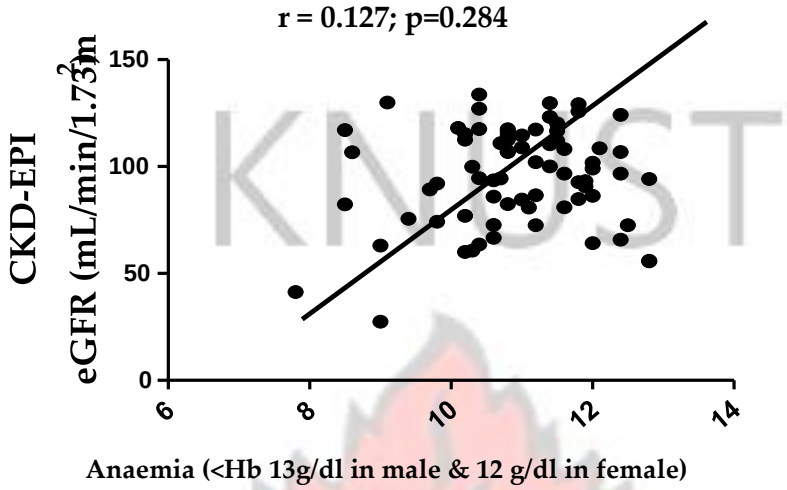


Figure 15 Scatterplot of Pearson correlation between CKD-EPI and anaemia

As shown in **Figure 15**, there was non-statistically significant positive correlation between eGFR and anaemia (r=0.127; p=0.284).

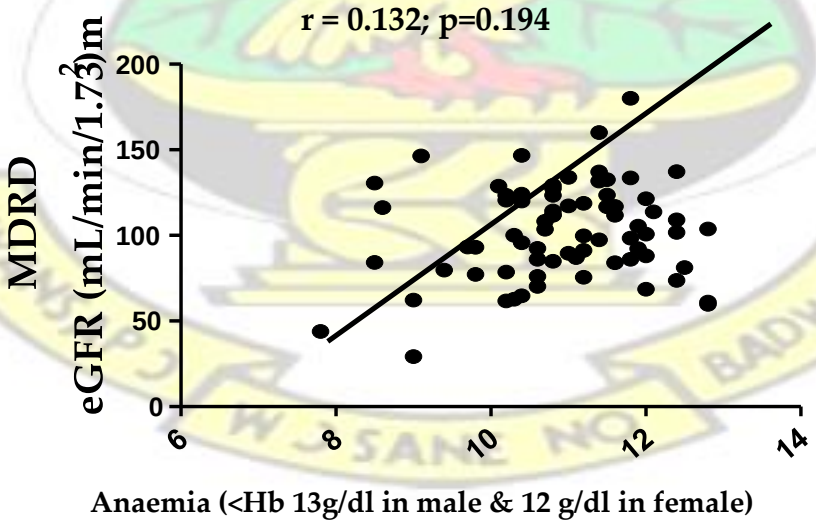


Figure 16 Scatterplot of Pearson correlation between MDRD and anaemia

As shown in **Figure 16**, there was non-statistically significant positive correlation between eGFR and anaemia (r=0.132; p=0.194).

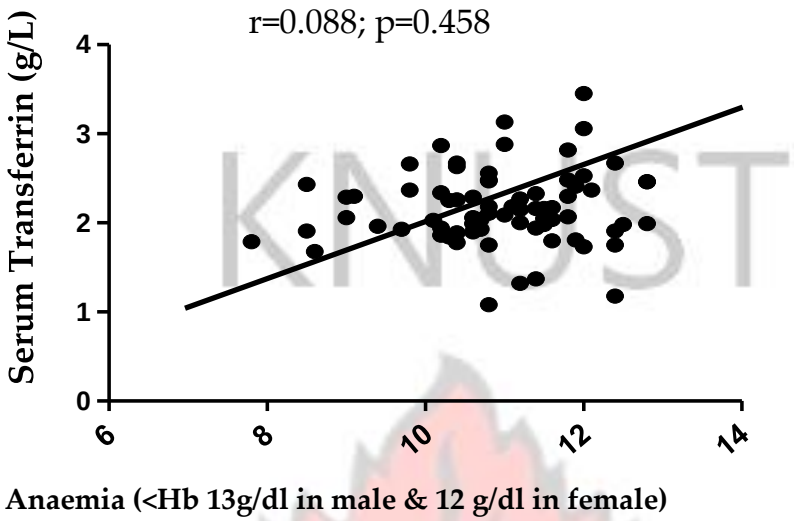


Figure 17 Scatterplot of Pearson correlation between serum transferrin and anaemia

As shown in **Figure 17**, there was non-statistically significant positive correlation between serum transferrin and anaemia (r=0.088; p=0.458).

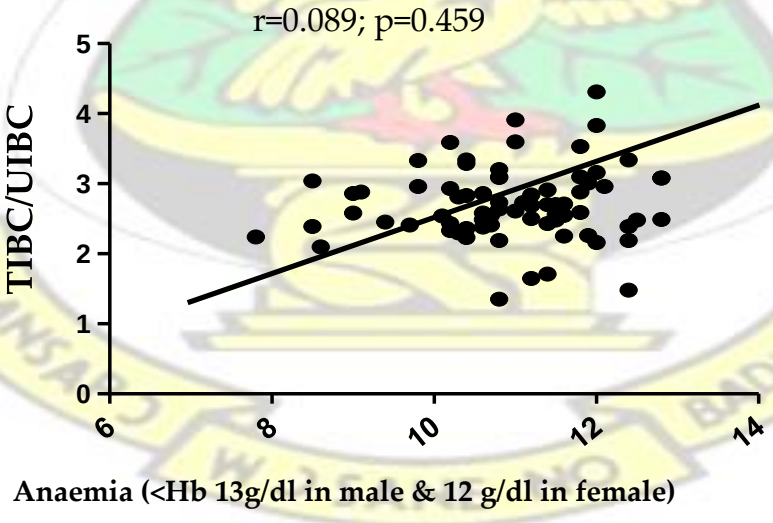


Figure 18 Scatterplot of Pearson correlation between serum TIBC/UIBC and anaemia

As shown in **Figure 18**, there was non-statistically significant positive correlation between TIBC/UIBC and anaemia (r=0.089; p=0.459).

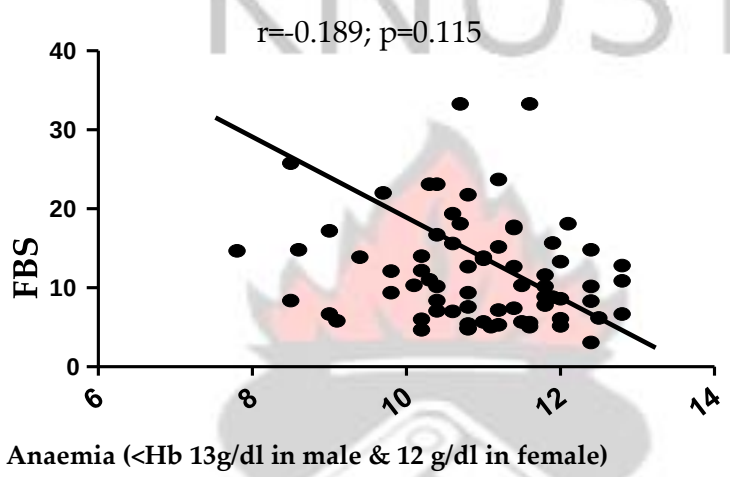


Figure 19 Scatterplot of Pearson correlation between FBS and anaemia

As shown in **Figure 19**, there was non-statistically significant negative correlation between FBS and anaemia (r=-0.189; p=0.115).

Chapter 5 DISCUSSION

Anaemia is common in diabetes and this may contribute to the pathogenesis of diabetic complications (Thomas *et al.,* 2004b; Stevens *et al.,* 2003). The prevalence of anaemia in this study is 70.9% with approximately a little over two in every three of the study participants having anaemia (73/103) as defined by the WHO gender specific guidelines. These results are about three times the prevalence rates established in previous studies

(19%-25%) (Thomas *et al.,* 2003; Kanapuru *et al.,* 2007). A study conducted at the University of Benin Teaching Hospital and the Igbinedion University Teaching Hospital in Nigeria also established an even lower prevalence rate of 15.3% (Adejumo *et al.,* 2012).

In this study, women constituted a higher percentage of study participants (Table 1). This observation is consistent with the findings of Wild *et al*. (2004) who have indicated that there are more women with diabetes than men. Women in the sub-region are more likely to be obese than men thus predisposing them to higher risk of developing DM (Hilawe *et al*. (2013). Women in Cameroon, South Africa and Uganda have been found to have higher prevalence of diabetes mellitus than men. The opposite was however observed in Ghana, Nigeria, Sierra Leon and rural areas of the United Republic of Tanzania (Wild et al., 2004).

Observations in this study indicates that study participants with both DM + HTN were significantly older (60.29 ± 1.26years) compared to those with only DM (53.93 ± 1.79 years) ($p=0.0035$). Participants with both DM + HTN had a significantly increased BMI, WHR and eGFR (using CKD-EPI) compared to those with only DM ($p<0.05$).

Haematobiochemical profile was however not statistically significantly different between the two groups ($p>0.05$).

This observation is consistent with the findings of Wild *et al.,* (2004) which indicates that even if the prevalence of obesity remains stable until 2030 though unlikely the incidence of diabetes will continue to increase as a consequence of population aging and urbanization. In the light of the importance of obesity as a risk factor for diabetes, obese individuals are at increased risk for diabetes, hypertension, renal failure and other cardiovascular disease. This assertion has also been made by Owiredu *et al*. (2008) in a study conducted among Penteco-charismatic Christians in Kumasi, Ghana. The relationship between obesity and hypertension has been widely described in research (Hall *et al.,* 2011). The complex relationship between aging and the development of hypertension as indicated by Aviv *et al.* (2001) supports the results of this study where participants with both DM + HTN were significantly older (60.29 ± 1.26years) compared to those with only DM (53.93 ± 1.79 years). The significant finding of an increase in the eGFR (using CKD-EPI) of participants with both DM+HTN compared to those with only DM ($p<0.05$) in this study is also consistent with observations made in other studies (Thomas *et al.,* 2004c; Stevens *et al.,* 2003). This observation however requires further scientific enquiry to help outline the basis for the disparity in eGFR for patients presenting with both HPT and DM on one hand and those presenting with only DM on the other hand. The CKD-EPI equation classifies fewer low-risk patients as having reduced eGFR, although it is also limited in detecting eGFR for definition of CKD stages (Murata *et al.,* 2011). This finding also makes it very necessary that the choice of measuring tools by researchers and clinicians should consider the clinical presentation of the patients.

In this study the incidence of anaemia in diabetic patients (Figures 7 and 8) did not corroborate with renal impairments as indicated in the studies of Stevens *et al.,* (2003). Out of the total number of anaemic diabetic participants in this study, the percentage of diabetic patients with GFR<60mL/min/1.73m^2 (5.5% and 4.1% respectively) for both (CKD-EPI and MDRD) was not comparable to the findings of other studies which indicated that anaemia may directly influence the development and progression of diabetic complications (Stevens *et al.,* 2003). In the work of Stevens *et al*. (2003), about 36.0% of the anaemic diabetic patients had GFR <60mL/min/1.73m^2. Contrary to the findings of this study, Thomas *et al*. (2006) have indicated that anaemia develops earlier and is more severe in patients with diabetes nephropathy compared to patients with renal impairment from other causes. However other studies have confirmed that the incidence of anaemia increases with decreasing renal function hence renal function of study participants strongly influences the total prevalence of anaemia in a study (Jones *et al.,* 2010). This has been supported by findings from the work of Bonakdaran *et al.* (2011). The duration of diabetes between the male and female study population was comparable with the numbers in both categories. Studies by Al-Khoury *et al.* (2006) shows that anaemia occurs earlier and is apparently more severe in chronic kidney disease (CKD) associated with diabetes rather than non-diabetic CKD. Also patients presenting with diabetes nephropathy were commonly shown to have a greater degree of anaemia than those presenting with other causes of renal failure with anaemia developing earlier in these patients than in those with renal impairment of other aetiology (Bosman *et al.,* 2001), This finding was in agreement with this study though anaemia had a higher prevalence of (70.9%) in the total population of diabetic it was though not largely as a result of renal impairment for which the majority of the study participants were anaemic.

The aetiology of anaemia in diabetes includes inflammation, concomitant autoimmune diseases, nutritional deficiencies, drugs, and hormonal changes in addition to kidney diseases (Abate *et al.,* 2013). This supports the argument that the high prevalence of anaemia in this study group (70.9%) could be as a result of other causes other than renal insufficiency. Iron deficiency anaemia is prevalent in patients with diabetes and chronic kidney disease (Mehdi *et al.*, 2009). In these patients, gastro-intestinal bleeding, dietary deficiency and low intestinal absorption may result in absolute iron-deficiency anaemia. Recent analysis of the National Health and Nutrition Examination Survey IV suggest that up to 50% of patients with CKD stages 2-5 have absolute or relative (functional) iron deficiency. Absolute and relative iron deficiency are common in CKD. The study site being the Dunkwa Metropolis is in the Central region which is classified as one of the poorest regions in Ghana. Ironically, the Dunkwa metropolis has increased mining activity and hence an increase in economic activity.

The major laboratory tests used in the diagnosis of anaemia and iron deficiency include the analysis of Hb, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), serum iron (Fe), total iron-binding capacity (TIBC), erythrocytes protoporphyrin (EP), and serum ferritin. The values for all these laboratory tests vary to some degree according to age and /or gender (Bothwell *et al.*, 1979; Yip *et al.*, 1984; Lewis *et al.*, 2004). Studies by Jolobe *et al.* (2000) indicated that MCH may be more reliable because it is less influenced by type of counting machine used and by storage. Raised red cell distribution width (RDW) predicts coexistent vitamin B_{12} or folate deficiency. In the absence of vitamin B_{12} or folate deficiency, microcytosis and hypochromia are sensitive indicators of iron deficiency. However, in sideroblastic anaemia and in some cases of anaemia of chronic disease microcytosis and hypochromia may be present. Hb electrophoresis is preferred when microcytosis is present in patients of suggestive

ethnicity to prevent unnecessary GI investigation. Biochemical markers of iron deficiency include low iron, low ferritin, low transferrin saturation, raised red cell zinc protoporphyrin, raised total iron-binding capacity and increased serum transferrin receptor (sTfR) with serum ferritin being the most useful test in the absence of inflammation.

The findings in this study are consistent with the diagnosis of iron deficiency anaemia using the WHO criteria and the findings of Lewis *et al.* (2004) and Jolobe *et al.* (2000). Although a significantly elevated red cell distribution width (RDW) was present in the Metformin treated diabetic participants compared to the non-metformin treated participants which ordinarily will often indicate a coexistent vitamin B_{12} or folate deficiency, their mean corpuscular cell volumes (MCV), Hb, HCT and Ferritin were reduced. The incidence of microcytosis which will reflect in the MCH and anisocytosis and poikilocytosis which will reflect in the RDW were comparable in this study. Additionally the correlation between serum iron and anaemia and serum ferritin and anaemia ($r=0.333$; $p=0.0038$), ($r=0.565$; $p<0.0001$) respectively were the only statistically significant correlations. The findings in this study also indicates that although there were statistically significant differences between the anaemic and non-anaemic diabetic participants and the metformin treated and metformin naive diabetics in the parameters of urea, creatinine, BMI, WC, eGFR, MDRD, Transferrin, TIBC/UIBC, FBS, platelets they all did not show statistically significant correlation with anaemia. Primarily dietary iron deficiency remains the major cause of anaemia although other factors including haemorrhage, infection, genetic disorders and chronic diseases contribute to the burden of anaemia (International Nutritional Anemia Consultative Group (INACG),

1979; 1989; Yip *et al.,* 1984; Hercberg and Galan, 1992).

It is interesting to note that no malaria infection was noticed in this study cohort after careful examination of their blood films. This could partly be as a result of the dry season within which the study was conducted. Additionally the Dunkwa metropolis is covered under the Anglo Gold Mass Malaria community spraying project and this could have accounted for the absence of malaria in this study participants.

A high prevalence of 70.9% of diabetics in this study have been identified to be anaemic and out of this proportion of anaemic diabetics there were (84.9%) identified to have mild anaemia and (15.1%) with moderate anaemia.

Chapter 6

6.1 CONCLUSIONS

Anaemia is a common complication with diabetes in this cohort of study participants and it occurs early even in the absence of renal impairment thus necessitating early screening for anaemia. The primary cause of the anaemia established in this study in the Dunkwaon-Offin metropolis is iron deficiency anaemia and thus further studies should be conducted to establish the cause of this presentation to ensure that remedial measures are implemented accordingly.

6. 2 LIMITATIONS OF THE STUDY

The comparison of the results of this study was done with countries whose sociodemographics could vary from this local setting due to paucity of data on prevalence of anaemia and cut offs for determining reference intervals.

The dietary intake of the study participants could not be assessed and their possible impact on the nutritional status needs further investigation.

6. 3 RECOMMENDATIONS

The management of diabetes should strongly include the screening for anaemia nationwide. Basic haematological assays of anaemia should be routinely added to the monitoring and management of diabetics since early management of anaemia will decrease if not prevent further complications.

The recruitment of future participants for such cohorts should be matched by gender to allow for wider comparable inferences.

Future studies in this research should include the assays of vitamin B_{12} and folate although their incidence are in the minority indicated by other indices.

REFERENCES

Abate, A., Birhan, W., and Alemu, A. (2013). Association of anaemia and renal function test among diabetes mellitus patients attending Fenote Selam Hospital, West Gojam, Northwest Ethiopia: a cross sectional study. *BMC hematology*, **13**(1): 6.

Adrogue, H. J., Wilson, H., Boyd, A. E., Suki, W. N. and Eknpyan, G. (1982). Plasma acid base patterns in diabetic ketoacidosis. *New England Journal of Medicine*, **307**: 1603 - 1610.

Agyei - Frempong, M. T., Titty, F. V., Owiredu, W. K. and Eghan, B. A. (2008). The prevalence of autoimmune diabetes among diabetes mellitus patients in Kumasi, Ghana. *Pakistan Journal of Biological Sciences*, **11**(19): 2320 - 2325.

Alberti, K. G. and Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic medicine : Journal of the British Diabetic Association*, **15**(7): 539 - 553.

Al-Khoury, S., Afzali, B., Shah, N., Covic, A., Thomas, S., and Goldsmith, D. J. (2006). Anaemia in diabetic patients with chronic kidney disease—prevalence and predictors. *Diabetologia*, **49**(6): 1183-1189.

American Diabetes Association (2003). Treatment of hypertension in adults with diabetes. *Diabetes Care*, **26**: 80 - 82.

American Diabetes Association (ADA). (2012). Executive summary: Standards of medical care in diabetes - 2012. *Diabetes care*, **35**(1): S4 – S10.

Amoah, A. G. (2002). Undiagnosed diabetes and impaired glucose regulation in adult Ghanaians using the ADA and WHO diagnostic criteria. *Acta Diabetologica*, **39**(1): 7 - 13.

Amoah, A. G., Owusu, S. K. and Adjei, S. (2002a). Diabetes in Ghana: a community based prevalence study in Greater Accra. *Diabetes Research in Clinical Practice* **56**(3): 197 - 205.

Amoah, A. G., Owusu, S. K., Acheapong, J. W., Agyenim - Boateng, K., Asare, H. R., Owusu, A. A., Mensah - Poku, M. F., Adamu, F. C., Amegashie, R.A., Saunde, J. T., Fang, W. L., Pastors, J. G., Sanborn, C., Barrett, E. J. and Woode, M. K. (2000). A national diabetes care and education programme: the Ghana model. *Diabetes Research in Clinical Practice*, **49**(2-3): 149 - 157.

Amoah, A. G., Owusu, S. K., Saunders, J. T., Fang, W. L., Asare, H. A., Pastors J. G., Sanborn, C., Barrette, E. J., Woode, M. K. and Osei, K. (1998). Facilities and resources for diabetes care at regional health facilities in Southern Ghana. *Diabetes Research in Clinical Practice*, **42**(2): 123 - 30.

Amoah, A.G., Owusu, S.K., Schuster, D.P. and Osei, K. (2002b). Pathogenic mechanism of type 2 diabetes in Ghanaians - the importance of beta cell secretion, insulin sensitivity and glucose effectiveness. *South African Medical Journal*, **92**(5): 377 - 84.

Atkinson, M. A. and Maclaren, N. K. (1994). The pathogenesis of insulin-dependent diabetes mellitus. *The New England journal of medicine*, **331**(21): 1428 - 1436.

Avagaro, A., Gnudi, I., Valerio, A., Maran, A., Miola, M., Opportuno, A., Tiengo, A. and Bier, D. E. (1993). Effects of different plasma glucose concentrations on

lipolytic and ketogenic responsiveness to epinephrine in type 1 diabetic subjects. *Journal of Clinical Endocrinology and Metabolism*, **76**: 845 - 850.

Aviv A. (2001). Pulse pressure and human longevity. *Hypertension*. **37**(4):1060-6.

Balasse, E. O. and Fery, F. (1989). Ketone body production and disposal: effects of fasting, diabetes and exercise. *Diabetes/Metabolism Research and Reviews*, **5**: 247 - 270.

Bao, W., Srinivasan, S. R. and Berenson, G. S. (1996). Persistent elevation of plasma insulin levels are associated with increased cardiovascular risk in children and young adults: the Bogalusa Heart Study. *Circulation*, **93**: 54 - 59.

Bardet, S., Rohmer, V. and Maugendre, D. (1991). Acute insulin response to intravenous glucose, glucagon and arginine in some subjects at risk for type 1 (insulindependent) diabetes mellitus. *Diabetologia* **34**: 648 - 654.

Basu, A., Close, C. F., Jenkins, D., Krentz, A. J., Nattrass, M. and Wright, A. D. (1992). Persisting mortality in diabetic ketoacidosis. *Diabetes Medicine*, **10**: 282 - 289.

Beers, M. H., Robert, S., Porter, Jones, T. V., Kaplan, J. L. and Berkwits, M. (2006) Abnormalities of Pregnancy. *The Merck Manual Professional online Medical Library*.

Beylot, M., Picard, S., Chambrier, C., Vidal H., Laville, M., Cohen, R., Cotisson, A. and Mornes, R. (1991). Effect of physiological concentrations of insulin and glucagon on the relationship between non esterified fatty acid availability and ketone body production in humans. *Metabolism*, **40**: 1138 - 1146.

Bonakdaran, S., Gharebaghi, M., and Vahedian, M. (2011). Prevalence of anaemia in type

2 diabetes and role of renal involvement. *Saudi Journal of Kidney Diseases and Transplantation*, **22**(2): 286.

Bosman, D. R., Osborne, C. A. and Marsden, J. T. (2002). Erythropoietin response to hypoxia in patients with diabetic autonomic neuropathy and non-diabetic chronic renal failure. *Diabetes Medicine*, **19**: 65 - 69.

Bosman, D. R., Winkler, A. S., Marsden, J. T., Macdougall, I. C., and Watkins, P. J. (2001). Anaemia with erythropoietin deficiency occurs early in diabetic nephropathy. *Diabetes care*, **24**(3): 495-499.

Bothwell, T. H., Charlton, R. W., Cook, J. D., and Finch, C. A. (1979). Iron metabolism in man. *[Available Online] http://www.cabdirect.org/abstracts/19801408535.html;jsessionid=2E5DFC098 68C0F48B1F9263563B9F2B2#* (Accessed) 30th August 2015 12:00GMT

Brenyah, R. C., Ephraim, R. K. D., Owiredu, W. B. K. A., Eghan, B. A. Jr. and Quaye, L. (2013). Prevalence and determinants of proteinuria among type 2 diabetics in Kumasi, Ghana. *Journal of Medical and Biomedical Sciences*, **2**(1): 13-21

Brucks, S., Little, W. C. and Chao, T. (2004). Relation of anaemia to diastolic heart failure and the effect on outcome. *American Journal of Cardiology* **93**: 1055 - 1057.

Buchanan, T. A. and Xiang, A. H. (2005). Gestational diabetes mellitus. *Journal of Clinical Investigation*, **115**(3): 485.

Carlson, M. G., Snead, W. L. and Campbell, P. J. (1993). Regulation of free fatty acid metabolism by glucagon. *Journal of Clinical Endocrinology and Metabolism*, **77**: 11 - 15.

Castellino, P., Luzi, L., Haymond, M., Simonson, D. and DeFronzo, R. A. (1987). Effect of insulin and plasma amino acid concentrations on leucine turnover in man. *Journal of Clinical Investigations*, **80**: 1784-1793.

Charles, M. A., Fontboune, A., Thibult, N., Warnet, J. M., Rosselin, G. E. and Eschwege, E. (1991). Risk factors for NIDDM in white population: Paris Prospective Study. *Diabetes*, **40**: 796 - 799.

Cody, J. C. M. C., Daly, C., Campbell, M., Donaldson, C., Grant, A., Khan, I. and MacLeod, A. (2001). Recombinant human erythropoietin for chronic renal failure anaemia in pre‐dialysis patients. *The Cochrane database of systematic reviews*, **3**: CD003266.

Cooper, M. E., Jandeleit - Dahm, K. and Thomas, M. C. (2005). Targets to retard the progression of diabetic nephropathy. *Kidney International*, **68**: 1439 - 1445.

Cowie, C. C., Rust, K. F., Ford, E. S., Eberhardt, M. S., Byrd - Holt, D. D., Li, C. and Geiss, L. S. (2009). Full accounting of diabetes and pre-diabetes in the US population in 1988–1994 and 2005 - 2006. *Diabetes care*, **32**(2): 287 - 294.

Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M. and Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase - 3 by insulin mediated by protein kinase B. *Nature* **378**: 785 -789.

Czech, M. P. and Corvera, S. (1999). Signalling mechanisms that regulate glucose transport. *Journal of Biological Chemistry*, **274**: 1865 - 1868.

DCCT, C. (2004). Follow-up Report on the Diagnosis of Diabetes Mellitus. *Diabetes Spectrum*, **17**(1), 51.

De - Graft, A. A. (2004). Strengthening quality and continuity of diabetes care in rural

Ghana: a critical social psychological approach. *Journal of Health and Psychology,* **9**(2): 295 - 309.

DeFronzo, R. A. (1997). Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes,* **5**: 177 - 269.

DeFronzo, R. A., Cooke, C. R., Andres, R., Faloona, G. R. and Davis P. J. (1975). The effect of insulin on renal handling of sodium, potassium, calcium and phosphate in man. *Journal of Clinical Investigation,* **55**: 845 - 855.

DeFronzo, R. A., Goldberg, M. and Agus, Z. S. (1976). The effects of glucose and insulin on renal electrolyte transport. *Journal of Clinical Investigation,* **58**: 83 - 90.

DeFronzo, R. A., Matsuda, M. and Barret, E. (1994). Diabetic ketoacidosis: a combined metabolic - nephrologic approach to therapy. *Diabetes Review,* **2**: 209 - 238.

Deicher, R. and Horl, W. H. (2003). Anaemia as a risk factor for the progression of chronic kidney disease. *Current Opinion in Nephrology and Hypertension,* **12**: 139 - 143.

Donnelly, S. (2001). Why is erythropoietin made in the kidney? The kidney functions as a critmeter. *American Journal of Kidney Diseases,* **38**: 415 - 425.

Dosoo, D. K., Rana, S. V., Offe - Amoyaw, K., Tete - Donkor, D. and Maddy, S. Q. (2001). Total antioxidant status in non - insulin - dependent diabetes mellitus patients in Ghana. *West African Journal of Medicine,* **20**(3): 184 - 86.

Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J. H., Masiarz, F., Kan, Y. A. N. and Goldfine, I. W. (1985). The human IR cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell*, **40**: 747 - 758.

Eghan, B. A. Jr. Frempong, M. T. and Adjei - Poku, M. (2007). Prevalence and predictors of microalbuminuria in patients with diabetes mellitus: a cross - sectional observational study in Kumasi, Ghana. *Ethnicity and Disease*, **17**(4): 726 - 730.

Ellemann, K., Soerensen, J. N., Pedersen, L., Edsberg, B. and Andersen, O. (1984). Epidemiology and treatment of diabetic ketoacidosis in a community population. *Diabetes care*, **7**: 528 - 532.

Engelgau, M. M., Thompson, T.J., Herman, W. H. Boyle, J. P., Aubert, R. E. and Kenny, S. J. (1997). Comparison of fasting and 2 - hour glucose and HbA1c levels for diagnosing diabetes: diagnostic criteria and performance revisited. *Diabetes care* **20**: 785 - 91.

Ennis, E. D., Stahl, E. J. V. B. and Kreisberg, R. A. (1994). The hyperosmolar hyperglycaemic syndrome. *Diabetes Review*, **2**: 115 - 212.

Erslev, A. J. and Besarab, A. (1997). Erythropoietin in the pathogenesis and treatment of the anaemia of chronic renal failure. *Kidney International*, **51**: 622 - 630.

Exton, J. H. (1987). Mechanism of hormonal regulation of hepatic glucose metabolism. *Diabetes/Metabolism Research and Reviews*, **3**: 163 - 183.

Ezekowitz, J A., McAlister, F. A. and Armstrong, P. W. (2003). Anaemia is common in heart failure and is associated with poor outcomes: Insights from a cohort of 12 065 patients with new-onset heart failure. *Circulation*, **107** (2): 223 - 225.

Feinstein, R., Kanety, H., Papa, M. Z., Lunenfeld, B. and Karasik, A. (1993). Tumour necrosis factor - α suppresses insulin-induced tyrosine phosphorylation of IR and its substrates. *Journal of Biological Chemistry*, **268**: 26055 - 26058.

Felig, P. and Wahren, J. (1971). Influence of endogenous insulin secretion on splanchnic glucose and amino acid metabolism in man. *Journal of Clinical Investigation*, **50**: 1702 - 1711.

Ferrannini, E. (1998). Insulin resistance vs. insulin deficiency in non - insulin - dependent diabetes mellitus: problems and prospects. *Endocrine Reviews,* **19**: 477 - 490.

Fine, L. G., Bandyopadhay, D. and Norman, J. T. (2000). Is there a common mechanism for the progression of different types of renal diseases other than proteinuria? Towards the unifying theme of chronic hypoxia. *Kidney International Supplements,* **75**: S22 - S26.

Finne, P. H. and Skoglund, R. W. (1970). Erythropoietin production in the rat following splanchnic neurectomy. *Journal of Laboratory Clinical Medicine,* **76**: 103 - 106.

Fishbane, S., Pollack, S., Feldman, H. I. and Joffe M. M. (2009). Iron indices in chronic kidney disease in the National Health and Nutritional Examination Survey 1988 - 2004. *Clinical Journal of American Society of Nephrology*, **4**: 57 - 61.

Foster, D. W. and McGarry, J. D. (1983). The metabolic derangements and treatment of diabetic ketoacidosis. *New England Journal of Medicine*, **309**: 159 - 169.

Frier, B. M., Ashby, J. P., Nairn, I. M. and Bairs, J. D. (1981). Plasma insulin, C - peptide and glucagon concentrations in patients with insulin independent diabetes treated with chlorpropamide. *Diabetes and Metabolism,* **7**(1): 45 - 49.

Goldstein, D. E. (1984). Is glycosylated haemoglobin clinically useful (Editorial)? *New England Journal of Medicine,* **310**: 384 - 385.

Goldstein, R. E., Wasserman, D. H., Reed, G. W., Lacy, D. B., Abumrad, N. N. and

Cherrington A. D. (1994). The effects of acute hypercortisolemia on β - hydroxybutyrate and metabolism during insulin deficiency. *Hormone and Metabolism Research*, **26**: 9 - 13.

Granner, D. and Pilkis, S. (1990). The genes of hepatic glucose metabolism. *Journal of Biological Chemistry*, **265**: 1173 - 1176.

Greene, D. A. (1986). Acute and chronic complications of diabetes mellitus in older patients. *The American journal of medicine*, **80**(5): 39-53.

Guariguata, L., *et al.* "Global estimates of diabetes prevalence for 2013 and projections for 2035." *Diabetes research and clinical practice* **103.2** (2014): 137-149.

Hall, V., Thomsen, R. W., Henriksen, O. and Lohse, N. (2011). Diabetes in Sub - Saharan Africa 1999 - 2011: Epidemiology and public health implications. A systematic review. *BMC Public Health*, **11**: 564.

Halperin, M. L. and Cheema - Dhadli, S. (1989). Renal and hepatic aspects of ketoacidosis: a quantitative analysis based on energy turnover. *Diabetes and Metabolism Reviews*, **5**: 321 - 336.

Harris, M.I. (1989). Impaired glucose tolerance in the U.S. population. *Diabetes Care*, **12**: 464 - 474.

Hayashi, T., Suzuki, A and Shoji, T. (2000). Cardiovascular effect of normalizing the haematocrit level during erythropoietin therapy in predialysis patients with chronic renal failure. *American Journal of Kidney Diseases*, **35**: 250 - 256.

Hercberg, S. and Galan, P. (1992). 7-Nutritional anaemias. *Baillière's Clinical Haematology*, **5**(1), 143-168.

Herzog, C. A., Littrell, K., Arko, C., Frederick, P. D., and Blaney, M. (2007). Clinical

Characteristics of Dialysis Patients With Acute Myocardial Infarction in the United States A Collaborative Project of the United States Renal Data System and the National Registry of Myocardial Infarction. *Circulation*, **116**(13): 1465 - 1472.

Hilawe, E. H., Yatsuya, H., Kawaguchi, L., and Aoyama, A. (2013). Differences by sex in the prevalence of diabetes mellitus, impaired fasting glycaemia and impaired glucose tolerance in sub-Saharan Africa: a systematic review and meta-analysis. *Bulletin of the World Health Organization*, **91**(9): 671-682D.

Hubbard, S. R. (1997). Crystal structure of the activated IR tyrosine kinase in complex with peptide substrate and ATP analog. *The EMBO Journal*, **16**: 5572 - 5581.

Hubbard, S. R., Wei, L., Ellis L. and Hendrickson, W. A. (1994). Crystal structure of the tyrosine kinase domain of the human IR. *Nature*, **372**: 746 - 754.

Hue, L. (1987). Gluconeogenesis and its regulation. *Diabetes/Metabolism Research and Reviews*, **3**: 111 - 126.

International Diabetes Federation (IDF) (2009). Diabetes Atlas, *Fourth Edition. Brussels, Belgium*.

International Nutritional Anemia Consultative Group (INACG). (1979). Iron deficiency in infancy and childhood. *Geneva, Switzerland: INACG, World Health Organization.*

International Nutritional Anemia Consultative Group (INACG). (1989). Iron deficiency in women. *Geneva, Switzerland: INACG, World Health Organization*.

Jensen, M. D., Caruso, M. and Heiling, V. (1989). Insulin regulation of lipolysis in non diabetic and IDDM subjects. *Diabetes*, **38**: 1595 - 1601.

Jolobe, O. M. P. (2000). Prevalence of hypochromia (without microcytosis) vs microcytosis (without hypochromia) in iron deficiency. *Clinical and Laboratory Haematology*, **22**(2): 79-80.

Jones, S. C., Smith, D., Nag, S., Bilous, M. T., Winship, S., Wood, A., and Bilous, R. W. (2010). Prevalence and nature of anaemia in a prospective, population based sample of people with diabetes: Teesside anaemia in diabetes (TAD) study. *Diabetic Medicine*, **27**(6): 655-659.

Jungers, P., Choukroun, G. and Oualim, Z. (2001). Beneficial influence of recombinant human erythropoietin therapy on the rate of progression of chronic renal failure in predialysis patients. *Nephrology Dialysis Transplantation*, **16**: 307 - 312.

Kalk, W. J., Huddle, K. R. and Raal, F. J. (1993). The age of onset and sex distribution of insulin - dependent diabetes mellitus in Africans in South Africa. *Postgraduate Medical Journal*, **69**(813): 552 - 556.

Kanapuru, B., Artz, A. S., and Ershler, W. B. (2007). Erythropoietin Deficiency and Late - Life Anaemia. In *Anaemia in the Elderly*, *Springer U. S.* 115-127.

Keane, W. F. and Lyle, P. A. (2003). Recent advances in management of type 2 diabetes and nephropathy: Lessons from the RENAAL study. *American Journal of Kidney Diseases*, **41**(1): S22 - S25.

Keller, U., Gerger, P. P. G. and Stauffacher, W. (1984). Stimulatory effect of norepinephrine on ketogenesis in normal and insulin deficient humans. *American Journal of Physiology*, **247**: E732 - E739.

Kilpatrick, E. S. (2004). HbA1c measurement. *Journal of Clinical Pathology*, **57**(4): 344 - 345.

Kitabchu, A. I., Umperrez, G. E., Miles, J. M. and Fisher, J. N. (2009). Hyperglycaemia cries in adult patients with diabetes. *Diabetes Care,* **32**(7): 1335 - 1343

Krolewski, A. S., Laffel, L. M. B., Krolewski, M., Quinn, M. and Warram, J. H. (1995). Glycosylated haemoglobin and the risk of microalbuminuria in patients with insulin-dependent diabetes mellitus. *New England Journal of Medicine*, **332**: 1251 - 1255.

Kuriyama, S., Tomonari, H. and Yoshida, H. (1997). Reversal of anaemia by erythropoietin therapy retards the progression of chronic renal failure, especially in nondiabetic patients. *Nephron* **77**: 176 - 185.

Kuzuya, T. and Matsuda, A. (1997). Classification of diabetes on the basis of aetiologies versus degree of insulin deficiency. *Diabetes Care*, **21**: 666-667.

Lapolla, A., Dalfrà, M. G. and Fedele, D. (2009). Management of gestational diabetes mellitus. *Diabetes, metabolic syndrome and obesity: targets and therapy*, **2**:73.

Lee, J., Pilch, P. F., Shoelson, S. E. and Scarlata, S. F. (1997). Conformational changes of the IR upon insulin binding and activation as monitored by fluorescence spectroscopy. *Biochemistry*, **36**: 2701 - 2708.

Levin, A, Thompson, C. R. and Ethier, J. (1999). Left ventricular mass index increase in early renal disease: Impact of decline in haemoglobin. *American Journal of Physiology*, **34**: 125 - 134.

Levin, A. (2002). Anaemia and left ventricular hypertrophy in chronic kidney disease populations: a review of the current state of knowledge. *Kidney International*, **61**: S35 - S38.

Lewis, S. M., Osei-Bimpong, A., and Bradshaw, A. (2004). Measurement of haemoglobin

as a screening test in general practice. *Journal of Medical screening*, **11**(2): 103105.

Macdougall, I. C., Eckardt, K. U. and Locatelli, F. (2007). Latest US KDOQI Anaemia Guidelines update – what are the implications for Europe? *Nephrology Dialysis Transplantation*, **22**: 2738 - 2742.

Malone, M. L., Gennis, V. and Goodwin, J. S. (1992). Characteristics of diabetic ketoacidosis in older versus young adults. *Journal of the American Geriatrics Society*, **40**: 1100 - 1104.

Mathers, C. D. and Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030". *PLoS Medicine.* **3** (11): e442.

Mayer - Davis, E. J., Bell, R. A. and Dabelea, D. (2009). The many faces of diabetes in American youth: type 1 and type 2 diabetes in five race and ethnic populations: the SEARCH for Diabetes in Youth Study. *Diabetes care*, **32**(2): 99 - 101.

Mbanya, J. C., Motala, A. A., Sobngwi, E., Assah, F. K. and Enoru, S. T. (2010). Diabetes in sub - Saharan Africa. *Lancet*, **375**: 2254 - 2266.

Mbanya, J. C., Kengne, A. P. and Assah, F. (2006). Diabetes care in Africa. *Lancet*, **368**: 1628 - 1629.

McCance, D. R., Hanson, R. L., Charles, M. A., Jacobsson, L. T. H., Pettitt, D. J. and Bennett, P. H. (1994). Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *BMJ*, **308**: 1323 - 1328.

McGarry, J. D. (1979). Lilly lecture: new perspectives in the regulation of ketogenesis. *Diabetes*, **28**: 517 - 523.

McGarry, J. D., Woeltje, K.F., Kuwajima, M. and Foster D. W. (1989). Regulation of ketogenesis and the renaissance of carnitine palmitoyl transferase. *Diabetes/Metabolism Research and Reviews*, **5**: 271 - 284.

Mehdi, U., and Toto, R. D. (2009). Anaemia, diabetes, and chronic kidney disease. *Diabetes Care*, **32**(7): 1320-1326.

Menz, H. B. and Lord, S. R. (1999). Foot problems, functional impairment, and falls in older people. *Journal of the American Podiatric Medical Association*, **89**(9): 458-467.

Meyer, C., Stumvoll, M., Nadkarni, V., Dostou, J., Mitrakou, A. and Gerich, J. (1998). Abnormal renal and hepatic glucose metabolism in type 2 diabetes mellitus. *Journal of Clinical Investigation*, **102**(3): 619.

Moeller, N., Schmitz, O., Moeller, J., Porksen, N. and Jorgensen, J. O. L. (1992). Dose - response studies on metabolic effects of a growth hormone pulse in humans. *Metabolism*, **41**: 172-175.

Morris, A. D., Boyle, D. I., McMahon, A. D., Greene, S. A., MacDonald, T. M., Newton, R. W. and DARTS/MEMO Collaboration. (1997). Adherence to insulin treatment, glycaemic control, and ketoacidosis in insulin-dependent diabetes mellitus. *The Lancet*, **350**(9090): 1505-1510.

Moss, S. E., Klein, R., Klein, B. E. K. and Meuer, M. S. (1994). The association of glycaemia and cause - specific mortality in a diabetic population. *Archives of Internal Medical*, **154**: 2473 - 2479.

Murata, K., Baumann, N. A., Saenger, A. K., Larson, T. S., Rule, A. D., and Lieske, J. C. (2011). Relative performance of the MDRD and CKD-EPI equations for estimating glomerular filtration rate among patients with varied clinical presentations. *Clinical Journal of the American Society of Nephrology*, CJN02300311.

Murphy, D. (1998). Acute complications of diabetes mellitus. In *Nurse practitioner forum* **9**(2): 69-73.

Musey, V. C., Lee, J. K., Crawford, R., Klatka, M. A., McAdams D. and Phillips, L. S. (1995). Diabetes in African - Americans. I. Cessation of insulin therapy is the major precipitating cause of diabetes ketoacidosis. *Diabetes Care*, **18**: 483 - 489.

Nathan, D. M., Cleary, P. A. and Backlund, J. Y. C. (2005). Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *The New England Journal of Medicine*, **353**(25): 2643 - 2653.

Nathan, D. M., Davidson, M. B., Defronzo, R. A., Heine, R. J., Henry, R. R., Pratley, R. and Zinman, B. (2007). Impaired fasting glucose and impaired glucose tolerance implications for care. *Diabetes care*, **30**(3): 753-759.

Nosadini, R., Avogaro, A., Doria, A., Fioretto, P., Trevisan, R. and Morocutti, A. (1989). Ketone body metabolism: a physiological and clinical overview. *Diabetes/Metabolism Research and Reviews*, **5**: 299 - 319.

Nurjhan, N., Consoli, A. and Gerich, J. (1992). Increased lipolysis and its consequences on gluconeogenesis in non - insulin - dependent diabetes mellitus. *Journal of Clinical Investigations*, **89**: 169-175.

Nyarko, A. K., Asare - Anane, H., Ofosuhene, M., Addy, M. E., Teye, K. and Addo, P. (2002). Aqueous extract of Ocimum canum decreases levels of fasting blood glucose and free radicals and increases antiatherogenic lipid levels in mice. *Vascular Pharmacology,* **36**(6): 273 - 279.

Nyarko, A., Adubofour, K., Ofei, F., Kpodonu, J. and Owusu, S. (1997). Serum lipid and lipoprotein levels in Ghanaians with diabetes mellitus and hypertension. *The Journal of the National Medical Association,* **89**(3): 191 - 196.

Orchard, T. J., Costacou, T., Kretowski, A. and Nesto, R. W. (2006). Type 1 diabetes and coronary artery disease. *Diabetes care*, **29**(11): 2528 - 2538.

Owiredu, W. K. B. A., Adamu, M. S., Amidu, N., Woode, E., Bam, V., Plange-Rhule, J., and Opoku-Okrah, C. (2008). Obesity and cardiovascular risk factors in a pentecostal population in Kumasi-Ghana. *Journal of Medical Sciences*, **8**: 682690.

Petzold, R., Trabert, C., Walther, A. and Schoffling, K. (1971). Aetiology and prognosis of diabetic coma: a retrospective study. *Verh Dtsch Ges Inn Med*, **77**: 637 - 640.

Picot, J., Jones, J., Colquitt, J, L., Gospodarevskaya, E., Loveman, E., Baxter, L. and Clegg, A. J. (2009). The clinical effectiveness and cost - effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation. *Health technology assessment (Winchester, England),* **13**(41): 1 - 190, 215 - 357.

Pilkis, S. J., El - Maghrabi, M. R. and Claus, T. H. (1990). Fructose - 2, 6 - bisphosphate in control of hepatic gluconeogenesis. *Diabetes Care*, **13**: 582 - 599.

Pinhas-Hamiel, O. and Zeitler, P. (2007). Acute and chronic complications of type 2 diabetes mellitus in children and adolescents. *The Lancet*, **369**(9575): 1823-1831.

Polonsky, W. H., Anderson, B. J., Lohrer, P. A., Aponte J. E., Jacobson, A. M. and Cole, C. F. (1994). Insulin omission in women with IDDM. *Diabetes Care*, **17**: 1178 - 1185.

Portoles, J., Torralbo, A. and Martin, P. (1997). Cardiovascular effects of recombinant human erythropoietin in predialysis patients. *American Journal of Kidney Diseases*, **29**: 541 - 548.

Press, M., Tamborlance, W. V. and Sherwin, R. S. (1984). Importance of raised growth hormone levels in mediating the metabolic derangements of diabetes. *New England Journal of Medicine*, **310**: 810 - 815.

Reddan, D. N., Szczech, L. A., Tuttle, R. H., Shaw, L. K., Jones, R. H., Schwab, S. J., and Owen, W. F. (2003). Chronic kidney disease, mortality, and treatment strategies among patients with clinically significant coronary artery disease. *Journal of the American Society of Nephrology*, **14**(9): 2373 - 2380.

Risérus, U., Willett, W. C. and Hu, F. B. (2009). Dietary fats and prevention of type 2 diabetes. *Progress in Lipid Research*, **48**(1): 44 - 51.

Rossing, P., Rossing, K., Gæde, P., Pedersen, O., and Parving, H. H. (2006).Monitoring kidney function in type 2 diabetic patients with incipient and overt diabetic nephropathy. *Diabetes care*, **29(**5): 1024 - 1030.

Rother, K. I. (2007). Diabetes treatment - bridging the divide. *The New England Journal of Medicine*, **356** (15): 1499 - 1501.

Salman, M. A. (2015). Anaemia in Patients with Diabetes Mellitus: Prevalence and Progression. *General Medicine*, **3**(1000162), 2.

Sandgren, P. E., Murray, A. M. and Herzog, C. A. (2005). Anaemia and new - onset

congestive heart failure in the general Medicare population. *Journal of Cardiac Failure*, **11**: 99 - 105.

Schade, D. S. and Eaton, R. P. (1980). The temporal relationship between endogenously secreted stress hormone and metabolic decompensation in diabetic man. The *Journal of Clinical Endocrinology and Metabolism*, **50**: 131 - 136.

Silverberg, D. S., Wexler, D. and Blum, M. (2003). The effect of correction of anaemia in diabetics and non-diabetics with severe resistant congestive heart failure and chronic renal failure by subcutaneous erythropoietin and intravenous iron. *Nephrology Dialysis Transplantation*, **18**: 141 - 146.

Silverberg, D. S., Wexler, D. and Sheps, D. (2001). The effect of correction of mild anaemia in severe, resistant congestive heart failure using subcutaneous erythropoietin and intravenous iron: A randomized controlled study. *Journal of the American College of Cardiology*, **37**: 1775 - 1780.

Siperstein, M. D. (1992). Diabetic ketoacidosis and hyperosmolar coma. Endocrinology *Metabolism Clinics of North America*, **21**: 415 - 432.

Srivastava, P. M., Thomas, M. C. and Calafiore, P. (2006). Diastolic dysfunction is associated with anaemia in patients with type II diabetes. *Clinical Science*, **110**: 109 - 116.

Stevens, P. E., O'Donoghue, D. J. and Lameire, N. R. (2003). Anaemia in patients with diabetes: Unrecognised, undetected and untreated? *Current Medical Research Opinion*, **19**: 395 - 401.

Thomas, M. C. (2007). Anaemia in diabetes: marker or mediator of microvascular disease? *Nature Clinical Practice Nephrology*, **3**: 20 - 30.

Thomas, M. C., Cooper, M. E., Rossing, K., and Parving, H. H. (2006). Anaemia in diabetes: is there a rationale to TREAT?. *Diabetologia*, **49**(6): 1151-1157.

Thomas, M. C., MacIsaac, R. J., Tsalamandris, C., and Jerums, G. (2004a). Elevated iron indices in patients with diabetes. *Diabetic medicine*, **21**(7): 798 - 802.

Thomas, M. C., MacIsaac, R. J., Tsalamandris, C., Molyneaux, L., Goubina, I., Fulcher, G., and Jerums, G. (2004b). The burden of anaemia in type 2 diabetes and the role of nephropathy: a cross-sectional audit. *Nephrology Dialysis Transplantation*, **19**(7): 1792-1797.

Thomas, M. C., MacIsaac, R. J., Tsalamandris, C., Power, D., and Jerums, G. (2003). Unrecognized Anaemia in Patients with Diabetes: A cross - sectional survey. *Diabetes care*, **26**(4): 1164 - 1169.

Thomas, M. C., Tsalamandris, C. and MacIsaac, R. (2004c). Low - molecular-weight AGEs are associated with GFR and anaemia in patients with type 2 diabetes. *Kidney International*, **66**: 1167 - 1172.

Turok, D. K., Ratcliffe, S. D. and Baxley, E. G. (2003). Management of gestational diabetes mellitus. *American family physician*, **68**(9): 1767-1772.

Ueda, H., Ishimura, E. and Shoji, T. (2003). Factors affecting progression of renal failure in patients with type 2 diabetes. *Diabetes Care*, **26**: 1530 - 1534.

Umpierrez, G. E., Kelly, J. P., Navarrete, J. E., Casals, M. M. C. and Kitabchi, A. E. (1997). Hyperglycaemic crisis in urban blacks. *Archives of Internal Medicine*, **157**: 669 - 675.

Vaag, A., Hother - Nielsen, O., Skott, P., Anderson, P., Richter, E. A. and Beck - Nielsen, H. (1992). Effect of acute hyperglycaemia on glucose metabolism in skeletal muscles in IDDM patients. *Diabetes*, **41**: 174 - 182.

Wachtel, T. J., Silliman, R. A. and Lamberton, P. (1987). Predisposing factors for the diabetic hyperosmolar state. *Archives of Internal Medicine,* **147**: 499 - 501.

Wasserman, D. H. and Vranic, M. (1986). Interaction between insulin and counter regulatory hormones in control of substrate utilization in health and diabetes during exercise. *Diabetes/Metabolism Research and Reviews*, **1**: 359 - 384.

Weiner, D. E., Tighiouart, H., Vlagopoulos, P. T., Griffith, J. L., Salem, D. N., Levey, A. S., and Sarnak, M. J. (2005). Effects of anaemia and left ventricular hypertrophy on cardiovascular disease in patients with chronic kidney disease. *Journal of the American Society of Nephrology*, **16**(6): 1803 - 1810.

Welsh, G. I. and Proud C. G. (1993). Glycogen synthase kinase - 3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. *Biochemical Journal*, **294**: 625 - 629.

Weyer, C., Tataranni, P. A., Bogardus, C. and Pratley, R. E. (2001). Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. *Diabetes Care*, **24**: 84 - 88.

Wild, S., Roglic, G., Green, A., Sicree, R., and King, H. (2004). Global prevalence of diabetes estimates for the year 2000 and projections for 2030. *Diabetes care*, **27**(5): 1047-1053.

Winkler, A. S., Marsden, J. and Chaudhuri, K. R. (1999). Erythropoietin depletion and anaemia in diabetes mellitus. *Diabetic Medicine,* **16**: 813 - 819.

World Health Organisation (WHO) (1999). Report Part 1: Diagnosis and classification of diabetes mellitus. WHO, Geneva.

World Health Organisation (WHO) (2013). The top 10 causes of death Fact sheet N°310. WHO, Geneva.

World Health Organization (1968). Nutritional anaemias: report of a WHO scientific group

[meeting held in Geneva from 13 to 17 March 1967].

Yip, R., Johnson, C., and Dallman, P. R. (1984). Age-related changes in laboratory values used in the diagnosis of anaemia and iron deficiency. *The American journal of Clinical Nutrition*, **39**(3): 427-436.

Yokoyama, H., Tomonaga, O. and Hirayama, M. (1997). Predictors of the progression of diabetic nephropathy and the beneficial effect of angiotensin - converting enzyme inhibitors in NIDDM patients. *Diabetologia*, **40**: 405 - 411.

Zammit, V. A. (1994). Regulation of ketone body metabolism, a cellular perspective. *Diabetes Review*, **2**: 132-155.

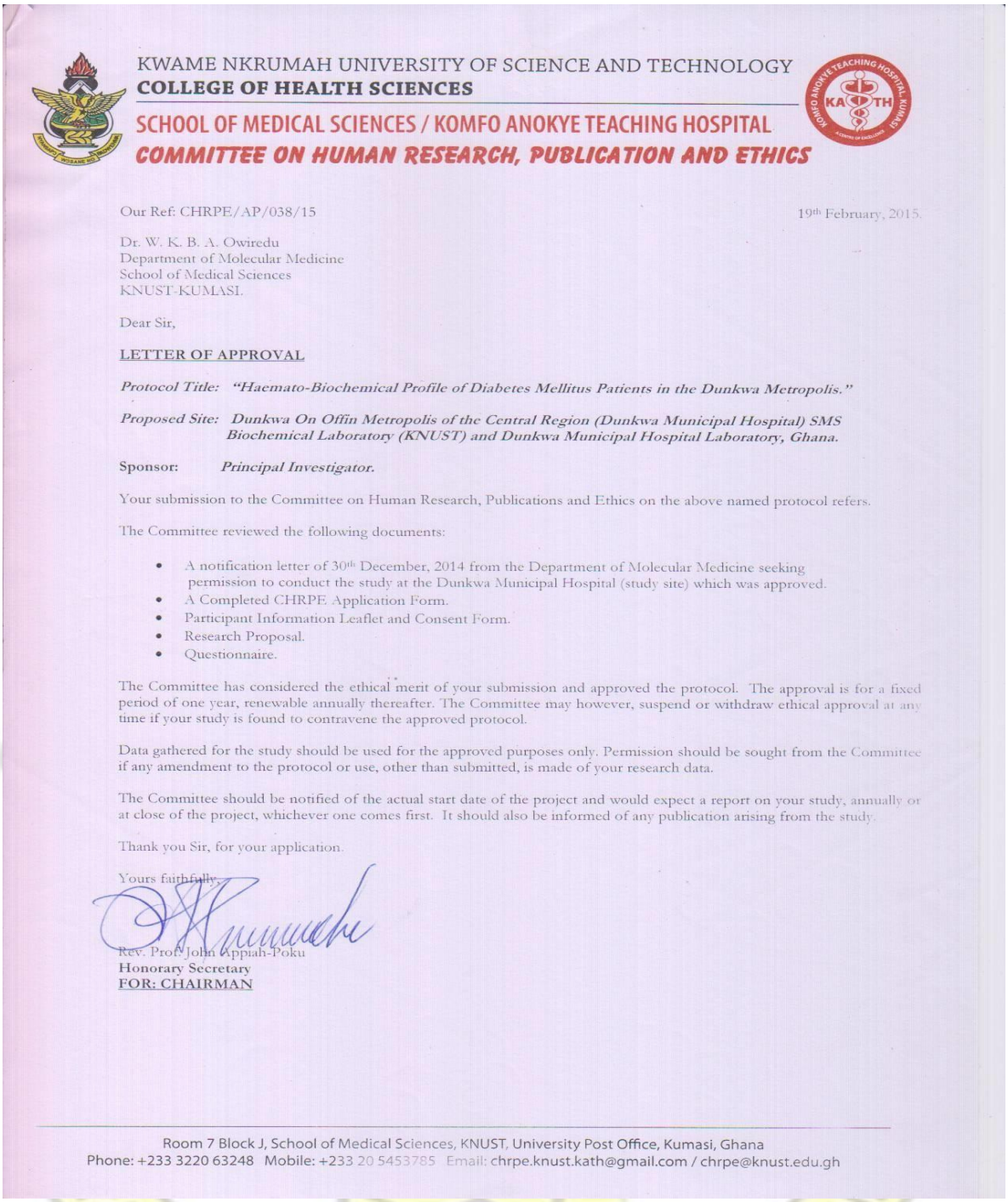
Zeidman, A., Fradin, Z. and Blecher, A. (2004). Anaemia as a risk factor for ischemic heart disease. *Israel Medical Association Journal*, **6**: 16 - 18.

Zou, A. P. and Cowley, A. W. J. (2003). Reactive oxygen species and molecular regulation of renal oxygenation. *Acta Physiologica Scandinavia*, **179**: 233 - 241.

APPENDIX

Appendix A

Ethical Approval Letter



KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/038/15

19th February, 2015.

Dr. W. K. B. A. Owiredu
Department of Molecular Medicine
School of Medical Sciences
KNUST-KUMASI.

Dear Sir,

LETTER OF APPROVAL

Protocol Title: "Haemato-Biochemical Profile of Diabetes Mellitus Patients in the Dunkwa Metropolis."

Proposed Site: Dunkwa On Offin Metropolis of the Central Region (Dunkwa Municipal Hospital) SMS Biochemical Laboratory (KNUST) and Dunkwa Municipal Hospital Laboratory, Ghana.

Sponsor: *Principal Investigator.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 30th December, 2014 from the Department of Molecular Medicine seeking permission to conduct the study at the Dunkwa Municipal Hospital (study site) which was approved.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Proposal.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Rev. Prof. John Appiah-Poku
Honorary Secretary
FOR: CHAIRMAN

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

APPENDIX B

Letter of Permit to Carry Out Research at the Dunkwa Municipal Hospital

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES
DEPARTMENT OF MOLECULAR MEDICINE

30th December, 2014

The Medical Superintendent
Dunkwa Municipal Hospital
Dunkwa-

Dear Sir

The bearer, Mr. Ernest Gockah-Adapoe is an M.Phil Chemical Pathology student of the School of Medical Sciences , KNUST. His research entitled `Haemato-Biochemical profile of Diabetes Mellitus patients in the Dunkwa metropolis' is under the supervision of Profs. George Bedu-Addo, Benjamin A. Eghan Jnr of the Department of Medicine as well as Dr. W.K.B.A. Owiredu of the Departments of Molecular Medicine, SMS and Clinical Biochemistry, KATH.

Sir, I shall be most grateful if you could please indicate in writing whether or not Diabetic patients in your hospital may be enrolled into the study. Please find attached a copy of the study synopsis for your perusal and necessary action.

Thanking you in anticipation of your cooperation.

Yours sincerely,

Dr. W.K.B.A. OWIREDU
(Principal Supervisor)

Permission granted to enrol diabetic patients in our hospital

MEDICAL SUPERINTENDENT
MUNICIPAL HOSPITAL
DUNKWA-OFFIN

16/1/2015

Dr. Abraham Wolay

Private Mail Bag, University Post Office, Kumasi, Ghana. Phone: 233-3220-63252. Fax 233-51-60302.
E-mail: molecularmedicine.sms@knust.edu.gh * secretary.sms@knust.edu.gh * Website: www.knust.edu.gh

APPENDIX C QUESTIONNAIRE

This project is being conducted at the Department of Molecular Medicine KNUST with the aim of determining the Haemato-Biochemical profile of

Diabetes Mellitus patients in the Dunkwa metropolis. The information that you provide will contribute to our knowledge of the prevalence of unrecognized anaemia in patients with diabetes mellitus among individuals in your age group.

Participation in this study would involve completing this questionnaire

SOCIAL DATA

1. Name…….
2. Sex : M ☐ F ☐
3. Age: 20-29 ☐ 30-39 ☐ 40-49 ☐ 50-59 ☐ 60-69 ☐ >69 ☐
4. Marital Status: Single ☐ Married ☐ Divorced ☐ Widowed ☐ Separated ☐ Cohabitating ☐
5. Current occupation? ……
6. Educational level? Primary ☐ Secondary ☐ Tertiary ☐
7. Are you a diabetic? ☐ Yes ☐ No

   Duration of diabetes:
8. <5years ☐ Yes ☐ No
9. >5years ☐ Yes ☐ No
10. Are you hypertensive? ☐ Yes ☐ No
11. Are you on any medication? ☐ Yes ☐ No
12. If yes what? .....
13. How long have you been on oral Folic Acid……Fesolate…….
14. How long have you been on Metformin ……Daonil…….
15. Are you on Lisinopril……Insulin.....

16. If yes how long : 1-3months ☐ 4-6months ☐ 7-9months ☐ 10-12months ☐ 2years ☐ Over 2years ☐

17. Are you on any antioxidant medications ☐ Yes ☐ No

18. If yes what? .....

19. If yes how long : 1-3months ☐ 4-6months ☐ 7-9months ☐ 10-12months ☐ 2years ☐ Over 2years ☐

20. Are you taking any herbal medications ☐ Yes ☐ No

21. If yes what? .....

22. If yes how long : 1-3months ☐ 4-6months ☐ 7-9months ☐ 10-12months ☐ 2years ☐ Over 2years ☐

23. **Do you smoke?** ☐ ☐ Yes ☐ No **24. Do you take Alcohol?** Yes No

25. **Quantities taken**

Once in a while ☐ once in a month ☐ once in two weeks ☐ once a week ☐ once a day ☐ twice a day ☐

26. Do you exercise? ☐ Yes ☐ No

27. If Yes type of exercise? .....

28. **How often do you Exercises?**

Once in a while/Rarely? ☐ Once in a month ☐ once in Two weeks ☐ Once a week ☐ twice a day ☐ once a day ☐

29. **Duration of brisk exercise?**

Ten minutes (10mins) ☐ Twenty minutes (20mins) ☐ Thirty minutes (30mins) ☐
Forty minutes (40mins) ☐ Fifty minutes (50mins) ☐ Sixty minutes (60mins) ☐

Biochemical markers

Urea ……mmol/L Creatinine ……mmol/L Na+ ……mmol/L K+…… Cl^- ……mmol/L

Fasting Blood Sugar……mmol/L Serum transferrin……..

Serum ferritin…… TIBC/UIBC……., Serum Iron…….

Hematological Assay

Full blood count Blood Film comment **Anthropometric**

Indices

Waist circumference …… (cm) Hip circumference …….. (cm) Wc/Hc

……

Weight ……. (Kg) Height…..(m) BMI …… BP ……mmHg

Pulse……

Arm circumference…….(cm)

APPENDIX D

Participant Information Leaflet

Title of Research: Haemato-Biochemical profile of Diabetes Mellitus patients in the Dunkwa metropolis.

Name(s) and affiliation(s) of researcher(s): This study is being conducted by Dr. William Kwame Boakye Ansah Owiredu of the Department of Molecular Medicine, SMS KNUST, Mr. Ernest Gockah-Adapoe of the Department of Molecular Medicine,

SMS KNUST, Prof. Benjamin Ackon Eghan Jnr. of the Department of Medicine, SMS/KATH and Prof. George Bedu-Addo of the Department of Medicine, SMS/KATH

Purpose(s) of research: To inform and enhance better management of patients with diabetes who may have unrecognized anaemia visiting Dunkwa Municipal Hospital.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research: Blood sample would be taken (about 6ml) from 102 patients for the estimation of biochemical (4mls) and hematological (2mls) assays under sterile conditions. **Biochemical assays**: Fasting Blood Sugar, Blood Urea and Electrolytes (Na^+, K^+,Cl^-) Serum Creatinine, Serum Transferrin, Serum Ferritin, TIBC/UIBC, Serum iron. Hematological and other assays: Full blood count (FBC), Blood Film comment.

Anthropometric data of the subjects would be taken; the indices being:

- Waist circumference
- Hip circumference
- Arm circumference

This will help to estimate the Waist and Hip ratio (WHR)

- Weight
- Height

This is to calculate the Body mass index (BMI) and Weight to height ratio (WtHR).

Risk(s): The sample taking will be done by qualified personnel who permit virtually little or even no discomfort to the patients. With qualified personnel taking the sample, there will be assurance of only minimal risk to the subjects. Aseptic techniques would be employed to prevent HIV, Hepatitis B etc infections.

Participants would benefit from this exercise by knowing their biochemical and hematological markers. Participants would also be informed of their anthropometric indices and its implication.

Benefit(s): The outcome of this research we hope will help inform the scientific community on the prevalence of unrecognized anaemia in diabetic patients visiting this facility. Knowledge in this area will inform and enhance better management of patients with unrecognized anaemia in diabetes. We cannot be certain about this; this is research)

Confidentiality: Any information collected will be treated with strict confidentiality; this will be ensured by giving code numbers to the samples and results

Voluntariness: Taking part in this study should be out of your own free will. You are not under obligation to. Research is entirely voluntary. If you choose not to participate, this will not affect your treatment in this hospital/institution in any way. You will still enjoy all services provided by this facility/hospital.)

Withdrawal from the research: If you agree to participate and later change your mind, you may withdraw from the research at anytime without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private).

Consequences of Withdrawal: You can also choose to withdraw from the research at anytime with no consequence. Please note that some of the information that has been obtained about you before you chose to withdraw may have been modified or used in reports and publications. These cannot be removed anymore. No compensation will be given.

Costs/Compensation: No compensation will be given

Contacts: If you have any question concerning this study, please do not hesitate to contact (For example: Dr. William Kwame Boakye Ansah Owiredu of the Department Of Molecular Medicine KNUST 0244 228667

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

The Chairman
Committee on Human Research and Publications Ethics
Kumasi
Tel: 22301-4 ext 1098 or 020 5453785

APPENDIX E

CONSENT FORM

Statement of person obtaining informed consent:
I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ SIGNATURE: _____

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 (optional). I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this information leaflet and consent form to keep for myself.

NAME OF PARTICIPANT: _____

DATE: _____ SIGNATURE_____

THUMB PRINT:_____
(For all non-literate participants, a thumbprint is required as well as a witness' signature)

To all PIs, please select and use as appropriate: (delete whichever provision below that does not apply to your study)

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

APPENDIX F

Justification of sample size

The sample size was determined using the formula

$$N=z^2pq/d^2$$

Where N= sample size Z= standard normal distribution p=prevalence in the population q=1-p d=delta (precision or degree of accuracy)

The following assumptions were made for unrecognized anaemia in diabetes mellitus Z= (standard normal distribution) =1.96 d (precision)=0.09

p(prevalence rate)= 0.25 based on ratio of estimates of unrecognized anaemia in diabetes mellitus. Nearly 1 in 4 (23%) patients with type 1 or type 2diabetes had anaemia using this definition (Kanapuru *et al.*, 2007). Also diabetes is a major health problem, affecting approximately 20.8million people in the United States (Cowie *et al.*, 2006). Also for these patients, anaemia is a common concern and can occur in 25% of people with the disease (Thomas *et al.*, 2003).

p(prevalence rate is being adjusted to 30% on the assumption of the dietary differences and the presence of some endemic diseases eg Malaria)

Substituting in the formula:

$N=(1.96)^2(0.3)(0.7)/(0.09)^2$

N= 0.80674/0.0081

N=99.6

Assuming a response rate of 98%

Sample size=99.6/0.98

Sample size =102 persons