ERYTHROPOIETIN AND HbA1c LEVELS IN PATIENTS WITH COMPLICATED DIABETES MELLITUS IN UNIVERSITY OF BENIN TEACHING HOSPITAL, BENIN CITY, EDO STATE

BY

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BENIN CITY.

SETEMBER, 2023

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE (B.M.L.S Hons) IN MEDICAL LABORATORY SCIENCE

SUPERVISED BY

DR. B.I.G. ADEJUMO

SEPTEMBER, 2023

CERTIFICATION

This is to certify that **OMOREGIE RUTH OSASENAGA (MISS)** with matriculation number BMS1708718, under the supervision of DR. B.I.G. ADEJUMO and co-supervision of PROF. M.E. ENOSOLEASE, carried out this research work and it was submitted to the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin in partial fulfillment for the requirement of Bachelor in Medical Laboratory Science (BMLS) degree in the Department.

DR. B.I.G. ADEJUMO Project Supervisor	**DATE**
PROF. M.E. ENOSOLEASE Co-Project Supervisor	**DATE**
DR. B.I.G. ADEJUMO Ag. Head Of Department	**DATE**
External Supervisor	**DATE**

DEDICATION

This project work is dedicated to God Almighty for his presence, faithfulness, strength and love during the course of this work.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude and appreciation to the following individuals and organizations who have contributed to the successful completion of this research project.

First and foremost, I thank GOD Almighty, who enabled me with ideas, philosophy, perception and motivation to present this work, after so many hurdles and obstacles. Without HIM, all efforts will account to nothing.

My heartfelt gratitude goes to my highly esteemed supervisor **DR. B. I. G. ADEJUMO** for his invaluable guidance, continuous support, and insightful feedback throughout the entire research process. His expertise and dedication have been instrumental in shaping the direction of this study.

I am deeply thankful to my co-supervisor, **PROF. M.E. ENOSOLEASE** for creating time despite his busy schedule to supervise and inspect my work. I really appreciate his fatherly concern, usefulness and supportive ideas.

Furthermore, I extend my appreciation to the participants of this study who dedicated their time and willingly shared their data. Their involvement was crucial in gathering valuable data and insights that formed the foundation of this research.

I am indebted to **Mr. Abdulazeez** from the University of Benin Teaching Hospital Chemical Pathology Laboratory for his collaboration and assistance in conducting various experiments and analyses. His willingness to collaborate and support significantly enhanced the quality of this research.

Lastly, I would like to express my deepest gratitude to my family, friends, and colleagues for their unwavering support and encouragement throughout this research journey. Their understanding, patience, and belief in my abilities have been a constant source of motivation.

In conclusion, the successful completion of this research project would not have been possible without the contributions and support of the aforementioned individuals. Their involvement and assistance have truly been invaluable, and I am honoured to have had the opportunity to work with them.

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ABSTRACT

Chronic diabetes mellitus is a metabolic disorder characterized by persistent hyperglycaemia and associated complications. Assessing erythropoietin (EPO) levels and glycated haemoglobin (HbA1c) levels in diabetic patients can provide valuable insights into the management and progression of the disease. This study aims to assess the EPO levels and HbA1c levels in patients with complications of diabetes mellitus and evaluate the clinical implications of these assessments. A case-control study was conducted on a sample of 81 consenting participants including 21 patients presenting complications of diabetes mellitus, 30 diabetic patients without any complications, and 30 environmental control persons. EPO levels were measured using enzyme-linked immunosorbent assay (ELISA), and HbA1c levels were also determined through modified enzymatic reaction. Demographic and clinical data, including age, gender, diabetes duration, and medication history, were collected via questionnaires. Statistical analysis was performed to determine the correlation between EPO levels and HbA1c levels. Values obtained in this study was presented as mean ± standard deviation (levels of significance were accepted at $p<0.001$). In this study, high HbA1c levels among diabetic patients with complications were associated with a diet rich in protein and carbohydrates. Additionally, the increased erythropoietin levels were attributed to the absence of nephropathy, a condition typically linked to lower erythropoietin levels, in these patients. Further research is warranted to explore the clinical implications of these assessments in predicting diabetes complications and guiding therapeutic interventions.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Diabetes mellitus (DM) is a carbohydrate metabolism disorder which is triggered due to impairment in insulin secretion and/or the action of insulin, resulting to prolonged hyperglycaemia with defective carbohydrate, fat and protein metabolism (Lebovitz, 2000). DM could as well be referred to as a diverse group of metabolic conditions with related high disease burden in developing countries, for instance Nigeria (Ogbera and Ekpebegh, 2014). It is identified as a leading global health problem, and risk factor for impaired vision, vascular brain diseases, renal failure, as well as limb amputations, among others (Chobanian *et al*., 2003). In Nigeria, the recent prevalence of DM amongst adults aged 20-69 years is 1.7% (International Diabetes Federation, 2017).

In patients with diabetes mellitus, years of poorly treated diabetes mellitus are linked with a myriad of microvascular (affects small vessels) and macrovascular (affects large vessels) complications (Brutsaert, 2023). The most frequently documented microvascular complications of diabetes mellitus are neuropathy, nephropathy, and retinopathy (Cade, 2008) whereas macrovascular complications consist of myocardial infarction, peripheral arterial disease, and stroke (Brutsaert, 2023). Diabetic retinopathy, characterized at the outset by retinal capillary microaneurysms (background retinopathy) and later by neovascularization (proliferative retinopathy) and macular edema, is a common cause of adult loss of sight (or blindness), with no initial symptoms or signs; however, focal blurring, vitreous or retinal detachment, and partial or total vision loss sooner or later develop, and the degree of progression is vastly variable (Brutsaert, 2023). Diabetic nephropathy, a leading cause of chronic kidney disease, is

characterized by thickening of the glomerular basement membrane, mesangial expansion, and glomerular sclerosis, leading to glomerular hypertension and a progressive decline in glomerular filtration rate; its progression may be accelerated by systemic hypertension. The disease is typically asymptomatic until nephrotic syndrome or renal failure advance. (Brutsaert, 2023). Diabetic neuropathy is the end result of nerve ischemia caused by microvascular complications, direct effects of hyperglycemia on neurons, and intracellular metabolic changes that impair nerve function (Brutsaert, 2023).

Anemia is the most common blood disorder as well as a common outcome in patients with diabetes (Thomas *et al*., 2004). The advancement and progression of microvascular and macrovascular complications in diabetes, which is connected with anemia, is typically observed in diabetes mellitus patients with renal damage, where anemia can be considered as a pointer of kidney injury, posing a greater risk of mortality in diabetics (Anjaly *et al*., 2022). Evidence points out that the presence of anemia among type 2 diabetes mellitus is characteristically linked with the failure of the kidney to produce functional erythropoietin (Thomas *et al*., 2006). Diabetic neuropathy, chronic inflammatory activity, increased levels of advanced glycation end products (AGEs), erythropoietin hypo-responsiveness, effects of oxidative stress, as well as anti-diabetic medications are other likely cause of anemia in DM patients (Craig *et al.,* 2005; Antwi-Bafour *et al*., 2016; Baisakhiya *et al*., 2017). Increasing evidence shows that the anemia in type 2 diabetes mellitus cases is a strong and independent index of increased threat for diabetes-related macrovascular and microvascular complications (Thomas *et al*., 2006, Periasamy *et al*., 2016; AlDallal and Jena, 2018; Samuel *et al*., 2018). It causes early incidence and swift development of complications like diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, end-stage renal diseases, ischemic heart disease, and non-healing diabetic foot ulcers (Samuel *et al*., 2018).

The earlier onset of anemia in patients can be attributed to several elements, including severe symptomatic autonomic neuropathy leading to efferent sympathetic denervation of the kidney and loss of functional erythropoietin, injury to the renal interstitium, systemic inflammation, and inhibition of erythropoietin release. Additionally, damage to the peritubular fibroblasts can occur, resulting in erythropoietin insufficiency and anemia even before the loss of filtration. (Craig *et al*., 2005). Promptly diagnosing and handling anemia in patients with diabetes not only leads to reduced tiredness, better exercise tolerance, and enhanced quality of life but also results in a decrease in mortality and hospital admissions for congestive heart failure (CHF), thereby contributing to lessened morbidity and improved overall well-being (Craig *et al*., 2005).

1.2 Justification of study

The importance of conducting research on individuals living with diabetes mellitus in Edo State, specifically focusing on the health risks associated with reduced erythropoietin levels, cannot be underestimated. There is insufficient awareness regarding the impact of untreated diabetes on kidney function, potentially leading to a deficiency of erythropoietin and subsequent development of anemia. Moreover, the detection of this condition may only occur at an advanced stage when kidney impairment has already taken place. Therefore, this study aims to investigate the levels of erythropoietin and HbA1c in diabetic patients with complications, potentially generating valuable data that could prompt government or relevant organizations to take measures aimed at mitigating the consequences of diabetes-related anemia on the population. Furthermore, it aims to raise awareness about the significance of erythropoietin and HbA1c levels among individuals affected by diabetes.

1.3 Scope of study

To collect data, questionnaires was given to both diabetic patients at the University of Benin Teaching Hospital (UBTH) and the control group from the University of Benin in Benin City. Subsequently, blood samples was acquired using intravenous collection and dispensed into various containers such as Ethylene Diamine Tetra-acetic Acid (EDTA), Flouride Oxalate, Lithium Heparin, as well as clean and dry plain containers. This process was carried out for both the diabetic patients at UBTH and the control group at the University of Benin.

1.4 Hypothesis

Null hypothesis

1. No Significant elevation in the levels of HbA1c in patients with complications of diabetes
2. No Significant reduction in the levels of Erythropoietin in patients with complications of diabetes

Alternate hypothesis

1. There is Significant elevation in the levels of HbA1c in patients with complications of diabetes
2. There is Significant reduction in the levels of Erythropoietin in patients with complications of diabetes

1.5 Aim of study

This study aims to determine the erythropoietin (EPO) levels and glycated haemoglobin (HbA1c) levels in patients with chronic diabetes mellitus.

1.6 Research questions

1. Does diabetes mellitus have any effect on Erythropoietin level in diabetic patients with complications in Benin City?
2. Is HbA1c level increased or decreased in chronic diabetes mellitus patients with complications?
3. Is there a relationship between erythropoietin and HbA1c level in patients with complications of diabetes mellitus in Benin City?
4. Is the Packed Cell Volume level of patients with chronic diabetes mellitus increased or decreased?
5. Is Haemoglobin level increased or decreased in patients with chronic diabetes mellitus?
6. Is Urea level increased or decreased in patients with chronic diabetes mellitus?
7. Is Creatinine level increased or decreased in patients with chronic diabetes mellitus?

1.7 Specific objectives

The specific objective of this study is to:

1. To estimate the Erythropoietin level in patients with chronic diabetes mellitus and in the control group.
2. To estimate the HbA1c level in patients with chronic diabetes mellitus and in the control group.
3. To estimate the Packed Cell Volume (PCV) in patients with chronic diabetes mellitus and in the control group.
4. To estimate the Haemoglobin level in patients with chronic diabetes mellitus and in the control group.

5. To estimate the Urea level in patients with chronic diabetes mellitus and in the control group.
6. To estimate the Creatinine level in patients with chronic diabetes mellitus and in the control group.
7. To determine if there is a prominent relationship among these biomarkers (erythropoietin, HbA1C, PCV, haemoglobin, urea, and creatinine levels) in patients with chronic diabetes mellitus and the control group.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes mellitus

Diabetes, also referred to as diabetes mellitus, is a collection of common endocrine disorders characterized by persistently elevated levels of glucose in the bloodstream (Galicia-Garcia *et al*., 2020; Yadav *et al*., 2021). Common symptoms embrace frequent urination, excessive thirst, as well as increased appetite; if left untreated, diabetes can give rise to various health complications (Goyal *et al.,* 2023). Immediate complications may involve diabetic ketoacidosis, hyperglycemic state, or even death, while long-term complications encompass cardiovascular disease, stroke, chronic kidney disease, foot ulcers, nerve damage, vision impairment, and cognitive impairment (Razaq *et al*., 2020). The condition arises from insufficient insulin production by the pancreas or inadequate response of the body's cells to the produced insulin (Shoback and Gardner, 2011). Patients with diabetes are at higher risk for atherosclerotic cardiovascular disease, peripheral arterial disease, cerebrovascular disease, and hypertension (Razaq et al., 2020).

As of 2019, it was estimated that 463 million people globally (8.8% of the adult population) had diabetes, with type 2 diabetes constituting about 90% of all cases (Razaq *et al*., 2020). The prevalence of diabetes continues to escalate, particularly in low- and middle-income countries (De Silva *et al*., 2018). The rates of occurrence are similar among men and women, and diabetes ranks as the ninth-leading cause of death worldwide (Khan *et al.,* 2020). The global expenditure on healthcare related to diabetes amounts to an estimated USD 760 billion annually (Bommer *et al*., 2018).

2.2 History of diabetes mellitus

Diabetes, an ailment dating back to ancient times, was among the earliest diseases documented (Lakhtakia, 2013; Karamanou *et al.,* 2016). In a manuscript from ancient Egypt dated back to 1500 BC, there was a reference to the sign of increased urination (Karamanou *et al.,* 2016; Metwaly *et al.,* 2021). Furthermore, the Ebers papyrus contained a suggestion for a beverage to alleviate this condition (Roberts, 2015). The initial reported instances of diabetes are believed to have been cases of type 1 diabetes (Mobasseri *et al.,* 2020). Around the same period, Indian physicians identified the disease and referred to it as madhumeha, meaning "honey urine," noting that ants were attracted to the urine (Lakhtakia, 2013; Roberts, 2015).

The Greek physician Apollonius of Memphis introduced the term "diabetes" or "to pass through" in 230 BC (Roberts, 2015; Trikkalinou *et al.,* 2017). During the time of the Roman Empire, diabetes was considered rare, with Galen mentioning that he had encountered only two cases throughout his career (Roberts, 2015; Karamanou *et al.,* 2016). The scarcity of recorded cases during the time of the Roman Empire may have been influenced by the dietary and lifestyle factors of ancient people, or because clinical symptoms were only observed in advanced stages of the disease, so, Galen referred to the condition as "diarrhea of the urine" (diarrhea urinosa) (Laios *et al*., 2012).

Dating back to the 2nd or early 3rd century AD, the earliest existing comprehensive account of diabetes, attributed to Aretaeus of Cappadocia, describes the symptoms and progression of the disease, linking it to moisture and coldness, which aligned with the beliefs of the "Pneumatic School" (Karamanou *et al.,* 2016). He also proposed a connection between diabetes and other illnesses and discussed how to distinguish it from snakebites, which also cause excessive thirst.

Aretaeus' work remained unknown in the Western world until 1552 when the first Latin edition was published in Venice (Laios *et al*., 2012).

In 400-500 CE, Indian physicians Sushruta and Charaka identified two types of diabetes as separate conditions, with one type associated with youth and the other type associated with being overweight (Lakhtakia, 2013; Trikkalinou *et al.,* 2017). In the early 20th century, notable advancements in treatment took place when Canadian scientists Frederick Banting and Charles Herbert Best achieved the successful isolation and purification of insulin in 1921 and 1922 (Vecchio *et al.,* 2018). Subsequently, the development of long-acting insulin NPH took place in the 1940s (Hirsch *et al.,* 2020; Bolli *et al.,* 2022).

2.3 Signs and symptoms of diabetes mellitus

The classic indications of untreated diabetes encompass unintended weight loss, increased urination (polyuria), heightened thirst (polydipsia), and increased hunger (polyphagia) (Cooke and Plotnick, 2008). These symptoms may manifest rapidly (within weeks or months) in type 1 diabetes, while they tend to develop more gradually and may be subtle or even absent in type 2 diabetes (Razaq *et al*., 2020).

Furthermore, there are several other non-specific signs and symptoms that can accompany the onset of diabetes which may include blurred vision, headaches, fatigue, slow wound healing, and itchy skin (Razaq *et al*., 2020). Prolonged elevation of blood glucose levels can lead to the absorption of glucose in the lens of the eye, causing changes in its shape and resulting in vision alterations (Calvo-Maroto *et al.,* 2014). Diabetic retinopathy can also result in long-term vision loss. Additionally, there is a group of skin rashes associated with diabetes collectively known as diabetic dermadromes (Nentwich and Ulbig, 2015).

2.4 Classification of diabetes mellitus

Diabetes is not a singular disease but rather a syndrome characterized by high blood sugar levels (hyperglycemia) and, over time, an increased risk of complications affecting the eyes, kidneys, nerves, heart, and medium to large blood vessels (American Diabetes Association, 2010). It can be classified into four main types: type 1, type 2, gestational, and secondary or other specific types of diabetes (American Diabetes Association, 2013). The majority of patients fall under the first two types, and various names have been used over the years, such as juvenile-onset/adult-onset, ketosis-prone/non-ketosis-prone, and insulin-dependent/non-insulin-dependent (Vellanki and Umpierrez, 2017). Despite the challenges in categorizing the specific type of diabetes in individual patients based on their phenotypic features, the preferred nomenclature now includes type 1 diabetes and type 2 diabetes (Genuth *et al.,* 2018). Gestational diabetes refers to diabetes induced by pregnancy. Secondary or other specific types of diabetes encompass a wide range of specific causes, including genetic defects in beta cell function, genetic defects in insulin action or structure, pancreatic diseases like pancreatitis and hemochromatosis, endocrinopathies, drug/chemical and surgically induced diabetes, infections, and rare immune-mediated and other genetic syndromes sometimes associated with diabetes (Genuth *et al.,* 2018).

2.4.1 Type 1 (insulin dependent) diabetes mellitus

This form of diabetes mellitus is referred to as autoimmune diabetes, previously recognized as juvenile-onset or ketosis-prone diabetes (Yadav *et al*., 2021). Individuals with this type of \diabetes may also experience other autoimmune conditions like Graves's disease, Hashimoto's thyroiditis, and Addison's disease (Nishi, 2018; Li *et al.,* 2020). Type 1 diabetes mellitus is also commonly known as insulin-dependent diabetes mellitus (IDDM), typically occurring in children and young adults, with the onset often abrupt and potentially life-threatening (Yadav *et al*., 2021).

Type 1 diabetes, which accounts for approximately 5% of all diabetes cases, is characterized by the main pathophysiological feature of an autoimmune attack on the pancreatic beta cells, resulting in a severe deficiency of insulin (Kahanovitz *et al.,* 2017; DiMeglio *et al.,* 2018). In type 1 diabetes, the damage to beta cells is primarily caused by T cells; however, autoantibodies produced by B cells against islet antigens are used as markers for the disease and may play a role in its development, with the presence of one or more of these autoantibodies often required for the diagnosis of the condition in research studies (Burrack *et al.,* 2017; Vandamme and Kinnunen, 2020; Zhu, 2022). Additionally, there may be non-immune-mediated causes of beta cell damage and destruction, and a specific type of diabetes called fulminant diabetes has been observed, particularly in Asians (Brawerman and Thompson, 2020; Choy *et al.,* 2022).

At the time of diagnosis, individuals with type 1 diabetes are typically of peripubertal age, Caucasian, lean, and have a short duration of symptoms such as increased urination, excessive thirst, and weight loss (Genuth *et al.,* 2018). It is common for there to be no family history of type 1 diabetes, although a family history of other autoimmune diseases like Grave's disease or Hashimoto's thyroiditis may be present (Frommer and Kahaly, 2021). No single clinical characteristic, such as age at diagnosis, body mass, or the presence of ketoacidosis, is sufficiently sensitive or specific to effectively distinguish between different forms of diabetes (American Diabetes Association, 2010; Genuth *et al.,* 2018). For example, due to the increasing prevalence of childhood and adolescent obesity, children with type 1 diabetes often have similar weight distributions to their age group (Kansra *et al.,* 2021; Ciężki *et al.,* 2022). In studies focused on the prevention and natural progression of type 1 diabetes, where individuals at high risk are closely monitored, over 50% of cases are detected through asymptomatic hyperglycemia, as the glucose levels are not yet high enough to cause symptoms like increased urination and weight

loss (Chiang *et al.,* 2018; Genuth *et al.,* 2018). This differs from the seemingly sudden onset of symptoms when individuals are diagnosed in a clinical setting (Welch and Carson, 2018).

Although severe insulin deficiency is a central characteristic of type 1 diabetes, it may not be sufficient to differentiate it from type 2 diabetes, especially in the early stages of the disease (Thomas *et al.,* 2019). Early on, insulin and C-peptide levels may not be significantly low in type 1 diabetes, and there is a "honeymoon period" shortly after diagnosis when the symptoms of diabetes appear to temporarily subside for several months to a year (Maddaloni *et al.,* 2022). On the other hand, some individuals with type 2 diabetes may experience severe insulin deficiency with very low levels of insulin and C-peptide in later stages, which can overlap with the levels seen in type 1 diabetes (Leighton *et al.,* 2017). The most reliable laboratory tests for distinguishing between type 1 and type 2 diabetes are the presence of autoantibodies to glutamic acid decarboxylase (GAD), insulin, insulinoma-associated protein 2 (IA-2), and zinc transporter 8 (ZnT8), especially when multiple antibodies are present and their titers are relatively high (Kawasaki *et al.,* 2020).

Type 1 diabetes exhibits heterogeneity in various aspects, with several genes that predispose or protect against the condition having been identified; however, individual patients display a wide range of genotypes (Genuth *et al.,* 2018). Many environmental factors can trigger or influence the severity of the autoimmune attack on beta cells, and the specific immune mechanisms involved can vary among patients (Petrelli *et al.,* 2021). At the time of diagnosis, patients may present with severe insulin deficiency accompanied by pronounced hyperglycemia and ketoacidosis, or they may have asymptomatic, mild postprandial hyperglycaemia (Genuth *et al.,* 2018). The rate of beta cell function decline before and after diagnosis varies greatly among

individuals with type 1 diabetes (Oram *et al.,* 2019; Sims *et al.,* 2020). In Caucasians, beta cell function declines over months to years, and this decline is slower in individuals who develop diabetes at an older age (Greenbaum *et al.,* 2012). More sensitive assays for C-peptide have detected measurable levels even after many years of type 1 diabetes (Oram *et al.,* 2014; Davis *et al.,* 2015). In fact, circulating C-peptide has been observed in Joslin Medalists who have lived with type 1 diabetes for over 50 years (Leete *et al.,* 2020). The frequency of end-organ complications over a lifetime with type 1 diabetes varies greatly among patients, even though it is influenced by glycemic control (Genuth *et al.,* 2018).

The American Diabetes Association (ADA) recognizes two forms of type 1 diabetes: type 1a and type 1b diabetes (American Diabetes Association, 2013). If antibodies are present along with insulin deficiency and ketosis, the diagnosis is autoimmune type 1 diabetes or type 1a diabetes (Genuth *et al.,* 2018). If individuals exhibit clinical features consistent with type 1 diabetes but do not have detectable antibodies, the ADA recognizes a category known as type 1b diabetes (or idiopathic type 1 diabetes) (Genuth *et al.,* 2018). Patients in this latter group may have a different underlying pathology or may possess autoantibodies that are not detectable with common assays (Pisetsky, 2023). Unless specifically indicated, in *Diabetes in America, 3rd edition*, the term "type 1 diabetes" primarily denotes the autoimmune variant (type 1a) (Genuth *et al.,* 2018).

2.4.2 Type 2 (non-insulin dependent) diabetes mellitus

Type 2 diabetes, accounting for approximately 90% to 95% of diabetes cases worldwide, is the other prominent type of diabetes (Genuth *et al.,* 2018). It results from a combination of insulin resistance, largely associated with obesity, and insufficient insulin secretion, which is believed to be the primary factor in the development of type 2 diabetes (Galicia-Garcia *et al.,* 2020). This

inadequate insulin secretion, despite the presence of insulin resistance, is referred to as relative insulin deficiency (American Diabetes Associatiom, 2009). The underlying cause of this defect in insulin secretion is likely multifactorial, typically attributed to metabolic factors rather than autoimmunity (American Diabetes Associatiom, 2009). Studies conducted on Native American Pima Indians, for instance, demonstrate a progressive and significant decline in insulin secretion as individuals transition from normal glucose tolerance to impaired glucose tolerance (IGT) and ultimately to diabetes (Weyer *et al.,* 1999).

Type 2 diabetes primarily results from a combination of lifestyle factors and genetic predisposition (Wu *et al.,* 2014; Galicia-Garcia *et al.,* 2020). The presence of excess body fat is associated with around 30% of cases in individuals of Chinese and Japanese descent, 60-80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders (Lesser *et al.,* 2013; Sun *et al.,* 2021). It is worth noting that even individuals who are not classified as obese may still exhibit a high waist-hip ratio (Ross *et al.,* 2020).

Dietary factors also play a role, as the consumption of sugar-sweetened beverages has been linked to an increased risk of developing type 2 diabetes (Malik and Hu, 2022). The type of fats included in the diet is also significant, with saturated and trans fats increasing the risk, while polyunsaturated and monounsaturated fats decrease the risk (Risérus *et al*., 2009). Excessive consumption of white rice may contribute to a higher risk of diabetes, particularly among Chinese and Japanese populations (Bhavadharini *et al.,* 2020). Additionally, a lack of physical activity can increase the likelihood of developing diabetes in certain individuals (Anjana and Mohan, 2016).

Adverse experiences during childhood, including abuse, neglect, and household difficulties, have been found to elevate the risk of developing type 2 diabetes later in life by 32%, with neglect having the most substantial impact (Huang *et al*., 2015). The use of antipsychotic medications and their side effects, such as metabolic abnormalities, dyslipidemia, and weight gain, along with unhealthy lifestyles characterized by poor diet and reduced physical activity, are potential risk factors for type 2 diabetes (Zhang *et al*., 2017).

When diagnosing type 2 diabetes, it is assumed that the patient does not have any of the specific causes of diabetes listed under secondary or other specific types of diabetes (American Diabetes Association, 2010; Genuth *et al.,* 2018). Classic clinical features of type 2 diabetes include obesity, onset in middle to late adulthood, a positive family history of type 2 diabetes among first-degree relatives, and a gradual rise in blood sugar levels that often presents with minimal symptoms (Arslanian *et al.,* 2018). Unlike type 1 diabetes, which predominantly affects Caucasians of northern European descent, type 2 diabetes is more prevalent among ethnic minorities such as African Americans, Hispanics, Asians, and Native Americans compared to Caucasians (Spanakis and Golden, 2013). However, similar to type 1 diabetes, no clinical characteristics can definitively confirm type 2 diabetes (American Diabetes Association, 2010). Except for the absence of autoimmune markers associated with type 1 diabetes, there are no specific laboratory tests for type 2 diabetes beyond glucose levels (Morran *et al.,* 2015).

Similar to type 1 diabetes, type 2 diabetes also displays significant heterogeneity, as it can manifest in individuals of various age groups, including children, adolescents, and adults, and can affect both lean and obese individuals (Genuth *et al.,* 2018). Patients with type 2 diabetes can vary widely, ranging from being asymptomatic to experiencing ketoacidosis or nonketotic

hyperosmolar coma; furthermore, the occurrence of diabetic complications can vary considerably among individuals over their lifetimes (American Diabetes Association, 2010; Genuth *et al.,* 2018). Diabetic microvascular complications, such as retinopathy and nephropathy, as well as neuropathy, share similarities between type 1 and type 2 diabetes, with factors like diabetes duration and glycemic levels playing major roles in their development (Chawla *et al.,* 2016; Mansour *et al.,* 2023). Both types of diabetes also increase the risk of macrovascular complications associated with atherosclerosis, with the advanced age of most type 2 diabetes patients contributing to an elevated absolute risk (Chawla *et al.,* 2016; Huang *et al.,* 2017).

2.4.3 Gestational diabetes mellitus

Gestational diabetes refers to the diagnosis of diabetes during pregnancy (Rani and Begum, 2016). Aiming to minimize perinatal complications through proactive treatment, screening for gestational diabetes is strongly recommended during pregnancy (Rani and Begum, 2016). Typically, during pregnancy, the onset of diabetes is a result of insufficient insulin secretion to counterbalance the significant increase in insulin resistance, particularly in the second and third trimesters, and as a result, gestational diabetes often improves or resolves after delivery (Plows *et al.,* 2018). The development of gestational diabetes indicates an underlying beta cell issue in these women, placing them at a very high risk (over 50%) of developing permanent type 2 diabetes over time (Plows *et al.,* 2018; Choudhury and Rajeswari, 2021; Ornoy *et al.,* 2021).

On rare occasions during pregnancy, type 1 diabetes or type 2 diabetes may be diagnosed, unrelated to the metabolic changes associated with pregnancy, and in such cases, the diabetes persists after delivery (Plows *et al.,* 2018). Customarily, these patients are diagnosed with type 1 diabetes or type 2 diabetes rather than gestational diabetes (Genuth *et al.,* 2018). The increased

prevalence and earlier onset of type 2 diabetes, coupled with the obesity epidemic, have made type 2 diabetes more prevalent among women of reproductive age (Bhupathiraju and Hu, 2016).

In rare instances, type 1 diabetes or type 2 diabetes can be diagnosed during pregnancy, independent of the metabolic changes typically associated with pregnancy (Plows *et al.,* 2018). In such cases, the diabetes persists after delivery, and these patients are typically diagnosed with type 1 diabetes or type 2 diabetes rather than gestational diabetes (Genuth *et al.,* 2018). The prevalence of type 2 diabetes has increased among women of reproductive age, coinciding with the rise in obesity rates (Chivese *et al.,* 2016; Rezai *et al.,* 2016).

Gestational diabetes shares similarities with type 2 diabetes, involving a combination of inadequate insulin secretion and responsiveness; this occurs in approximately 2-10% of pregnancies and may improve or disappear after delivery (Razaq *et al*., 2020). It is recommended that all pregnant women undergo testing around 24-28 weeks of gestation (Soldavini, 2019). Typically, gestational diabetes is diagnosed during the second or third trimester when insulin-antagonist hormone levels are elevated (Soldavini, 2019). However, after pregnancy, about 5-10% of women with gestational diabetes are found to have another form of diabetes, most commonly type 2; gestational diabetes is treatable but requires close medical supervision throughout the pregnancy (Razaq *et al*., 2020). Management may involve dietary changes, blood glucose monitoring, and, in some cases, insulin administration (Welson, 2018).

Untreated gestational diabetes, even if transient, can pose risks to the health of both the fetus and the mother. Risks to the baby include macrosomia (high birth weight), congenital heart and central nervous system abnormalities, and skeletal muscle malformations (Mitanchez *et al.,* 2015). Elevated insulin levels in the fetus's blood may hinder the production of surfactant,

leading to infant respiratory distress syndrome (Atar *et al.,* 2021). Increased bilirubin levels in the blood can result from the destruction of red blood cells. In severe cases, poor placental perfusion due to vascular impairment can lead to perinatal death (Roescher *et al.,* 2014). Induction of labor may be necessary if placental function is diminished, and a cesarean section may be performed if there is significant fetal distress or an increased risk of injury due to macrosomia, such as shoulder dystocia (Tarvonen *et al*., 2021).

2.4.4 Secondary or other specific (monogenic) types of diabetes

The secondary or other specific types of diabetes constitute the fourth category of diabetes. These encompass various classifications such as monogenic defects affecting beta cell function, genetic defects related to insulin action, exocrine pancreatic diseases, endocrinopathies, drug or chemical-induced diabetes, infectious causes, and less common immune-mediated and genetic syndromes associated with diabetes (American Diabetes Association, 2009). Previously, the term "maturity-onset diabetes of youth" (MODY) was used to describe monogenic defects of beta cell function (Arslanian *et al.,* 2020; Younis *et al.,* 2022). However, with advancements in research, specific gene defects underlying these conditions have been identified and are now described accordingly. For instance, MODY1 involves the hepatocyte nuclear factor 4-alpha (HNF4α) gene located on chromosome 20, while MODY2 is associated with the glucokinase gene on chromosome 7 (Firdous *et al.,* 2018). As further investigations progress, it is anticipated that the list of specific genetic causes contributing to diabetes will continue to expand (Greeley *et al.,* 2011; Ali, 2013).

The following is a list of disorders that may increase the risk of diabetes (Sheppard *et al*., 2007).

- Genetic defects of β-cell function

    - Maturity onset diabetes of the young
    - Mitochondrial DNA mutations

- Genetic defects in insulin processing or insulin action
    - Defects in proinsulin conversion
    - Insulin gene mutations
    - Insulin receptor mutations

- Exocrine pancreatic defects
    - Chronic pancreatitis
    - Pancreatectomy
    - Pancreatic neoplasia
    - Cystic fibrosis
    - Hemochromatosis
    - Fibrocalculous pancreatopathy

- Endocrinopathies
    - Growth hormone excess (acromegaly)
    - Cushing syndrome
    - Hyperthyroidism
    - Hypothyroidism
    - Pheochromocytoma
    - glucagonoma

- Infections
    - Cytomegalovirus infection
    - Coxsackievirus B
- Drugs
    - Glucocorticoids
    - Thyroid hormone
    - B-adrenergic agonists
    - Statins

(Sattar *et al*., 2010)

2.4.5 Combined type 1 and 2 diabetes

While type 1 diabetes and type 2 diabetes are typically considered as distinct conditions, characterized by high blood sugar levels, it is possible for these diseases, or certain aspects of each, to coexist within individual patients (American Diabetes Association, 2010). For instance, having type 1 diabetes does not prevent the development of obesity and insulin resistance, which are commonly associated with type 2 diabetes (Al-Goblan *et al.,* 2014; Wondmkun, 2020). Therefore, in the context of the obesity epidemic, an increasing number of individuals with type 1 diabetes may exhibit features of type 2 diabetes as well (Wondmkun, 2020). On the other hand, the discovery of islet cell antibodies as markers for the autoimmune process underlying type 1 diabetes revealed a higher prevalence of these autoantibodies (5%-10%) in patients with phenotypic type 2 diabetes compared to individuals without diabetes (1%) (Brooks-Worrell *et al.,* 2013; Kawasaki, 2023). Subsequent testing for GAD autoantibodies led to the identification of a

subgroup of phenotypic type 2 diabetes patients with latent autoimmune diabetes of adults (LADA) (Jones *et al.,* 2021; Manisha, 2022). Various terms, such as double diabetes and type 1.5 diabetes, have been introduced to describe the combination of type 1 and type 2 diabetes characteristics within individual patients (Khawandanah, 2019; Hamed *et al.,* 2021). In fact, numerous names have been used to categorize autoantibody-positive individuals with phenotypic type 2 diabetes (Brooks-Worrell and Palmer, 2012). The most consistent feature observed in these patients is a more rapid decline in beta cell function compared to autoantibody-negative type 2 diabetes patients, resulting in the need for earlier insulin treatment in autoantibody-positive phenotypic type 2 diabetes patients (Genuth *et al.,* 2018).

While the main cause of beta cell damage in type 1 diabetes is autoimmune-related, it is possible that some of the metabolic factors contributing to beta cell dysfunction in type 2 diabetes may also play a role in patients with type 1 diabetes (Genuth *et al.,* 2018). In cases where type 1 diabetes patients receive intensive treatment to achieve A1c levels close to normal, some individuals may experience weight gain and the insulin resistance typically associated with being overweight (Genuth *et al.,* 2018). Alongside this, these patients often develop additional features of the metabolic syndrome, which could potentially increase their risk of macrovascular complications compared to nonobese individuals with type 1 diabetes (Asghar *et al.,* 2023).

2.4.6 Prediabetes

Prediabetes is a term employed to describe individuals who have a heightened risk of developing either type 1 diabetes or type 2 diabetes in the future. It is important to note that not all individuals meeting the criteria for prediabetes will progress to diabetes (Genuth *et al.,* 2018). Prediabetes criteria for type 2 diabetes encompass individuals with elevated fasting glucose

levels that are below the diabetic range (referred to as "impaired fasting glucose" or IFG), those with impaired glucose tolerance after meals ("impaired glucose tolerance" or IGT), A1c levels ranging from 5.7% to 6.4% (39-46 mmol/mol), and individuals with a history of gestational diabetes (ElSayed *et al.,* 2023). While there is no universally accepted definition of prediabetes for type 1 diabetes, a combination of genetic, immune, and metabolic markers can be employed to accurately evaluate the risk of developing type 1 diabetes in the future (Genuth *et al.,* 2018). Utilizing this information to estimate the risk of future type 1 diabetes has been validated and effectively utilized in prevention trials focused on type 1 diabetes (Gale *et al.,* 2004; Greenbaum, 2021).

2.5 Problems with current classifications

The current classifications of diabetes present several problems and limitations. Distinguishing between type 1 and type 2 diabetes often relies on phenotypic characteristics that lack specificity for either type (Genuth *et al.,* 2018). Ideally, a more ideal classification system would be based on the underlying pathoetiology rather than relying on descriptive features (Basso, 2021). In type 1 diabetes, the presence of autoantibodies to GAD, insulin, IA-2, and ZnT8 indicates an autoimmune cause, therefore, it could be appropriate to categorize diabetes as autoimmune versus non-autoimmune (Genuth *et al.,* 2018). Alternatively, the presence of absolute insulin deficiency holds clinical significance and could serve as a useful basis for classification, as previously done with "insulin-dependent" versus "non-insulin-dependent" diabetes (American Diabetes Association, 2010). However, there are no specific markers exclusive to type 2 diabetes, making the absence of autoimmune markers an unreliable diagnostic criterion for type 2 diabetes (Genuth *et al.,* 2018; ElSayed *et al.,* 2023).

Type 1 and type 2 diabetes are not mutually exclusive and can coexist in many patients, or at least share some components of both diseases. For instance, the occurrence of islet autoantibodies is higher than expected in obese children with phenotypic type 2 diabetes (Badaru and Pihoker, 2012). Moreover, the characteristics of type 1 and type 2 diabetes in individual patients can change over time, for example, lean patients initially classified as having type 1 diabetes may develop obesity, while individuals with phenotypic type 2 diabetes who were previously negative for autoantibodies may later show the presence of islet autoantibodies (Genuth *et al.,* 2018; Galicia-Garcia *et al.,* 2020).

Additionally, the role of autoimmunity, as detected by autoantibodies, may differ between type 1 and type 2 diabetes (Genuth *et al.,* 2018). Autoimmunity is believed to be the primary cause of beta cell damage in type 1 diabetes, whereas other mechanisms such as oxidative stress, islet amyloid polypeptide toxicity, and glucotoxicity may initiate beta cell damage in type 2 diabetes (Cerf, 2013). This damage may subsequently lead to secondary beta cell autoimmunity, further accelerating beta cell deterioration (Kim *et al.,* 2021).

The field is reaching a point where the classification of most diabetes cases as either type 1 or type 2 needs to be reconsidered. The obesity epidemic has intensified the need to redefine diabetes in terms of type 1 versus type 2 (Genuth *et al.,* 2018).

2.6 Causes of diabetes mellitus

Impairments or abnormalities in the gluco-receptors of ß cells can lead to their altered response to higher glucose levels, resulting in either relative ß cell deficiency or impaired insulin secretion, which may progress to ß cell failure (Kumar and Clark, 2002). The theory behind microvascular

disease suggests that it causes neural hypoxia and that hyperglycemia directly affects neuronal metabolism (Ciofeta *et al.,* 1999).

1. Reduced sensitivity of peripheral tissues to insulin: This involves a decrease in the number of insulin receptors or their "down regulation." Many individuals with this condition exhibit hypersensitivity, hyperinsulinemia, and normal glycemic levels, along with associated dyslipidemia, hyperuricemia, and abdominal obesity. Consequently, there is a relative insulin resistance, particularly in the liver, muscle, and fat tissues. Hyperinsulinemia has been linked to angiopathy (National Institutes of Health, 1995).

2. Excess of hyperglycemic hormone (glucagon) and obesity: This leads to a relative insulin deficiency where ß cells fail to keep pace. Two theories propose abnormalities in nitric oxide metabolism, which result in altered perineural blood flow and nerve damage (Kumar and Clark, 2002).

3. Other less common forms of diabetes mellitus include those caused by specific genetic defects (type 3), such as "maturity-onset diabetes of the young" (MODY), as well as other endocrine disorders, pancreatectomy, and gestational diabetes mellitus (GDM) (National Institutes of Health, 1995).

4. Imbalances in specific receptors can also cause diabetes mellitus. Examples include the Glucagon-like peptide-1 (GLP-1) receptor, peroxisome proliferator-activated receptor gamma (PPARγ), beta3 (ß3) adrenergic receptor, and certain enzymes like α-glycosidase and dipeptidyl peptidase IV enzyme (National Institutes of Health, 1995).

5. Current research on diabetic neuropathy focuses on oxidative stress, advanced glycation end products, protein kinase C, and the polyol pathway (Ciofeta *et al.,* 1999).

2.6 Pathogenesis and pathophysiology of diabetes mellitus

Hyperglycemia is directly linked to physiological and behavioral responses, as the brain detects elevated blood glucose levels and communicates this information through nerve impulses to the pancreas and other organs, triggering a response to counteract its effects (Baynest, 2015).

2.7.1 Type 1 diabetes mellitus

Type 1 diabetes is characterized by the autoimmune destruction of insulin-producing cells in the pancreas, caused by the infiltration of CD4+ and CD8+ T cells and macrophages into the islets (Yoon and Jun, 2005; Burrack *et al.,* 2017). Several distinct features define type 1 diabetes mellitus as an autoimmune disease (Baynest, 2015):

1. Infiltration of immune-competent and accessory cells in the pancreatic islets.

2. Association of disease susceptibility with class II genes of the major histocompatibility complex (MHC) known as human leukocyte antigens (HLA).

3. Presence of autoantibodies specific to islet cells.

4. Abnormalities in T cell-mediated immunoregulation, particularly within the CD4+ T cell compartment.

5. Involvement of monokines and TH1 cells producing interleukins in the disease process.

6. Responsiveness to immunotherapy.

7. Frequent occurrence of other organ-specific autoimmune diseases in affected individuals or their family members.

8. Development of diabetic ketoacidosis (DKA).

(Baynest, 2015)

Approximately 85% of patients with type 1 diabetes have circulating islet cell antibodies, with many also showing detectable anti-insulin antibodies prior to receiving insulin therapy (Lutale *et al.,* 2007; DiMeglio *et al.,* 2018). Most islet cell antibodies target glutamic acid decarboxylase (GAD) found within pancreatic β cells (Raju and Raju, 2010).

The autoimmune destruction of pancreatic β cells leads to insulin secretion deficiency, resulting in metabolic disturbances associated with type 1 diabetes mellitus (American Diabetes Association, 2009). In addition to insulin deficiency, there are abnormalities in the function of pancreatic α cells, leading to excessive glucagon secretion in patients with type 1 diabetes mellitus (Adeva-Andany *et al.,* 2018). Normally, hyperglycemia suppresses glucagon secretion, but in individuals with type 1 diabetes mellitus, glucagon secretion is not properly regulated due to reduced levels of the counteracting hormone, insulin (Baynest, 2015). Elevated glucagon levels exacerbate the metabolic defects caused by insulin deficiency (American Diabetes Association, 2009; Li and Zhuo, 2014).

While insulin deficiency is the primary defect in type 1 diabetes mellitus, there is also a defect in insulin action. Insulin deficiency leads to uncontrolled lipolysis and increased levels of free fatty acids in the bloodstream, which suppress glucose metabolism in peripheral tissues like skeletal muscle (Holt, 2004). This impairs glucose utilization, and insulin deficiency also decreases the

expression of various genes necessary for target tissues to respond normally to insulin, such as glucokinase in the liver and the GLUT4 glucose transporters in adipose tissue (Holt, 2004). As Holt (2004) explains, the major metabolic abnormalities resulting from insulin deficiency in type 1 diabetes mellitus affect glucose, lipid, and protein metabolism. This leads to the development of diabetic ketoacidosis, a condition characterized by elevated ketone bodies in the blood.

2.7.2 Type 2 diabetes mellitus

In type 2 diabetes, the normal mechanisms involved in glucose regulation become dysfunctional, leading to two main pathological defects: impaired insulin secretion due to dysfunction in the pancreatic β-cells, and impaired insulin action caused by insulin resistance (American Diabetes Association, 2010). When insulin resistance is prominent, the β-cell mass undergoes changes that increase insulin production and compensate for the excessive and abnormal demand (Baynest, 2015). While the absolute concentration of plasma insulin (both fasting and after meals) is usually increased, it is insufficient relative to the severity of insulin resistance to maintain normal glucose levels (Baynest, 2015). Given the complex interplay between insulin secretion and sensitivity in regulating glucose homeostasis, it is practically impossible to separate the contribution of each in the development of type 2 diabetes (Kumar and Clark, 2002).

The primary events in type 2 diabetes are believed to involve an initial deficiency in insulin secretion and, in many patients, a relative insulin deficiency in conjunction with peripheral insulin resistance (American Disease Association, 2009; Galicia-Garcia *et al.,* 2020). Insulin resistance results in impaired glucose uptake in peripheral tissues such as muscle and fat, incomplete suppression of hepatic glucose output, and impaired fat uptake (Baynest, 2015). To compensate for insulin resistance, the islet cells increase insulin secretion. Endogenous glucose

production is accelerated in individuals with type 2 diabetes or impaired fasting glucose, and since this increase occurs alongside hyperinsulinemia, particularly in the early and intermediate stages of the disease, hepatic insulin resistance drives hyperglycemia in type 2 diabetes (Galicia-Garcia *et al.,* 2020). Over time, insulin resistance and hyperinsulinemia lead to impaired glucose tolerance (Wondmkun, 2020). A major complication of type 2 diabetes mellitus is the hyperglycemic hyperosmolar state.

2.8 Diagnostic tests for diabetes mellitus

Diabetes can be diagnosed using either HbA1c criteria or plasma glucose criteria, which include fasting plasma glucose (FPG) or the 2-hour plasma glucose (2-h PG) value obtained after a 75-gram oral glucose tolerance test (OGTT) (American Diabetes Association, 2014; The International Expert Committee, 2009). These tests serve the dual purpose of screening for and diagnosing diabetes. Diabetes can be detected in various clinical scenarios, spanning from seemingly low-risk individuals who undergo glucose testing to symptomatic patients and higher-risk individuals who are tested based on suspicion of diabetes (American Diabetes Association, 2015). Additionally, the same tests can identify individuals with prediabetes.

2.8.1 HbA1c

For the HbA1c test, it is essential to use a method that is certified by the NGSP and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay (Little *et al.,* 2019). While point-of-care (POC) HbA1c assays may have NGSP certification, proficiency testing is not mandatory for conducting the test. Therefore, the use of POC assays for diagnostic purposes may pose challenges and is not recommended (American Diabetes Association, 2015; Little *et al.,* 2019).

Compared to fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT), the HbA1c test offers several advantages, such as greater convenience since fasting is not required, enhanced preanalytical stability, and reduced day-to-day variations during periods of stress and illness (Florkowski, 2013). However, these benefits need to be balanced against higher costs, limited availability of HbA1c testing in certain regions of the developing world, and the incomplete correlation between HbA1c and average glucose levels in specific individuals (American Diabetes Association, 2015).

When utilizing HbA1c for diabetes diagnosis, it is crucial to consider factors such as age, race/ethnicity, and the presence of anemia or hemoglobinopathies (Klonoff, 2020).

2.8.1.1 Age

The foundation for recommending HbA1c as a diagnostic tool for diabetes is based on epidemiological studies conducted exclusively on adult populations (Florkowski, 2013). As a result, it is still uncertain whether HbA1c, along with the same HbA1c threshold, should be employed to diagnose diabetes in children and adolescents (Garcia de Guadiana *et al.,* 2012;).

2.8.1.2 Race/ethnicity

HbA1c levels can vary among individuals based on their race/ethnicity (Kumar *et al.,* 2010; Ziemer *et al.,* 2010). For instance, African Americans might exhibit higher HbA1c levels compared to non-Hispanic whites, even if their fasting and post-glucose load glucose levels are similar (American Diabetes Association, 2015). A recent study in epidemiology discovered that African Americans, both with and without diabetes, had elevated HbA1c levels compared to non-Hispanic whites when matched for FPG (American Diabetes Association, 2015). Additionally, African Americans showed higher levels of fructosamine and glycated albumin,

along with lower levels of 1,5-anhydroglucitol. These findings suggest that African Americans may carry a greater glycemic burden, particularly after meals (Selvin *et al.,* 2011).

2.8.1.3 Hemoglobinopathies/anemias

Understanding HbA1c levels can be challenging when certain hemoglobinopathies and anemia are present. For individuals with abnormal hemoglobin but regular red cell turnover, like those with the sickle cell trait, it is recommended to employ an HbA1c assay that is not affected by abnormal hemoglobin (American Diabetes Association, 2015). In cases involving increased red cell turnover, such as pregnancy during the second and third trimesters, recent blood loss or transfusion, erythropoietin therapy, or hemolysis, only blood glucose criteria should be utilized for diabetes diagnosis (American Diabetes Association, 2015).

2.8.2 Fasting and 2-hour plasma glucose

Apart from the HbA1c test, the fasting plasma glucose (FPG) and 2-hour plasma glucose (2-h PG) tests can also be used for diabetes diagnosis (American Disease Association, 2010). However, the agreement between these tests is not perfect, nor is the agreement between HbA1c and either of the glucose-based tests (American Diabetes Association, 2015). Data from the National Health and Nutrition Examination Survey (NHANES) suggest that using an HbA1c cutoff of ≥6.5% identifies one-third fewer cases of undiagnosed diabetes compared to using a fasting glucose cutoff of ≥126 mg/dL (7.0 mmol/L) (Picón *et al.,* 2012). Multiple studies have shown that the 2-h PG test detects more cases of diabetes compared to these HbA1c and FPG cutoffs. It's important to note that although the sensitivity of HbA1c is lower at the designated cutoff, its ease of use and potential for broader testing compensate for this limitation (American Diabetes Association, 2015).

Unless there is a clear clinical diagnosis, such as a patient experiencing a hyperglycemic crisis or exhibiting classic symptoms of hyperglycemia with a random plasma glucose ≥200 mg/dL, it is recommended to repeat the same test immediately using a new blood sample for confirmation (American Disease Association, 2011). This increases the likelihood of obtaining consistent results (American Diabetes Association, 2015). For instance, if the initial HbA1c result is 7.0% and the repeat result is 6.8%, the diagnosis of diabetes is confirmed. If two different tests (such as HbA1c and FPG) both yield values above the diagnostic threshold, this also confirms the diagnosis (American Diabetes Association, 2015). Conversely, if a patient shows discordant results from two different tests, the test result that exceeds the diagnostic cutoff should be repeated. The final diagnosis is based on the confirmed test. For example, if a patient meets the diabetes criterion of HbA1c (two results ≥6.5%) but not FPG (<126 mg/dL [7.0 mmol/L]), they should still be considered to have diabetes (American Diabetes Association, 2015).

Since all tests exhibit variability in the pre-analytic and analytic phases, it is possible for an abnormal result (i.e., above the diagnostic threshold) to produce a value below the cutoff when repeated (American Diabetes Association, 2015). This scenario is least likely for HbA1c, more likely for FPG, and most likely for the 2-h PG, especially if glucose samples are collected at room temperature and not promptly centrifuged (Sherwani *et al.,* 2016). Barring any laboratory errors, such patients are likely to have test results near the margins of the diagnostic threshold. Healthcare professionals should closely monitor these patients and repeat the test within 3-6 months (American Diabetes Association, 2015).

2.9 Complications of diabetes mellitus

Complications arising from diabetes mellitus can manifest as either acute problems that develop rapidly or chronic issues that progress gradually, affecting multiple organ systems (Banday *et al.,* 2020). These complications have the potential to significantly diminish the quality of life and result in long-term disabilities. However, individuals with well-managed blood sugar levels typically experience fewer and less severe complications (Adu *et al.,* 2019). Certain risk factors, such as age at diabetes onset, diabetes type, gender, and genetic predisposition, may play a role and are beyond a person's control. Moreover, lifestyle factors like smoking, obesity, high blood pressure, elevated cholesterol levels, and lack of regular exercise can exacerbate the chronic complications of diabetes (Wu *et al.,* 2014). It's worth noting that complications associated with diabetes increase the risk of severe COVID-19 illness (Kompaniyets *et al.,* 2021).

2.9.1 Acute complications of diabetes mellitus

2.9.1.1. Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) is a critical and immediate complication that requires urgent medical attention. It occurs due to low insulin levels, causing the liver to convert fatty acids into ketones for energy, leading to ketosis (Fletcher *et al.,* 2019). While ketones are normally produced periodically, sustained elevation can lead to serious issues. Elevated ketone levels in the bloodstream lower the blood's pH, leading to DKA. When a patient with DKA arrives at the hospital, they typically exhibit signs of dehydration, rapid and deep breathing, and may experience severe abdominal pain (Xu *et al.,* 2021). Initially, their level of consciousness is usually normal, but as the condition progresses, lethargy can advance to coma. Severe DKA can also cause hypotension, shock, and even death (Poovazhagi, 2014). Urine analysis often detects high levels of ketones, appearing before other obvious symptoms emerge. Proper and timely

treatment usually leads to complete recovery, but inadequate or delayed care, as well as complications like brain edema, can result in death (Shah and Kimberly, 2017). DKA is more prevalent in type 1 diabetes compared to type 2 diabetes.

2.9.1.2 Hyperglycemia hyperosmolar state

Nonketotic hyperosmolar coma (HNS) is an acute complication that shares many symptoms with DKA but has a completely different origin and requires different treatment (Mustafa *et al.,* 2023). When a person experiences very high blood glucose levels (usually exceeding 300 mg/dl or 16 mmol/L), water is drawn out of cells into the bloodstream, and the kidneys start expelling glucose into the urine (Mustafa *et al.,* 2023). This leads to water loss and an increase in blood osmolarity, eventually causing dehydration. Without fluid replacement (either orally or intravenously), the osmotic effect of high glucose levels, combined with water loss, results in progressive cell dehydration (Khetarpal *et al.,* 2016). Electrolyte imbalances are also common and pose significant risks. Similar to DKA, urgent medical attention is essential, typically starting with fluid replacement to address the dehydration. In some cases, lethargy may escalate into a coma, with type 2 diabetes being more susceptible to this outcome compared to type 1 (Khetarpal *et al.,* 2016).

2.9.2 Chronic complications of diabetes mellitus

2.9.2.1 Microvascular disease (microangiopathy)

Microangiopathy, also known as microvascular disease, is a condition that affects the small blood vessels in the microcirculation, as opposed to larger vessels impacted by coronary heart disease (Genuth *et al.,* 2018). Long-term diabetes mellitus is one of the causes of microangiopathy. In the case of diabetes, elevated blood glucose levels prompt the endothelial

cells lining the blood vessels to take in more glucose than usual, independent of insulin (Horton and Barrett, 2021). Consequently, these cells produce excessive glycoproteins on their surface and cause abnormal thickening and weakening of the vessel wall's basement membrane, and this leads to bleeding, protein leakage, and reduced blood flow throughout the body. As a consequence, certain organs and tissues may suffer from insufficient blood supply, resulting in damage, such as diabetic retinopathy affecting the retina or diabetic nephropathy affecting the kidneys (Xu and Shi, 2014). Additionally, nerves and neurons can also be harmed due to inadequate blood supply, resulting in diabetic neuropathy, particularly peripheral neuropathy. In severe cases, microangiopathy may lead to microangiopathic hemolytic anemia (MAHA) (Feldman *et al.,* 2019).

The damage to the small blood vessels can result in various complications, including but not limited to the following.

2.9.2.2 Diabetic nephropathy

Diabetic nephropathy, also known as diabetic kidney disease (Kittell, 2012), is a chronic condition characterized by the gradual decline of kidney function in individuals with diabetes mellitus. It stands as a primary cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide. Common features shared with many forms of CKD include protein leakage into the urine (proteinuria or albuminuria), hypertension or elevated blood pressure, and declining renal function (Longo *et al.,* 2013). The disease may progress slowly over several years (Afkarian *et al.,* 2016).

The pathophysiologic abnormalities of diabetic nephropathy usually stem from prolonged and poorly controlled blood glucose levels (Lim, 2014). These lead to changes in the filtration units

of the kidneys, the nephrons, which are instrumental in kidney function (Scott and Quaggin, 2015). Initially, the efferent arterioles constrict while the afferent arterioles dilate, resulting in glomerular capillary hypertension and hyperfiltration. This process, however, causes damage to the delicate glomerular capillaries, leading to further proteinuria, rising blood pressure, and a vicious cycle of additional nephron damage and decline in overall renal function (Yang and Xu, 2022). Simultaneously, changes within the glomerulus itself, such as basement membrane thickening and increased mesangial cells and matrix, contribute to the formation of Kimmelstiel-Wilson nodules that interfere with filtration (Conti *et al.,* 2018).

The status of diabetic nephropathy can be monitored through two values: the amount of protein in the urine (proteinuria) and a blood test called serum creatinine (Dabla, 2010). Proteinuria reflects the extent of impairment in operational glomeruli, whereas serum creatinine aids in the estimation of the glomerular filtration rate (eGFR), which mirrors the proportion of glomeruli not engaged in filtration. Treatment with certain medications, such as angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, may slow the progression of the disease by reducing blood pressure within the glomerular capillaries (Baltatzi *et al.,* 2011). Additionally, specific classes of diabetes medications, including GLP-1 agonists, DPP-4 inhibitors, and SGLT2 inhibitors, are believed to have potential in slowing diabetic nephropathy progression (deBoer, 2017).

Diabetic nephropathy constitutes the most prevalent cause of end-stage renal disease and is a serious complication affecting around a quarter of adults with diabetes in the United States (Mora-Fernández *et al.,* 2014; Ding and Choi, 2015). Individuals with end-stage kidney disease often require hemodialysis or kidney transplantation to replace lost kidney function. Moreover,

diabetic nephropathy is associated with an increased risk of death, particularly from cardiovascular disease (Mora-Fernández *et al.,* 2014; Pálsson and Patei, 2014).

The onset of symptoms typically occurs 5 to 10 years after the disease begins, with frequent urination at night (nocturia) being a common initial symptom. Other symptoms may include tiredness, headaches, general feelings of illness, nausea, vomiting, frequent daytime urination, lack of appetite, itchy skin, and leg swelling (Leslie *et al.,* 2023). As diabetic nephropathy's clinical presentation involves proteinuria, hypertension, and progressive loss of kidney function, regular screening for this condition is crucial in individuals with diabetes mellitus (Lim, 2014).

2.9.2.3 Diabetic neuropathy

Diabetic neuropathy refers to various types of nerve damage that occur in association with diabetes mellitus (Baynest, 2015). The symptoms experienced by individuals depend on the location of the nerve damage and can manifest as motor changes such as weakness, sensory symptoms like numbness, tingling, or pain, or autonomic changes, including urinary symptoms (Shin and Robinson-Papp, 2013). These alterations are believed to arise from microvascular injuries affecting the small blood vessels that supply the nerves (vasa nervorum). Common conditions that may be linked to diabetic neuropathy encompass distal symmetric polyneuropathy, third, fourth, or sixth cranial nerve palsy, mononeuropathy, mononeuropathy multiplex, diabetic amyotrophy, and autonomic neuropathy (Pasnoor *et al.,* 2013).

2.9. 2..2 Diabetic retinopathy

Diabetic retinopathy, also known as diabetic eye disease, is a medical condition characterized by damage to the retina resulting from diabetes mellitus. It stands as a leading cause of blindness in developed countries and affects approximately 80 percent of individuals who have had both type

1 and type 2 diabetes for 20 years or more. Proper treatment and regular eye monitoring can reduce the progression to more aggressive forms of sight-threatening retinopathy and maculopathy in at least 90% of new cases (Genuth *et al.,* 2018).

The risk of developing diabetic retinopathy increases with the duration of diabetes. Over several decades, nearly all people with diabetes experience some level of retina damage, which may go undetected and have no noticeable impact on vision. As time passes, a retinal exam may reveal progressive damage, starting with small bulges called microaneurysms in retinal blood vessels. Subsequently, larger abnormalities such as cotton wool spots, hemorrhages, hard exudates (lipid deposits), intraretinal microvascular abnormalities, and abnormal-looking retinal veins may appear. In some cases, new blood vessels grow throughout the retina, and when these vessels break and bleed, they can lead to vision problems, from minor dark spots obstructing vision to complete vision loss (Horton and Barrett, 2021).

Around half of individuals with diabetic retinopathy develop macular edema, which is swelling of the macula, and this can occur at any time. When the swelling affects the center of the macula, it can cause disruptions to vision, ranging from mild blurriness to severe loss of the central visual field. Without treatment, approximately 30% of those with macular edema may experience vision disruption within the next 3–5 years. Macular edema is the most common cause of vision loss in people with diabetic retinopathy. The repeated processes of blood vessel growth, swelling, and scarring may eventually lead to retinal detachment, which presents as sudden dark floating spots, flashes of light, or blurred vision (Genuth *et al.,* 2018).

2.9.2.4 Diabetic cardiomyopathy

Diabetic cardiomyopathy is a condition affecting the heart muscle in individuals with diabetes, leading to impaired blood circulation and heart failure (HF) (Kobayashi and Liang, 2015), which can result in fluid accumulation in the lungs (pulmonary edema) or legs (peripheral edema). It is primarily linked to coronary artery disease, and the term "diabetic cardiomyopathy" is used when there is no coronary artery disease present to explain the heart muscle disorder.

The development of diabetic cardiomyopathy is believed to involve defects in cellular processes like autophagy and mitophagy (Kobayashi and Liang, 2015). Functionally, it is characterized by ventricular dilation, enlargement of heart cells, prominent interstitial fibrosis, and either decreased or preserved systolic function alongside diastolic dysfunction. The disease's pathogenesis is attributed to several factors, including microangiopathy and related endothelial dysfunction, autonomic neuropathy, metabolic alterations involving abnormal glucose use and increased fatty acid oxidation, generation and accumulation of free radicals, disturbances in ion homeostasis (especially calcium transients), inflammation, and upregulation of local angiotensin systems. Diabetic cardiomyopathy can manifest as restrictive (HFPEF) or dilated (HFREF) phenotypes, with each having distinct underlying causes and contributing factors (Seferović and Paulus, 2015).

A unique characteristic of diabetic cardiomyopathy is its prolonged latent phase, during which the disease progresses without exhibiting symptoms. It is often detected alongside hypertension or coronary artery disease. In the early stages, signs may include mild left ventricular diastolic dysfunction with minimal impact on ventricular filling, decreased left ventricular compliance, left ventricular hypertrophy, or a combination of both. Subtle changes in the jugular venous pulse,

such as a prominent "a" wave, and an overactive or sustained cardiac apical impulse may also be observed. As the disease progresses and systolic dysfunction and heart failure develop, the jugular venous pressure may rise, the apical impulse may be displaced downward and to the left, and systolic mitral murmurs may occur. Electrocardiographic changes are also evident in about 60% of patients without structural heart disease, becoming more pronounced later in the disease progression with a prolonged QT interval indicative of fibrosis. Perfusion and atrial natriuretic peptide levels remain unchanged until the advanced stages when hypertrophy and fibrosis become more significant.

2.9.2.5 Macrovascular disease

Macrovascular disease is a condition affecting large (macro) blood vessels throughout the body, including the coronary arteries, aorta, and sizable arteries in the brain and limbs. It can occur after a prolonged period of diabetes. In this disease, fat and blood clots accumulate in the large blood vessels and adhere to the vessel walls. The three common forms of macrovascular disease are coronary disease (affecting the heart), cerebrovascular disease (involving the brain), and peripheral vascular disease (affecting the limbs). Macrovascular disease is synonymous with macroangiopathy, which is essentially a type of atherosclerosis. Atherosclerosis is a specific form of arteriosclerosis characterized by plaque deposits consisting of lipids, fibrous connective tissue, calcium, and other blood substances in medium and large arteries (excluding arterioles). The presence of macrovascular disease is associated with the development of coronary artery disease, peripheral vascular disease, stroke (brain attack), and an increased risk of infection. Type 2 diabetes is more closely linked to macrovascular diseases compared to type 1 diabetes. The presence of peripheral vascular disease and the heightened risk of infection hold significant implications for the care of acutely ill patients.

Macrovascular disease contributes to the development of cardiovascular disease, with accelerated atherosclerosis playing a role in this process. This involves conditions like:

- Coronary artery disease, which can lead to angina or myocardial infarction (commonly known as a "heart attack").
- Diabetic myonecrosis, often referred to as 'muscle wasting'.
- Peripheral vascular disease, contributing to exertion-related leg and foot pain known as intermittent claudication, as well as diabetic foot issues (Scott, 2013).
- Stroke (primarily the ischemic type).
- Carotid artery stenosis isn't significantly higher in diabetes, and there seems to be a lower prevalence of abdominal aortic aneurysm. However, diabetes does lead to increased morbidity, mortality, and surgical risks in these conditions.
- Diabetic foot problems often arise due to a combination of sensory neuropathy (resulting in numbness or insensitivity) and vascular damage. This combination elevates the chances of skin ulcers, infections, and, in severe cases, necrosis and gangrene. Consequently, healing from leg and foot wounds takes longer in diabetics, and they are more prone to infections. In developed countries, this issue is the leading cause of non-traumatic adult amputations, frequently involving toes and/or feet (Scott, 2013).
- In women with type 1 diabetes, female infertility is more prevalent. Despite modern treatment methods, delayed puberty, menarche, menstrual irregularities (especially oligomenorrhea), mild hyperandrogenism, polycystic ovarian syndrome, fewer live births, and potentially earlier menopause are commonly observed. Animal models suggest that at

a molecular level, diabetes disrupts leptin, insulin, and kisspeptin signaling (Codner *et al.,* 2012).

2.10 Kidneys

In humans, the kidneys are two kidney-shaped blood-filtering organs that possess a multilobar multipapillary structure characteristic of mammalian kidneys, typically lacking external lobulation (Haschek *et al.,* 2013; Zhou *et al.,* 2017). These organs are situated on the left and right sides within the retroperitoneal space, measuring around 12 centimeters (approximately 4+1⁄2 inches) in length in adult humans (Mescher, 2016). Blood is supplied to them through the paired renal arteries, and blood exits through the paired renal veins. A ureter, a tube responsible for conveying excreted urine to the bladder, is connected to each kidney.

The kidney plays a pivotal role in regulating the volume of different bodily fluids, fluid osmolality, acid-base balance, diverse electrolyte concentrations, and the elimination of toxins (Gounden *et al*., 2023). Filtration takes place in the glomerulus, where roughly one-fifth of the blood volume entering the kidneys undergoes filtration. Noteworthy substances like solute-free water, sodium, bicarbonate, glucose, and amino acids are reabsorbed, while substances such as hydrogen, ammonium, potassium, and uric acid are secreted (Breshears and Confer, 2017). The nephron constitutes the kidney's fundamental structural and functional unit. In adult human kidneys, there are approximately 1 million nephrons, while a mouse kidney contains only about 12,500 nephrons. Apart from nephron-related functions, the kidneys also perform additional roles. For instance, they convert a precursor of vitamin D into its active form, calcitriol, and synthesize hormones like erythropoietin and renin (Santoro *et al*., 2015).

2.10.1 Structure of the kidneys

In humans, the kidneys are situated in the upper abdominal cavity, flanking each side of the spine, and maintain a retroperitoneal position with a slight oblique angle. The liver's placement in the abdominal cavity introduces an asymmetry, leading to the right kidney being marginally smaller and lower than the left, and being positioned somewhat more centrally compared to the left kidney (Yoganandan *et al*., 2000). The left kidney is approximately located between the vertebral levels T12 to L3, while the right kidney is positioned a bit lower. The right kidney is situated just below the diaphragm and behind the liver, while the left kidney is located beneath the diaphragm and posterior to the spleen (Soriano *et al*., 2023). Atop each kidney rests an adrenal gland. The upper regions of the kidneys receive partial protection from the 11th and 12th ribs. Each kidney, together with its adrenal gland, is enveloped by two layers of fat: perirenal fat, located between the renal fascia and renal capsule, and pararenal fat situated above the renal fascia (Soriano *et al*., 2023).

The human kidney exhibits a bean-like structure with a convex border and a concave border. The renal hilum, a recessed region on the concave border, serves as the entry point for the renal artery and the exit point for the renal vein and ureter. The kidney is encased by durable fibrous tissue known as the renal capsule, which is encompassed by perirenal fat, renal fascia, and pararenal fat. The peritoneum lines the front surface of these tissues, while the transversalis fascia covers the rear surface (Frias *et al*., 2013).

The upper pole of the right kidney is adjacent to the liver, and for the left kidney, it abuts the spleen. Consequently, both kidneys shift downward during inhalation (Soriano *et al*., 2023).

2.10.2 Gross anatomy

The functional component, known as the parenchyma, of the human kidney is divided into two primary regions: the outer renal cortex and the inner renal medulla. These regions are visibly organized into eight to 18 cone-shaped renal lobes, each encompassing renal cortex that encircles a segment of the medulla referred to as a renal pyramid (Hansen *et al*., 2015; Tang *et al*., 2021). Renal columns, extensions of the cortex, extend between the renal pyramids. Nephrons, the essential structures responsible for producing urine, extend across both the cortex and the medulla. The initial filtration segment of a nephron is the renal corpuscle, situated within the cortex. This is followed by a renal tubule that extends from the cortex into the deep regions of the medullary pyramids. Within the renal cortex, a medullary ray is a cluster of renal tubules that drain into a singular collecting duct (Breshears and Confer, 2017).

At the apex, or papilla, of each pyramid, urine is discharged into a minor calyx; minor calyces merge into major calyces, and major calyces then lead into the renal pelvis. The renal pelvis serves as the origin of the ureter (Breshears and Confer, 2017). At the kidney's hilum, the renal vein and ureter exit, while the renal artery enters. These structures are enveloped by hilar fat and lymphatic tissue, which contains lymph nodes (Soriano *et al*., 2023). The hilar fat is connected to a cavity filled with fat known as the renal sinus. The renal sinus houses the renal pelvis and calyces and creates a separation between these components and the renal medullary tissue (Moritz *et al*., 2022).

2.10.3 Blood supply

The renal arteries, left and right, arise directly from the abdominal aorta, delivering blood to the kidneys. In adults, these organs receive around 20–25% of the heart's output. Each renal artery

branches into segmental arteries, which then further divide into interlobar arteries. These interlobar arteries pass through the renal capsule and traverse the renal columns situated between the renal pyramids. Moving on, the interlobar arteries supply blood to the arcuate arteries, which straddle the boundary between the cortex and the medulla. Each arcuate artery then provides nourishment to multiple interlobular arteries, which in turn feed the afferent arterioles responsible for supplying blood to the glomeruli (Leslie and Sajjad, 2023).

After the process of filtration, blood exits the kidneys, eventually reaching the inferior vena cava. Following filtration, the blood flows through a network of small veins (venules) that merge to form interlobular veins. Similar to the arteriole distribution, the veins follow a similar pattern: blood is transported from the interlobular veins to the arcuate veins and then to the interlobar veins, which eventually merge to form the renal veins that exit the kidneys (Dalal *et al*., 2023).

2.10.4 Nerve supply

The communication between the kidney and the nervous system occurs through the renal plexus, with its nerve fibers running alongside the renal arteries to connect with each kidney. Activation of the sympathetic nervous system prompts constriction of the blood vessels within the kidney, leading to a decrease in renal blood flow (Tanaka and Okusa, 2020). While the exact purpose of this input remains uncertain, it's worth noting that the parasympathetic nervous system also provides input to the kidney through the renal branches of the vagus nerve.. Sensory information from the kidney travels to the T10–11 spinal cord levels and is perceived in the corresponding dermatome. As a result, discomfort in the flank area might originate from the corresponding kidney.

2.9 Functions of the kidneys

2.9.1 Formation of urine

The kidneys expel a range of metabolic waste products into the urine. The nephron, which is the fundamental unit of the kidney both structurally and functionally, carries out this process through filtration, reabsorption, secretion, and excretion, resulting in urine production . These waste products encompass urea, produced from protein breakdown, and uric acid, originating from nucleic acid metabolism (Breshears and Confer, 2017). The capacity of certain mammals and some birds to concentrate waste products into a much smaller urine volume compared to the blood volume they come from relies on a complex countercurrent multiplication mechanism. This mechanism requires specific nephron characteristics to be operational, such as the intricate looping of tubules, water and ion permeability in the descending tubule segment, water impermeability in the ascending segment, and active ion transport in most of the ascending segment. Additionally, effective passive exchange through the vessels carrying the blood supply to the nephron is essential for enabling this function (Breshears and Confer, 2017).

The kidney plays a crucial role in maintaining overall body equilibrium by regulating aspects like acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. These homeostatic functions are achieved both independently by the kidney and in coordination with other organs, especially those within the endocrine system. Several endocrine hormones collaborate to manage these functions, including renin, angiotensin II, aldosterone, antidiuretic hormone, and atrial natriuretic peptide, among others (Hamm *et al.,* 2015).

2.10.5 Filtration

Filtration occurs within the renal corpuscle and involves separating larger cells and proteins for retention, while allowing smaller molecules to pass through from the blood, forming an ultrafiltrate that transforms into urine. In adults, the human kidney produces about 180 liters of filtrate daily. The standard range for a 24-hour urine volume collection spans from 800 to 2,000 milliliters per day. This process is also referred to as hydrostatic filtration due to the hydrostatic pressure exerted on the walls of the capillaries (Breshears and Confer, 2017).

2.10. 6. Reabsorption

Reabsorption involves moving molecules from the ultrafiltrate back into the peritubular capillary. This process is facilitated by selective receptors found on the luminal cell membrane. Around 55% of water is reabsorbed in the proximal tubule. Under normal conditions, glucose is entirely reabsorbed in the proximal tubule due to the action of the Na+/glucose cotransporter. If the plasma glucose level reaches 350 mg/dL, the transporters become fully saturated, leading to glucose being excreted in the urine. A plasma glucose level of approximately 160 is enough to trigger glucosuria, which is a significant sign of diabetes mellitus.

Amino acids are reabsorbed via sodium-dependent transporters in the proximal tubule. Hartnup disease, characterized by a deficiency in the tryptophan amino acid transporter, leads to pellagra as a result (Gattineni and Baum, 2015)

2.10.7 Secretion

Secretion is the opposite of reabsorption, involving the movement of molecules from the peritubular capillary through the interstitial fluid, then across the renal tubular cell and into the ultrafiltrate (Boron and Walter, 2006).

2.10.8 Excretion

The final stage in the handling of the ultrafiltrate is excretion: the ultrafiltrate exits the nephron and moves through a structure known as the collecting duct, which is a component of the collecting duct system. It then proceeds to the ureters, where it transforms into urine. Alongside its transport role, the collecting duct also plays a role in reabsorption (Breshears and Confer, 2017).

2.10.9 Blood pressure regulation

While the kidney lacks direct blood sensing capability, it significantly influences the long-term regulation of blood pressure. This primarily occurs by managing the extracellular fluid compartment size, which is influenced by the concentration of sodium in the blood plasma. Renin initiates a crucial sequence of chemical messengers within the renin–angiotensin system. Alterations in renin levels lead to changes in this system's output, mainly involving the hormones angiotensin II and aldosterone. Both hormones impact the kidney's absorption of sodium chloride, thus expanding the extracellular fluid compartment and elevating blood pressure. Elevated renin levels lead to higher angiotensin II and aldosterone concentrations, resulting in increased sodium chloride reabsorption, expanded extracellular fluid compartment, and raised blood pressure. Conversely, decreased renin levels lead to reduced angiotensin II and aldosterone levels, causing

contraction of the extracellular fluid compartment and lowering blood pressure (Van Beusecum and Inscho, 2015).

2.10.10 Acid-base balance

The body's acid–base balance is regulated by two major organ systems: the kidneys and the lungs. Acid–base homeostasis involves maintaining a pH around 7.4. The respiratory system, which includes the lungs, plays a key role in this balance by controlling the concentration of carbon dioxide (CO2) in the blood. It acts as the primary defense mechanism when the body encounters an acid–base issue. To bring the body's pH back to 7.4, the respiratory rate is adjusted. When the body becomes more acidic, the respiratory rate increases, expelling CO2 and reducing H+ concentration, thus raising pH. Conversely, in alkaline conditions, the respiratory rate decreases to retain more CO2, increasing H+ concentration and lowering pH.

The kidneys also contribute to acid–base balance through intercalated A and B cells. Intercalated A cells are activated in acidic conditions. High blood CO2 concentration in acidity prompts CO2 movement into cells, shifting the reaction $HCO3 + H \leftrightarrow H2CO3 \leftrightarrow CO2 + H2O$ to the left. On the cell's luminal side, H+ pumps and H/K exchangers operate against gradients, requiring ATP. These cells remove H+ from the blood to filtrate, elevating blood pH. On the basal side, a HCO3/Cl exchanger and Cl/K co-transporter move HCO3 into the blood, further raising pH. Similarly, intercalated B cells respond in a similar way but with reversed membrane protein orientation: proton pumps are on the basal side and HCO3/Cl exchangers and K/Cl co-transporters are on the luminal side. These cells release protons into the blood to lower Ph (Weiner and Verlander, 2015).

2.10.11 Regulation of osmolality

The kidneys play a role in regulating the body's water and salt balance. The hypothalamus detects substantial increases in plasma osmolality and directly communicates with the posterior pituitary gland. When osmolality rises, the gland releases antidiuretic hormone (ADH), leading to water reabsorption by the kidneys and a rise in urine concentration. These two factors collaborate to restore plasma osmolality to its typical levels (Cuzzo *et al.,* 2023).

2.10.12 Hormone secretion

The kidneys produce several hormones, such as erythropoietin, calcitriol, and renin. Erythropoietin is discharged when there's hypoxia (insufficient tissue oxygen levels) in the renal circulation, prompting the bone marrow to generate red blood cells. Calcitriol, the active form of vitamin D, enhances the absorption of calcium in the intestines and the reabsorption of phosphate in the kidneys. Renin functions as an enzyme that oversees the levels of angiotensin and aldosterone (Fu *et al.,* 2016).

2.10.13 Erythropoietin

Erythropoietin, a glycoprotein cytokine, is primarily secreted by the kidneys when cells experience a lack of oxygen, triggering the production of red blood cells in the bone marrow (Bhoopalan *et al.,* 2020). There is a continuous release of small amounts of EPO (around 10 mU/mL) to compensate for normal turnover of red blood cells. Conditions such as anemia and chronic lung disease can cause cellular oxygen deficiency, leading to higher EPO levels (up to 10,000 mU/mL) (Bhoopalan *et al.,* 2020).

Interstitial fibroblasts in the kidney, closely linked to the peritubular capillary and proximal convoluted tubule, along with perisinusoidal cells in the liver, are responsible for EPO production. Liver-produced EPO is more significant during fetal and perinatal stages, while kidney-produced EPO takes over in adulthood. Erythropoietin shares similarities with thrombopoietin (Bhoopalan *et al.,* 2020).

Recombinant human erythropoietin (rhEPO), known as exogenous erythropoietin, is produced the usage of recombinant DNA strategies inside mobileular cultures. These are collectively referred to as erythropoiesis-stimulating agents (ESA), including examples like epoetin alfa and epoetin beta. ESAs find application in treating anemia associated with chronic kidney disease, myelodysplasia, and anemia resulting from cancer chemotherapy (Bunn, 2013).

2.11. 3. Functions

2. 11.3.1 Red blood cell production

Erythropoietin stands as a crucial hormone vital for the generation of red blood cells. Its absence halts the process of definitive erythropoiesis. When faced with oxygen deficiency, the kidney responds by producing and releasing erythropoietin. This hormone directs its efforts towards enhancing the production of red blood cells by influencing specific subsets of cells involved in differentiation, including CFU-E, proerythroblast, and basophilic erythroblast groups. The primary impact of erythropoietin lies upon red blood cell precursors and progenitors, which are situated within the human bone marrow. It achieves this by bolstering their survival, shielding these cells from undergoing apoptosis, or cell demise (Bhoopalan *et al.,* 2020).

Erythropoietin takes on a leading role in the erythropoietic process, working alongside a variety of other growth factors like IL-3, IL-6, glucocorticoids, and SCF, which contribute to the

development of the erythroid lineage originating from multipotent progenitors. The initial stage, referred to as burst-forming unit-erythroid (BFU-E), initiates the expression of the erythropoietin receptor and displays sensitivity to erythropoietin. Progressing to the subsequent phase, the colony-forming unit-erythroid (CFU-E), exhibits peak levels of erythropoietin receptor density and relies exclusively on erythropoietin for continued differentiation. The precursors of red blood cells, namely proerythroblasts and basophilic erythroblasts, also bear the erythropoietin receptor and, as such, are influenced by its effects (Dzierzak and Philipsen, 2013).

2.11.3.2 Non-hematopoietic roles

Erythropoietin's effects extend beyond its role in promoting erythropoiesis. These include inducing hypertension through vasoconstriction, triggering angiogenesis, and enhancing cell survival by activating EPO receptors, leading to protective effects on ischemic tissues (Kimáková *et al.,* 2017). Nevertheless, this idea is met with controversy, as several studies have failed to establish any impact (Brar *et al.,* 2021). Moreover, this notion conflicts with the fact that these cells possess minimal EPO receptors. Human clinical trials involving ischemic heart, neural, and renal tissues have not replicated the favorable outcomes observed in animals (Sanchis-Gomer *et al.,* 2013). Furthermore, certain research investigations suggest its potential to safeguard against diabetic neuropathy, yet these findings have not been verified in clinical trials involving nerves such as the deep peroneal, superficial peroneal, tibial, and sural nerves (Sanchis-Gomer *et al.,* 2013).

2.11.3.3 Mechanism of action

Erythropoietin achieves its impacts by binding to the erythropoietin receptor (EpoR). EPO attaches to the erythropoietin receptor situated on the surface of red cell precursors, triggering a

signaling cascade involving JAK2 (Bhoopalan *et al.,* 2020). This sets off pathways like STAT5, PIK3, and Ras MAPK, culminating in the differentiation, survival, and multiplication of the erythroid cell. Negative regulatory elements of the cytokine signal, namely SOCS1, SOCS3, and CIS, are also produced (Bhoopalan *et al.,* 2020).

The erythropoietin receptor is notably prevalent in erythroid progenitor cells. Although there are reports of EPO receptors existing in various tissues like the heart, muscle, kidney, and nervous tissues, these findings are clouded by the lack of specificity in reagents such as anti-EpoR antibodies. Rigorous experiments do not identify a functional EPO receptor in those tissues. Within the bloodstream, red blood cells themselves lack the erythropoietin receptor, hence they cannot respond to EPO. Nonetheless, there are indications that the longevity of red blood cells in the bloodstream might indirectly depend on plasma erythropoietin levels, a phenomenon termed neocytolysis. Furthermore, there is solid proof indicating an increase in EPO receptor expression following brain injuries (Suresh *et al.,* 2019).

2.11.3.4 Synthesis and regulation

In the absence of anemia, erythropoietin levels in the bloodstream are relatively modest, approximately at 10 mU/mL. However, under conditions of low oxygen, the production of EPO can surge dramatically, escalating up to a thousand-fold and reaching 10,000 mU/mL in the blood. Among adults, the primary source of EPO synthesis is the interstitial cells situated within the peritubular capillaries of the renal cortex. Additionally, the liver and brain pericytes contribute to its production. The regulatory process is believed to hinge on a feedback mechanism that assesses blood oxygen saturation and the availability of iron. Transcription factors responsible for EPO synthesis, known as hypoxia-inducible factors, are continually

produced but undergo hydroxylation and proteosomal degradation when oxygen and iron are present. In periods of normal oxygen levels (normoxia), GATA2 acts to suppress the promoter region responsible for EPO. However, during times of oxygen deficiency (hypoxia), GATA2 levels decline, facilitating the activation of EPO production (Panjeta *et al.,* 2015).

2.9.2 Erythropoietin deficiency

This condition is often observed in patients who have been diagnosed with chronic kidney disease, which is usually caused by diabetic nephropathy (Gluba-Brzózka *et al.,* 2020). It occurs as the function of the kidneys declines, leading to a decrease in the production of erythropoietin by the kidneys. This reduction in erythropoietin production ultimately results in hypoproliferative anemia, which is a condition characterized by a decreased production of red blood cells by the bone marrow (Antwi-Bafour *et al.,* 2016). This may lead to a variety of symptoms, including fatigue, weakness, and shortness of breath (Gluba-Brzózka *et al.,* 2020).

2.12 Glycated haemoglobin

Glycated haemoglobin, also known as glycohaemoglobin or haemoglobin A1C (HbA1c), is a type of hemoglobin that forms a chemical bond with sugar (Sherwani *et al.,* 2016). When certain sugars like glucose, galactose, and fructose are present in the bloodstream, they spontaneously link with hemoglobin in a non-enzymatic manner (Sherwani *et al.,* 2016). However, glucose has a lower likelihood of bonding compared to galactose and fructose (only 13% compared to fructose and 21% compared to galactose). This could explain why glucose is the primary metabolic fuel in humans (Sherwani *et al.,* 2016).

The presence of the sugar-hemoglobin linkage indicates an excess of sugar in the bloodstream, which is often a sign of diabetes when its concentration is high (HbA1c > 6.4%) (Sherwani *et al.,*

2016). HbA1c is particularly noteworthy because it is easily detectable. The process of sugars attaching to hemoglobin is termed glycation, and the reference system is based on HbA1c, defined as beta-N-1-deoxy fructosyl hemoglobin as a component (Sherwani *et al.,* 2016).

HbA1c is primarily measured to determine the average blood sugar level over three months and can be used as a diagnostic test for diabetes mellitus. It is also employed as an assessment tool for glycemic control in people with diabetes (World Health Organization, 2011). The test covers a three-month average because red blood cells have an average lifespan of four months, and since they have varying lifespans, the test provides a limited measure for three months (Radin, 2014).

Normal glucose levels result in a normal amount of glycated hemoglobin. However, as the average plasma glucose levels rise, the proportion of glycated hemoglobin also increases in a predictable manner (Sherwani *et al.,* 2016). In diabetes, higher levels of glycated hemoglobin indicate poorer control of blood glucose levels and have been linked to cardiovascular disease, nephropathy, neuropathy, and retinopathy (Sherwani *et al.,* 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This is a case control study of patients with complicated diabetes mellitus who were evaluated for Erythropoietin and HbA1c level in their blood sample. A properly structured questionnaire was administered to every participant to obtain socio demographic details.

3.2 Study participants

The study participant consists of patients with complicated diabetes mellitus in Benin City, Edo State and the control groups were recruited from Benin City. Only subjects who gave informed consent and met the inclusion criteria were enrolled in this study.

3.3 Selection criteria

3.3.1 Inclusion criteria

Study Group - All adult males and females with complicated diabetic mellitus in Benin City who gave consent to participate in the study.

Control group 1 - Apparently healthy males and females with no history of diabetes mellitus in Benin City who gave consent to participate in the study.

Control group 2 - Adult males and females with diabetes mellitus (without complication) in Benin City who gave consent to participate in the study

3.3.2 Exclusion criteria

Individuals with underlying illness were excluded.

3.4 Sample Size

The sample size for this study was determined based on three factors:

1. The estimated prevalence of variable interest from literature review

2. Confidence interval of 95%
3. The acceptable margin of error

The sample size was calculated according to the following formula;

$$N = \frac{z^2 * p(1-p)}{d^2}$$

Where;

N= required sample size

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

p = estimated prevalence of diabetes mellitus of variable interest from literature review (2.1%)

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

$$N = \frac{1.92^2 * 0.021 (1- 0.021)}{0.05^2} = 30$$

N = 30

In a retrospective study carried out by Okoye *et al.,* 2011 in Benin City, Edo state, prevalence of diabetes mellitus in chronic kidney disease (CKD) patients was 2.1%. This was used to calculate the sample size for this study, (resulting to a sample size of 30). This finding also supports the WHO report (WHO, 1999) which classifies Nigeria as a highly endemic country.

3.5 Research setting

The hospital where this research was carried out is the University of Benin Teaching Hospital. The University of Benin Teaching Hospital (UBTH) is a tertiary healthcare institution located in Benin City, Edo State, Nigeria. It serves as a teaching hospital for the University of Benin's College of Medical Sciences, providing training and education for medical students and healthcare professionals. The establishment of UBTH can be traced back to the early 1970s when

there was a growing need for a teaching hospital to support medical education in the region. In 1973, the Federal Military Government of Nigeria under General Yakubu Gowon approved the construction of the teaching hospital. The aim was to improve the quality of healthcare services, advance medical research, and produce well-trained healthcare professionals. The teaching hospital has played a vital role in medical education, research, and healthcare delivery in the region. It offers a wide range of medical services and specialized care in various departments, including Surgery, Obstetrics and Gynecology, Internal Medicine, Pediatrics, Radiology, Pathology, and many others. UBTH has also been actively involved in medical research, collaborating with local and international institutions to address healthcare challenges, develop new treatment modalities, and contribute to scientific advancements.

The Internal Medicine Unit at the University of Benin Teaching Hospital (UBTH) is a specialized department that provides comprehensive healthcare services for the diagnosis, management, and treatment of a wide range of adult medical conditions. Under the Internal Medicine Department at the University of Benin Teaching Hospital (UBTH), there are several specialized units that focus on specific areas of medical care. These units work collaboratively to provide comprehensive healthcare services to patients. One of these units under the Internal Medicine Department is the Endocrinology Unit. The Endocrinology Unit specializes in the diagnosis and management of hormonal disorders and metabolic conditions, including diabetes mellitus, thyroid disorders, adrenal disorders, and metabolic bone diseases. Endocrinologists in this unit provide comprehensive care for patients with hormonal imbalances and work closely with other specialists for multidisciplinary management.

Due to the nature of this research, I worked with the Endocrinology Unit in the Department of Internal Medicine at the University of Benin, Benin City, Edo state for my data collection.

3.6 Ethical approval

Ethical approval with Reference number ADM/E22/A/VOL.VII/148301129(appendix) was obtained from the Health Research Ethics Committee, University of Benin Teaching Hospital, Benin City, Edo State to carry out this research in Benin City In addition, the recruited participants gave their consents after a thorough explanation of the rationale for the study and information on the nature of the research, benefits as well as confidentiality by making use of a questionnaire.

3.6 Sample collection by questionnaire administration

A validated questionnaire was administered to consenting participants to obtain information on:

- Age and sex
- State of origin
- Occupation
- Marital status
- Family history of diabetes mellitus
- Complication of diabetes mellitus
- Underlying disease conditions
- Types of diabetes mellitus
- Extent of smoking and extent of alcohol intake
- Exposure of sunlight
- Type of medication
- Duration of medication
- Intake of dietary supplements.

3.7 Sample collection

Under aseptic conditions, about 5 millimeters of venous blood samples was obtained from the ante-cubital vein of each subject using a sterile needle and syringe. Then, it was then dispensed into an Ethylene Diamine Tetra-acetic Acid (EDTA) container, fluoride oxalate container, and a clean dry plain container. The sample in the EDTA container was used to examine the HbA1c, hemoglobin, and PCV levels of the subject. The sample in the fluoride oxalate container was used to examine the fasting blood sugar of the subject. The sample in the plain container was left undisturbed for a few minutes to clot and centrifuged at 4000rpm for about 5 minutes to separate serum from the clot. The serum was then dispensed into another clean and dry plain container and used to examine erythropoietin, urea, and creatinine levels. The serum samples will be stored at -20 degrees Celsius prior analysis.

3.8 Analysis of parameters

3.8.1 Fasting Blood Sugar (FBS)

Principle of glucose oxidase method

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinonemine dye as indicator.

Procedure

To 1ml of glucose oxidase reagent, 10µl of sample/standard was added. It was mixed and incubated at 37^0C for 10 minutes. The absorbance was read at 490nm against a reagent blank.

Calculation of result

Glucose concentration = Absorbance of sample x Concentration of standard

Absorbance of standard

Reference range

70 – 100mg/dl

3.8.2 HbA1c

Principle

Proteases extensively digest the lysed whole blood sample releasing amino acids like glycated valines from the Hb beta chains. The recombinant fructosyl valine oxidase enzyme then acts on the glycated valines, cleaving them in the N-terminal sites producing hydrogen peroxide. This is then measured using a horseradish peroxidise catalyzed reaction and a suitable chromogen.

Procedure

Wavelength	**Temperature**	**Cuvette**	**Measurement**
660nm	37^0C	1cm light path	Against reagent blank
The following were pipetted into microtitre wells			
	Reagent Blank		**Sample/Control/Calibrator**
Sample/Control/Calibrator	-		6µl
R1 Buffer	150µl		150µl
It was mixed and incubated for 5 minutes, absorbance 1 was then read before proceeding			
R2 Enzyme/ Chromogen	50µl		50µl
It was mixed and incubated for another 5 minutes and absorbance absorbance 2 was measured			

N.B.: The final absorbance was calculated as follows:

A = A2 – A1

Calculation of result

HbA1c concentration = $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ x Concentration of standard

Reference range

4.0 – 6.0%

3.8.3 Hemoglobin

Hemoglobin concentration was determined by cyammethemoglobin method under standard protocol.

Reference range

Male: 13.2 – 16.6g/dl

Female: 11.6 – 15g/dl

3.8.3 Creatinine

Principle of Jaffe-Slot method

In the presence of alkaline picrate, creatinine forms an orange-red complex colour which is measured at 500nm in a spectrophotometer.

Creatinine + picric acid + NaOH ⟶ Orange-red colour complex

Procedure

To 1ml of alkaline solution, 1ml of picric acid solution and 100µl of sample/standard were added. It was mixed and incubated on the bench at room temperature for 10 minutes. The absorbance was read at 500nm against a reagent blank.

N.B: Alkaline solution consists of 0.4M NaOH and 50mM Sodium carbonate.

Calculation of result

Creatinine concentration = $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ x Concentration of standard

Reference range

Male: 0.9 – 1.3mg/dl

Female: 0.6 – 1.2mg/dl

3.8.4 Erythropoietin

Principle

The Elabscience ELISA kit was used in the estimation erythropoietin (EPO) level using sandwich ELISA principle. The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to Human EPO. Samples (or standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human EPO and Avidin-Horseradish peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human EPO, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in colour. The enzyme-substrate reaction is terminated by the addition of stop solution and the colour turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2nm. The OD value is proportional to the concentration of Human EPO. The concentration of human EPO in the samples can be calculated by comparing the OD of the samples to the standard curve.

Procedure

1. 100µl of standard or sample was added to each well and incubated for 90 minutes at 37^0C.
2. The liquid in the well were removed and 100µl of biotinylated detection Ab/Ag was added, which was incubated for an hour at 37^0C.
3. It was then aspirated and washed three times using wash buffer.
4. 100µl of HRP Conjugate was added to the well and incubated for 30 minutes at 37^0C.

5. It was then aspirated and washed three times using wash buffer.
6. 90µl of HRP Conjugate was added to the well and incubated for 15 minutes at 37^0C.
7. 50µl of HRP Conjugate was added to the well and the OD value was determined immediately at 450nm.

Calculation of result

The average absorbance value was calculated for each set of reference standards, controls and patient samples.

Reference range

5.8 – 9.9 IU/L

3.8.5 Packed Cell Volume (PCV)

Principle of Packed Cell Volume (Cheesbrough, 2006)

The word haematocrit means to separate the blood where the plasma and the blood cells are separated. This test gives information about the RBCs concentration and helpful to see haemoconcentration. This is basically a measurement of the total blood volume and RBCs ratio as a percentage.

Procedure of Packed Cell Volume

A well-mixed EDTA blood sample was drawn into a non-heparinized capillary tube with capillary action. The tube was filled about three-quarter length with whole blood. One end of the tube was sealed by heating with flame from a Bunsen burner, making sure that no blood cell gets burnt. The sealed tube was placed in a groove in the microhaematocrit centrifuge and balanced by placing another capillary tube opposite it after which the overhead lid of the microhaematocrit centrifuge was closed. It was centrifuged at 11,000 ± 2,000 rpm for 5 minutes. The capillary tube was removed from the centrifuge and it showed three layer. The top layer is the plasma layer, the

middle layer is the buffy coat layer while the last layer is the packed red cell layer. The microhaematocrit reader is used to read the value of PCV.

Microhaematocrit reader

The reader is used as a holder for the tube against a scale so that the bottom of the red cell is matched with zero line at the bottom of the card. Move the tube across until the plasma uppermost line come in contact with the 100% line. Check and confirm the bottom end is still on the zero line and the tube is straight and vertical. Adjust the moveable scale until it gets to the top of the packed red cells then read against the scale to obtain the PCV value.

Reference range

Male: 45 – 52%

Female: 37 – 48%

3.8.6 Urea

Principle of Urease method

Urea is hydrolyzed by urease to form ammonia from ammonium carbonate. The ammonia reacts with phenol in the presence of hypochlorite to form iodophenols which gives a blue colour in a alkaline medium and measured at 550nm spectrophotometrically.

Urea + NH_4CO_3 $\xrightarrow{\text{urease}}$ NH_3 + Phenol hypochloric iodophenols (blue colour)

Procedure

To 100µl of urease reagent, 10µl of the sample/standard was added and incubated in a water bath at 37^0C for 10 minutes. 2ml each of urea solution 2 and 3 were added and incubated in a water bath at 37^0C for 15 minutes. The absorbance was read at 550nm.

3.1.1.1 Calculation of result

Urea concentration = $\frac{\text{Absorbance of sample}}{}$ x Concentration of standard

Absorbance of standard

Reference range

10 – 55mg/dl

3.9 Statistical analysis

The questionnaires was screened for completeness, coded and data was entered into the SPSS version 25.0 software. Values obtained in this study was presented as mean ± standard deviation (levels of significance were accepted at $p<0.001$).

CHAPTER FOUR

RESULT

4.1 Socio-demographic characteristics of the study population

Table 1 shows the socio-demographic characteristics of the study population. The study population comprises 30 diabetes patients without complications, 21 diabetes patients with complications and 30 healthy non-diabetic controls. A greater percentage of the non-diabetic control were young adults (96.7%), females (96.7%), had no family history of diabetes (86.7%), were single (93.3%), unemployed (86.7%), had obtained tertiary education (100%) and of the Benin ethnic group (33.3%). A greater proportion of the diabetic patients without complications had suffered from the disease for 5 – 10 years (50%); were of the middle-age class (56.7%); were females (66.7%); had family history of diabetes mellitus (90%); were married (96.7%), retired (63.3%), had obtained tertiary education (80%) and from Benin city (70%). Majority of the diabetic patients with complications had suffered from diabetes for 5 – 10 years (66.7%); were of the elderly adult class (57.1%); were males (85.7%); had family history of diabetes mellitus (81%); were married (100%), retired (85.7%), had obtained tertiary education (85.7%) and from Benin city (52.4%).

Table 4.1. The socio-demographic characteristics of the study population

Characteristics		STUDY GROUPS			Total
		NON-DIABETIC	DIABETIC WITHOUT COMPLICATION	DIABETIC WITH COMPLICATION	
Duration of Diabetes Mellitus	>10yrs	0 (0)	8 (26.7)	7 (33.3)	15(18.5)
	0-5yrs	0 (0)	7 (23.3)	0 (0)	7 (8.6)
	5-10yrs	0 (0)	15 (50.0)	14 (66.7)	29(35.8)
	Nil	30 (100)	0 (0)	0 (0)	30(37.0)
Age Groups	Young	29 (96.7)	1 (3.3)	0 (0)	30(37.0)
	Middle-Age	1 (3.3)	17 (56.7)	9 (42.9)	27(33.3)
	Elderly	0 (0)	12 (40.0)	12 (57.1)	24(29.6)
Sex	Females	29 (96.7)	20 (66.7)	3 (14.3)	52(64.2)
	Males	1 (3.3)	10 (33.3)	18 (85.7)	29(35.8)
History of Diabetes Mellitus	No	26 (86.7)	3 (10.0)	4 (19.0)	33(40.7)
	Yes	4 (13.3)	27 (90.0)	17 (81.0)	48(59.3)
Marital Status	Married	2 (6.7)	29 (96.7)	21 (100.0)	52(64.2)
	Single	28 (93.3)	1 (3.3)	0 (0)	29(35.8)
Occupation	Employed	4 (13.3)	8 (26.7)	0 (0)	12(14.8)
	Retired	0 (0)	19 (63.3)	18 (85.7)	37(45.7)
	Unemployed	26 (86.7)	3 (10.0)	3 (14.3)	32(39.5)
Educational	Primary	0 (0)	5 (16.7)	3 (14.3)	8 (9.9)
	Secondary	0 (0)	1 (3.3)	0 (0)	1 (1.2)

Status	Tertiary	30(100.0)	24 (80.0)	18 (85.7)	72(88.9)
Ethnicity	Agbor	0 (0)	1 (3.3)	0 (0)	1 (1.2)
	Auchi	0 (0)	1 (3.3)	0 (0)	1 (1.2)
	Benin	10 (33.3)	21 (70.0)	11 (52.4)	42(51.9)
	Eshan	5 (16.7)	2 (6.7)	3 (14.3)	10(12.3)
	Etsako	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Ghana	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Igbo	6 (20.0)	1 (3.3)	0 (0)	7 (8.6)
	Ikan n	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Isoko	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Unknown	1 (3.3)	0 (0)	0 (0)	1 (1.2)
	Urhobo	2 (6.7)	3 (10.0)	0 (0)	5 (6.2)
	Yoruba	2 (6.7)	1 (3.3)	7 (33.3)	10(12.3)

4.2 Selected life-style profile of the study population

Table 2 shows selected life-style and clinical characteristics of the study population. Majority of the control subjects did not take nutritional supplements (96.7%); received daily sunlight (100%); performed daily exercise (96.7%); do not take alcohol (80%); do not smoke (100%); consume high carbohydrate, high protein diets (90%); indicated no blood loss (83.3%); and did not show any diabetes complications (100%). A greater percentage of the diabetes patients without complications had type 2 diabetes (80%); were taking diabetes medications (100%); were not taking nutritional supplements (86.7%); received daily sunlight (100%); performed daily exercise (100%); do not take alcohol (100%); do not smoke (100%); consume high carbohydrate, high protein diets (70%); indicated no blood loss (86.7%); and did not show any diabetes complications (100%). Most of the diabetes patients with complications had type 2 diabetes (51.9%); were taking diabetes medications (63%); were not taking nutritional supplements (93.8%); received daily sunlight (100%); performed daily exercise (98.8%); do not take alcohol (90.1%); do not smoke (100%); consume high carbohydrate, high protein diets (80.2%); indicated no blood loss (88.9%); and did not show any diabetes complications (74.1%).

Table 4. 2. Selected life-style and clinical characteristics of the study population

		GROUP			Total
		Control	DWOC	DWC	
Type of Diabetes Mellitus	Nil	30 (100)	0 (0)	0 (0)	30(37.)
	Type 1	0 (0)	6 (20.0)	3 (14.3)	9 (11.1)
	Type 2	0 (0)	24(80.0)	18(85.7)	42(51.9)
Diabetes Medication	Yes	0 (0)	30(100)	21(100)	51(63.0)
	None	30 (100)	0 (0)	0 (0)	30(37.0)
Nutritional Supplement	No	29(96.7)	26(86.7)	21 (100)	76(93.8)
	Yes	1 (3.3)	4 (13.3)	0 (0)	5 (6.2)
Daily Sunlight	No	0 (0)	(0)	(0)	(0)
	Yes	30 (100)	30 (100)	21 (100)	81 (100)
Daily Exercise	Yes	29(96.7)	30 (100)	21 (100)	80(98.8)
	No	1 (3.3)	0 (0)	0 (0)	1 (1.2)
Alcohol	No	24(80.0)	28(93.3)	21 (100)	73(90.1)
	Yes	6 (20.0)	2 (6.7)	0 (0)	8 (9.9)
Smoking	No	30 (100)	30 (100)	21 (100)	81 (100)
	Yes	(0)	(0)	(0)	(0)
Dietary Intake	High Carbohydrate, High Protein	27(90.0)	21(70.0)	17(81.0)	65 (80.2
	High Carbohydrate, Low Protein	1 (3.3)	0 (0)	0 (0)	1 (1.2)

	Low Carbohydrate, High Protein	2 (6.7)	9 (30.0)	4 (19.0)	15(18.5)
Blood Loss	Injury	0 (0)	4 (13.3)	0 (0)	4 (4.9)
	Menstruation	5 (16.7)	0 (0)	0 (0)	5 (6.2)
	None	25(83.3)	26(86.7)	21 (100)	72(88.9)
Complications	Foot Ulcer	0 (0)	0 (0)	4 (19.0)	4 (4.9)
	Retinopathy	0 (0)	0 (0)	13(61.9)	13(16.0)
	Neuropathy	0 (0)	0 (0)	4 (19.0)	4 (4.9)
	None	30 (100)	30 (100)	0 (0)	60(74.1)

Abbreviations: DWOC, diabetics without complications; DWC, diabetics with complications.

4.3 Mean glycated hemoglobin levels of the study population

The mean glycated hemoglobin levels of the study population are shown in Figure 1. Analysis of variance and a post-hoc analysis indicated that non-diabetic control had a significantly lower ($p < 0.001$) mean HBA1c (5.90 ± 1.28%) compared with diabetes patients without complications (8.57 ± 2.71%) and those with complications (12.08 ± 0.73%). Diabetic patients with complications also indicated significantly higher ($p < 0.001$) mean HBA1c level compared with patients without complications.

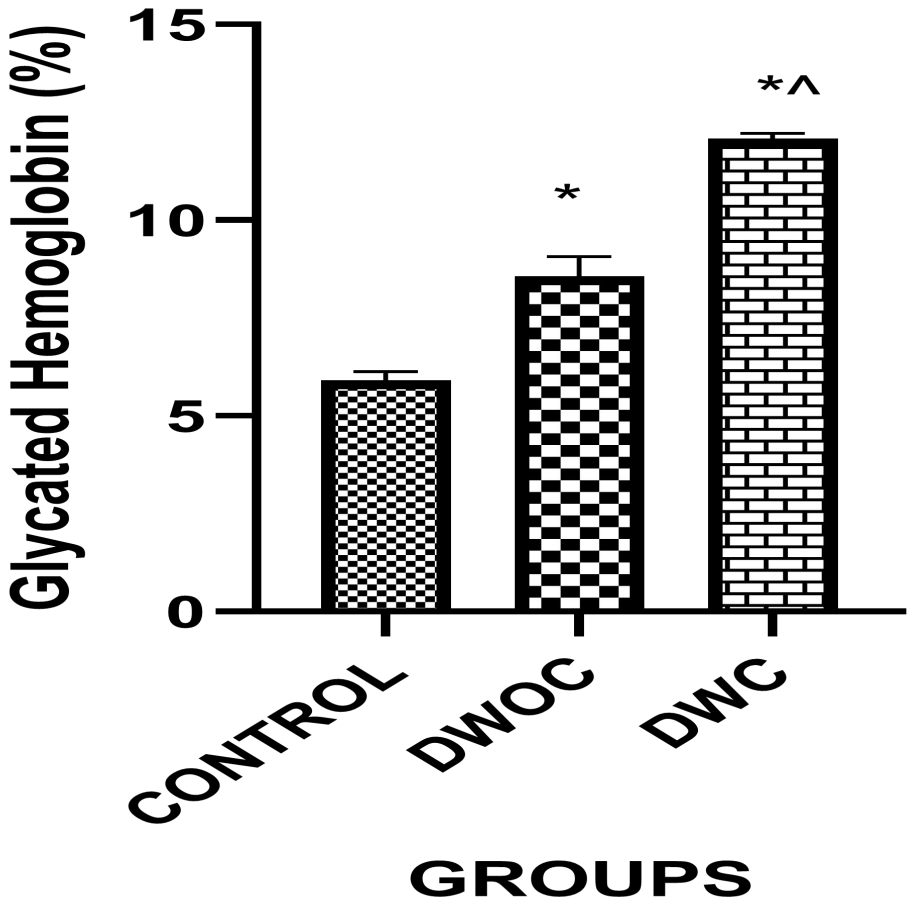

Figure 4.1. The mean glycated hemoglobin levels of the study population.

4.4 Mean fasting glucose level of the study population

Figure 2 shows the mean fasting glucose level of the study population. Data indicate significantly lower ($p < 0.001$) mean fasting blood sugar in non-diabetic control (92.60 ± 12.21 mmol/L) compared with diabetic patients without complications (166.16 ± 80.8 mmol/L) and those with complications (184.85 ± 83.43 mmol/L). There was no significant difference in mean FBS between diabetic patients with complications compared with patients without complications ($p = 0.319$).

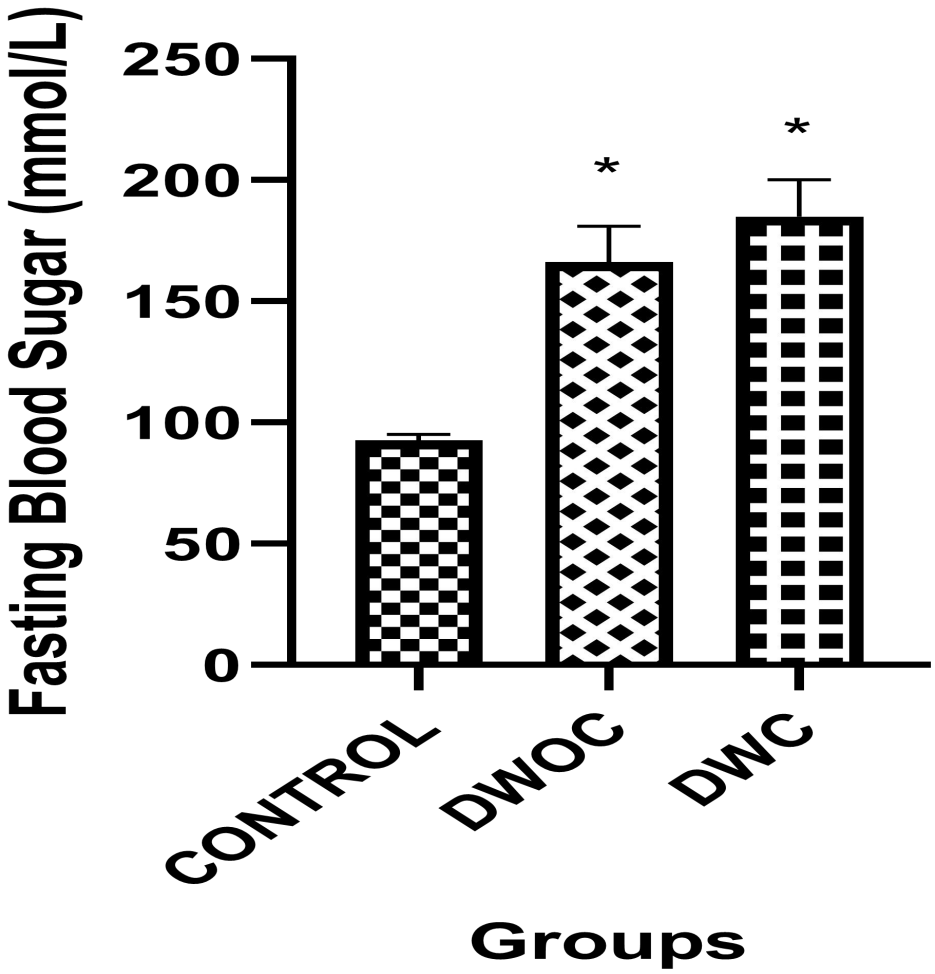

Figure 4.2. The mean fasting glucose level of the study population

4.5 Mean erythropoietin level of the study population

The mean erythropoietin level of the study population is shown in Figure 3. Data indicates a significantly higher mean erythropoietin level in the control group (332.27 ± 106.04 IU) compared with patients without complications (274.73 ± 52.19 IU) and those with complications (242.86 ± 23.44 IU). However, no significant differences were observed between diabetic patients with complications and those without complications ($p = 0.129$).

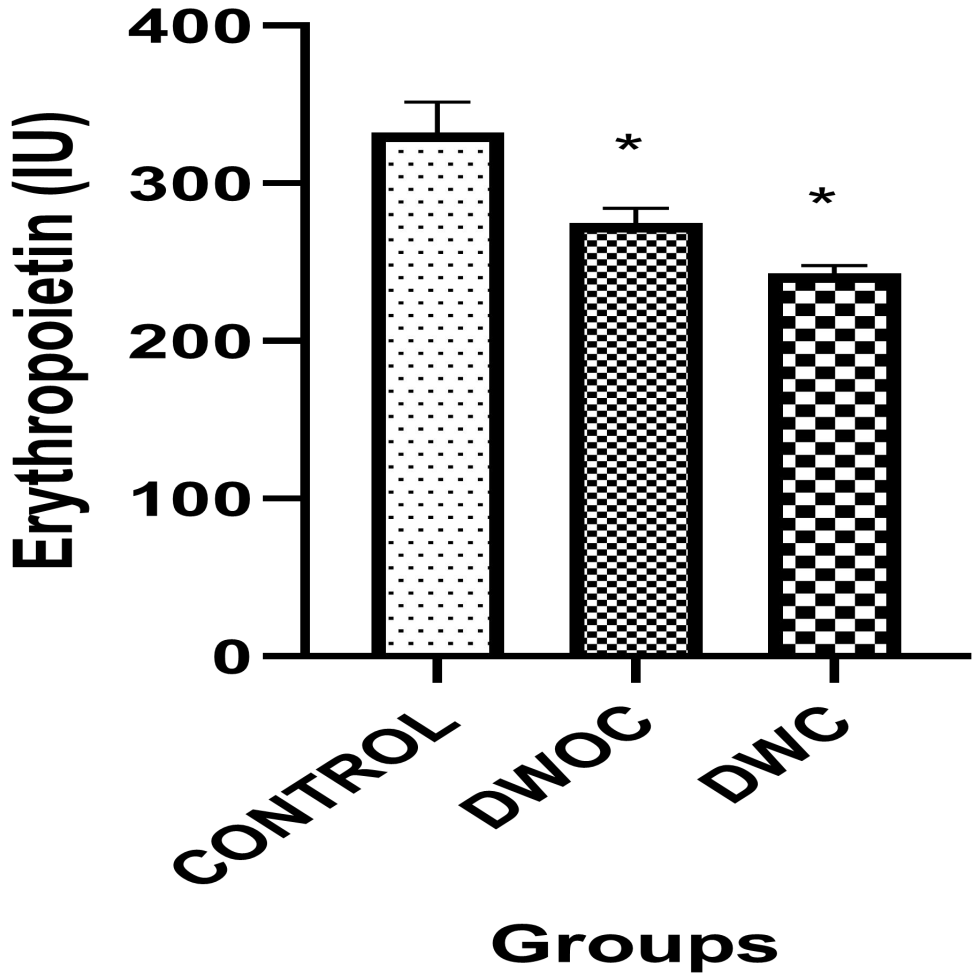

Figure 4.3. The mean erythropoietin level of the study population

4.6 Mean urea level of the study population

The mean urea level of the study population is presented in Figure 4. Data shows that the control group (17.46 ± 4.82 mmol/L) indicated significantly lower ($p < 0.001$) mean urea level compared with patients without complications (27.50 ± 10.94 mmol/L) and those with complications (31.71 ± 18.16 mmol/L). No significant differences were observed between diabetic patients with complications and those without complications ($p = 0.211$).

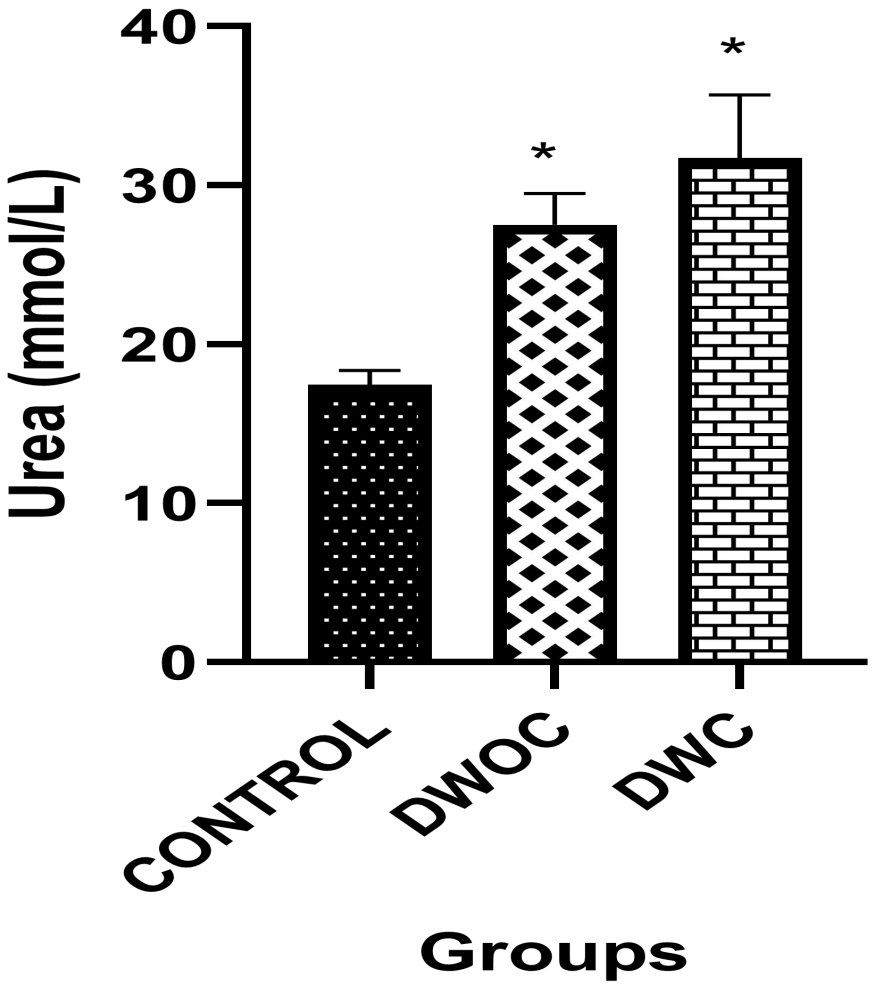

Figure.4 4. Mean urea level of the study population

4.7 Mean packed cell volume of the study population

The mean packed cell volume of the study groups is presented and compared among them in Figure 5. Analysis of variance shows that the control group (33.30 ± 3.55%) indicated significantly lower mean urea level compared with patients without complications (37.40 ± 5.49%) and patients with complications (36.66 ± 4.37%). No significant differences were observed between diabetic patients with complications and those without complications ($p = 0.514$).

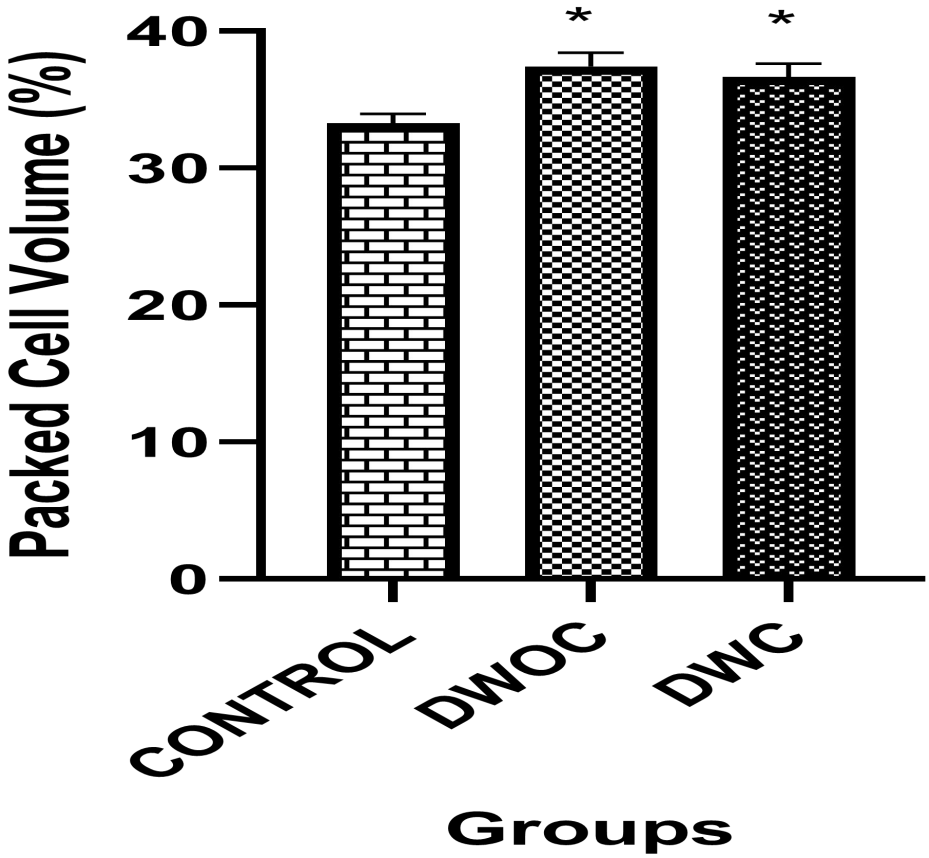

Figure 4.5. The mean packed cell volume of the study population

4.8 Mean hemoglobin concentration of the study population

The mean hemoglobin concentration of the study population is shown in Figure 6. There was no significant difference ($p = 0.067$) in hemoglobin concentration between the control group (11.26 ± 1.08 g/dl) and diabetic patients without complications (11.93 ± 1.50 g/dl). Similarly, no significant difference ($p = 0.214$) was observed in Hb concentration between diabetic patients with complications and those without complications (12.42 ± 1.58 g/dl). In contrast, the diabetic patients with complications indicated significantly greater ($p = 0.004$) level of hemoglobin compared with the non-diabetic controls.

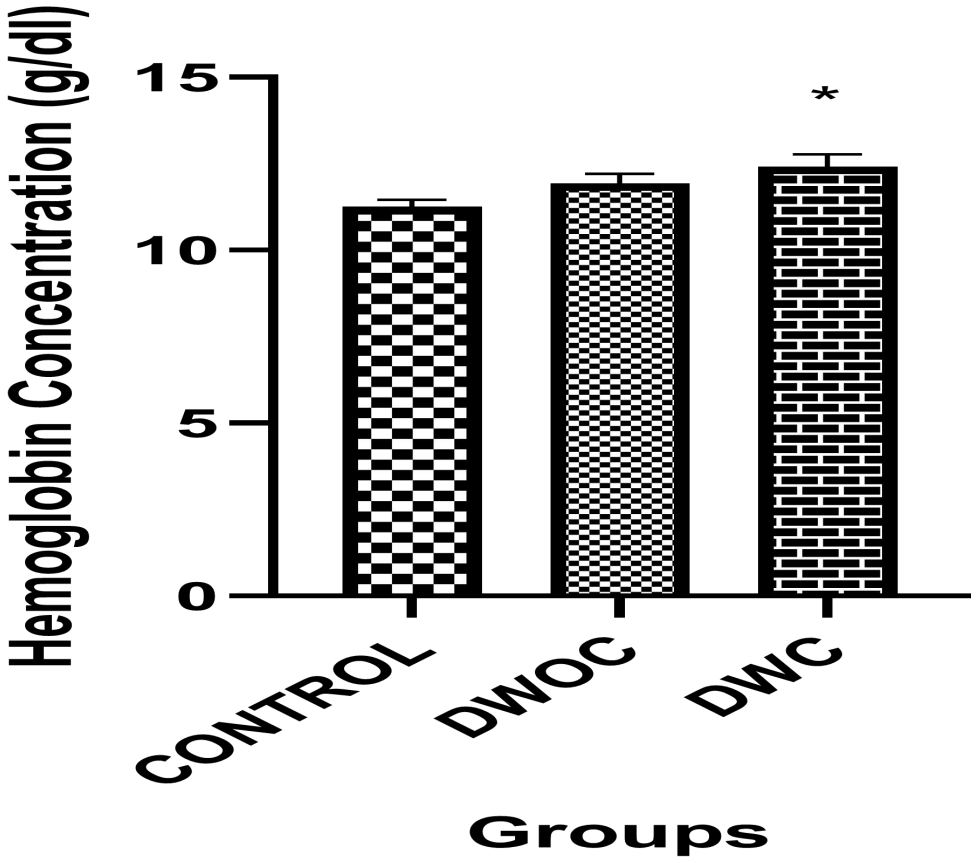

Figure 4.6. The mean hemoglobin concentration of the study population

4.9 Mean creatinine levels of the study population

Figure 7 shows the mean creatinine levels of the study population. Data shows that there were no significant differences in mean creatinine level between the control (4.27 ± 2.36 mg/dl) and patients without complications (3.78 ± 3.34 mg/dl; $p = 0.489$) and patients with complications (2.91 ± 2.19 mg/dl; $p = 0.084$). Similarly, no significant difference was found in creatinine level between the patients with complications and those without complications ($p = 0.267$).

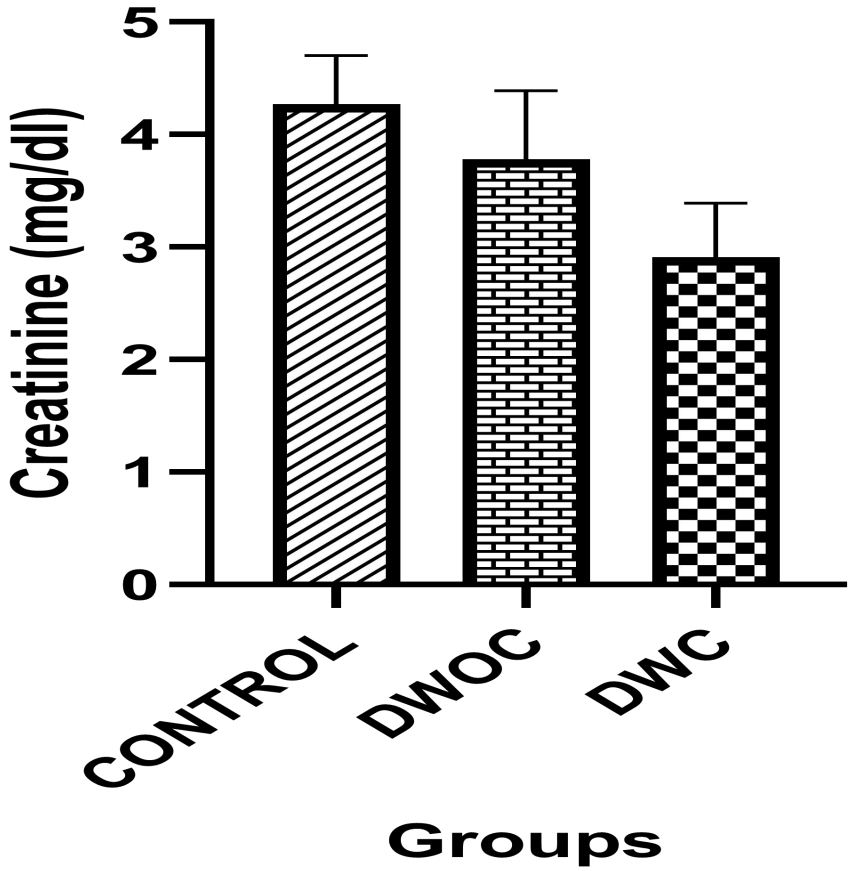

Figure 4.7. The mean creatinine levels of the study population

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion of Findings

This study investigated the levels of erythropoietin and HbA1c in patients with diabetic complications (both macrovascular and microvascular complications) in University of Benin Teaching Hospital, Benin City, Edo State. Diabetes, also referred to as diabetes mellitus, is a collection of common endocrine disorders characterized by persistently elevated levels of glucose in the bloodstream (Galicia-Garcia *et al.,* 2020; Yadav *et al*., 2021). Erythropoietin, a glycoprotein cytokine, is primarily secreted by the kidneys when cells experience a lack of oxygen, triggering the production of red blood cells in the bone marrow (Bhoopalan *et al.,* 2020). HbA1c is primarily measured to determine the average blood sugar level over three months and can be used as a diagnostic test for diabetes mellitus. It is also employed as an assessment tool for glycemic control in people with diabetes (World Health Organization, 2011).

In this study, the demographics, lifestyle and clinical characteristics is shown. Table 1 shows the socio-demographic characteristics of the study population. It shows that a greater proportion of patients living with complications of diabetes mellitus were of the elderly age range. This is in accordance with Bai, *et al*. (2021), that the prevalence of diabetes increased with advanced age with the highest prevalence for ages ≥65.

Table 1 show male have a higher percentage. This study is in disagreement with Khan, *et al*. (2020), which reported that the rates of occurrence of diabetes mellitus are similar among men and women.

Selected life-style and clinical characteristics of the study population as shown in Table 2, indicates that large percentage of patients with complication of diabetes mellitus have retinopathy. This is not in accordance with Tracey *et al*. (2015); the prevalence of diabetes complications ranged widely depending widely depending on study population and methodology used (6.5-25.2% retinopathy; 3.2-32.0% neuropathy; 2.5-5.2% nephropathy).

The mean glycated haemoglobin levels compared between patients living with complications of diabetes mellitus, without complications of diabetes mellitus and their healthy control as shown in Figure 1, indicated a significant difference in mean glycated haemoglobin levels between non-diabetic control compared with the diabetic patients without complications and those with complications. Similarly, diabetic patients with complications also indicated significantly higher mean HBA1c level compared with patients without complications. This is in agreement with Sherwani *et al*. (2016), which noted that higher levels of glycated haemoglobin have been linked to cardiovascular disease, nephropathy, neuropathy, and retinopathy, and indicate poorer control of blood glucose levels.

Figure 2, in the result shows that the mean fasting blood sugar in non-diabetic control compared with diabetic patients without complications and those with complications is statistically significant. However, There was no significant difference in mean FBS between diabetic patients with complications compared with patients without complications which implies that the fasting blood sugar level in both diabetic patients with complications and those without complications is not dependent on the patient having complications or not having complications.

Figure 3, in the result shows that the mean erythropoietin level of the study population indicates a significantly higher mean erythropoietin level in the control group compared with patients without complications and those with complications. However, no significant differences were

observed between diabetic patients with complications and those without complications. This finding is in disagreement with the study carried out by Gluba-Brzózka *et al*. (2020), which reported that erythropoietin level decreases as a result of complication (especially nephropathy) in diabetic patients.

Figure 4, in the result shows that the mean urea level of the control group indicated significantly lower mean urea level compared with patients without complications and those with complications. No significant differences were observed between diabetic patients with complications and those without complications. This implies that the level of urea was not dependent on whether the diabetic patient had any complication or not.

In this study, the mean packed cell volume level of the control group indicated significantly lower mean urea level compared with patients without complications and patients with complications, meaning there is a significant difference between the packed cell volume level in the healthy group and those with diabetes mellitus (both with complications and without complications). However, no significant differences were observed between diabetic patients with complications and those without complications, meaning the urea level is not dependent on whether the patient has any complication or not.

In this study, there was no significant difference in the mean hemoglobin level of the control group and diabetic patients without complications. Similarly, no significant difference was observed in Hb concentration between diabetic patients with complications and those without complications. In contrast, the diabetic patients with complications indicated significantly greater level of hemoglobin compared with the non-diabetic controls.

In this study, there were no significant differences in mean creatinine level between the control and patients without complication and patients with complications. Similarly, no significant

difference was found in creatinine level between the patients with complications and those without complications. This means that the creatinine level is not dependent on whether the patient is diabetic or not.

The study showed that higher percentage of diabetic patients with complications took high carbohydrate and high protein diet. This may have resulted in the high HbA1c levels gotten in this study.

5.2 Conclusion

The average erythropoietin levels and average HbA1c in diabetic patients with complications in the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria, were 242.86 IU/L and 12.08% respectively. These values were high than the reference ranges (5.8 – 9.9 IU/L for erythropoietin level and 4.0 – 6.0% for HbA1c levels). The elevated HbA1c levels observed in this study may be linked to the diabetic patients with complications consuming a diet rich in both protein and carbohydrates. Similarly, the higher erythropoietin levels found in this study may be connected to the absence of nephropathy among the diabetic patients with complications, as nephropathy is a primary factor contributing to reduced erythropoietin levels in these individuals.

5.3 Recommendation

1. There should be constant evaluation of erythropoietin and HbA1c levels among diabetic patients with complications.

2. Patients should be advised to take low carbohydrate diet in order to regulate their HbA1c level

5.4 Study limitations

1. Reluctant of patients to participate in the study
2. High cost of reagent

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APPENDIX

CONSENT FORM

Title of Research: Erythropoietin and HbA1c Levels in Patients with Complicated Diabetes Mellitus in **University of Benin Teaching Hospital,** Benin City, Edo State.

Name of Principal Investigator: Omoregie Ruth Osasenaga

Institution and Contact Address: Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City

Commencement Date of Research: 01/05/2023

Proposed Duration of Research: 4 months

Financial Sponsors: Parents

Conflict of Interest: None declared

Purpose of Research: To determine the Erythropoietin levels in controlled and uncontrolled diabetes mellitus in Benin City, Edo State.

Estimated Number of Participants: Sample size calculation shall be used to obtain the needed number of subjects whose samples shall be collected

Procedure Involved in the Study: Questionnaires and oral interview will be used for data collection and blood sample will be used for quantitative analysis.

Research Design and Methods: The study is a case-control study.

Risks to Participants: No risk envisaged aside the pain experienced while collecting blood sample from participants' cubital vein.

Benefits to Participants: No benefit (voluntary participation).

Compensation/ Inducement: None

Statement of Voluntariness and Circumstances for Withdrawal: Participants are allowed to withdraw from the research at any stage, and the withdrawal will have no adverse effect on the sujects in any form.

Statement on Use of Personal Data: Personal data shall be stored permanently with the research coordinator / principal investigator for further research purposes and no more than such.

Statement on Confidentiality: Strict and absolute confidentiality is guaranteed.

Measures to Take Care of Research-Related Injuries: Full COVID-19 personal protective wears / kits shall be provided.

I Have Fully explained this Research to the Participant(s) and Have Given Sufficient Information, Including Information about the Risks and Benefits, to make an Informed Decision.

Signature /Date:
NAME:

CONTACT: Omoregie Ruth Osasenaga (Principal Investigator)
If You have any Question about Your Participation in this Research, You can Contact the Principal Investigator. The Address is Department of Medical Laboratory Science (Clinical Chemistry Unit), School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City; Edo State, Nigeria.
GSM: 07056059666;
WhatsApp: 07056059666.

STATEMENT FROM PARTICIPANT(S)
I have Read the Description of the Research or have had it Translated to my Understanding. I have also Talked it over with the Researcher to my Satisfaction. I Understand that my Participation is Voluntary. 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 also understand that I may freely stop being Part of this Study at any Time if I so wish for any Reasons Best known to me. I have Received a Copy of this Consent Form and Additional Information Sheet to keep for myself.

Participant's Signature / Thumb Print: ---------- Date: ----------

Participant's Name / Or Number (If Applicable): ----------

Witness' Signature (if any): ----------**Date**: ----------

Witness' Name (If Applicable): ----------

PLEASE KEEP A COPY OF THE SIGNED INFORMED CONSENT
For Official Use Only

This Research has been approved by The University of Benin Teaching Hospital Health Research Ethics Committees and the Chair of the Committee can be contacted on:

_____

_____

_____

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN.

BENIN CITY.

QUESTIONNAIRE ON ESTIMATION OF ERYTHROPOIETIN AND HBA1C LEVELS IN PATIENTS WITH COMPLICATED DIABETES MELLITUS IN UNIVERSITY OF BENIN TEACHING HOSPITAL, BENIN CITY, EDO STATE

Dear Respondent,

This questionnaire is strictly to provide information regarding a research work on estimation of Erythropoietin level in diabetic patients. You are assured of full confidentiality of all information provided please. Please tick (√) or write your responses where appropriate.

Thank you.

Do you have your consent with the interview? YES [ ] NO [ ]

(Signature to certify that informed consent has been given by respondent)

_____

General Screening Questions

1. Age: _____
2. Sex: Male: [ ] Female: [ ]
3. Ethnicity: _____
4. State of residence: _____
5. State of origin: _____
6. Marital status: Single [ ] Married [ ] Divorced/Separated [ ]
7. Occupational status: Employed [ ] Unemployed [ ] Retired [ ]
8. Education level: Illiterate [ ] Primary [ ] Secondary [ ] Tertiary [ ]
9. Family history of diabetes: YES [ ] NO [ ]
10. Are you diabetic? YES [ ] NO [ ]
11. If question 10 is yes, what type of diabetes do you have? Type 1 [ ] Type 2 [ ] I don't know [ ]
12. How long have you had diabetes: 0-5 years [ ] 5-10 years [ ] >10 years [ ]

13. Are you on any medication? YES [ ] NO [ ]
14. If question 13 is yes, what kind of medication? Drugs [ ] Insulin [ ]
15. How long have you been under treatment/medication(s)? _____
16. Are you on dietary supplement? YES [ ] NO [ ]
17. If question 16 is yes, what kind of supplement(s)? _____
18. Are often are you exposed to sunlight? _____

19. Do you drink alcohol? YES [ ] NO [ ]
20. If question 19 is yes, how many bottles do you consume daily? _____
21. Do you smoke? YES [ ] NO [ ]
22. If question 21 is yes, how many sticks a day? _____
23. Do you exercise? YES [ ] NO[ ]
24. If question 23 is yes, how often do you exercise? Daily [ ] Weekly [ ] Occasionally [ ]
25. Are you conscious of your dietary intake? YES [ ] NO [ ]
26. Do you eat meat, fish, or eggs? YES [ ] NO [ ]
27. Do you often eat high carbohydrate foods? YES [ ] NO [ ]

28. Do you know diabetes can affect other organs? YES [ ] NO [ ] I don't know [ ]
29. Do you have any underlying disease? YES [ ] NO [ ]
30. If question 29 is yes, which disease? _____
31. Did you suffer blood loss recently? YES [ ] NO [ ]
32. If question 31 is yes, in what form? _____
33. Complications: Retinopathy [ ] Neuropathy [ ] Nephropathy [ ] None [ ]

THANK YOU