

Chapter one

Introduction and literature review

1.1 Introduction:

Most diseases irrespective of the organ systems of the body they primarily involve produce changes in the blood at sometime or after a period of time (Talwar and Srivastava, 2006).

One of the most common of this diseases is diabetes mellitus (DM) which may cause of end stage of renal disease (ESRD) due to the fact that diabetes particularly type 2 is increasing in prevalence (American diabetes Association, 2004).

Diabetes causes unique changes in kidney structure, classic glomerulosclerosis is characterized by increased glomerular basement membrane width, diffuse mesangial sclerosis, and hyaline arteriosclerosis (Mauer *et al .*, 1981)

One of the most important complication of diabetes mellitus is Diabetic nephropathy and is more prevalent among African Americans, Asians, and Native Americans than Caucasians (Young *et al .*, 2003).

Anemia of renal disease in most patients are normocytic normochromic ,it is principally due to reduced renal erythropoietin production for practical purposes , the kidney are responsible for approximately 90% of erythropoietin production in an individual , anemia considered when one or more of following are decreased Hb, hematocrit or RBCs count (Tkachuk and Hirschmann, 2007)

Anemia may occur in patients with diabetic nephropathy even before the onset of advanced renal failure (serum creatinine <1.8 mg/dl), and it has been related to erythropoietin deficiency (Bosman *et al .*, 2001).

The target Hb levels in diabetic patients should be 12–13 g/dl, and the potential risk of elevation of blood pressure levels with erythropoietin treatment should be taken into account (Sinclair *et al .*, 2003).

Anemia in diabetic patients with CKD may result from one or more mechanisms.. The major causes of anemia in CKD patients are iron and erythropoietin deficiencies and hyporesponsiveness to the actions of erythropoietin. The cause of erythropoietin deficiency in these patients is thought to be reduced renal mass with consequent depletion of the hormone (Mehdi and Toto, 2009).

The NKF recommends that physicians consider treating anemia in patients with diabetes and kidney disease when Hb is <11 g/dl in patients (Mehdi and Toto, 2009).

The kidney is a paired organ that performs a variety of important functions for the body. The most prominent functions are removal from plasma of unwanted substances (both waste and surplus), homeostasis (the maintenance of equilibrium) of the body water, electrolyte and acid-base status, and participation in endocrine regulation. Another important function is gluconeogenesis.

A complete blood count is a very important test for monitoring renal function and is routinely performed during annual physical examinations in some jurisdictions (Talwar and Srivastava, 2006).

Careful assessment of the blood is often the first step in assessment of hematological function and diagnosis of related diseases, and many hematological disorders are defined by specific blood tests. Examination of blood smears and hematological parameters yields important diagnostic information about cellular morphology, quantification of the blood cellular components, and evaluation of cellular size and shape that allows formation of broad differential diagnostic impressions, directing additional testing (Greer *et al.*, 2013).

1.2 Literature review:

Blood is a fluid connective tissue constituting about 7% of our total body weight (about 5 liters in the human) (Gartner and Hiatt, 2006).

The function of blood is the exchange of respiratory gases ,transport oxygen from the lung to tissue and deliver carbon dioxide from tissue leading the lung to be exchange and excreted ,blood also transport metabolic wastes to the lung ,kidney skin and intestine from removal , blood is also responsible for maintaining acid base balance (Young *et al.*, 2006).

Blood elements include erythrocytes or red cells, leukocytes or white cells, and platelets. Red blood cells (RBCs) are the most numerous blood cells in the blood and are required for tissue respiration. RBCs lack nuclei and contain hemoglobin, an ironcontaining protein that acts in the transport of oxygen and carbon dioxide. White blood cells (WBCs) serve an immune function and include a variety of cell types that have specific functions and characteristic morphologic appearances. In contrast to mature red cells, WBCs are nucleated and include neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Platelets are cytoplasmic fragments derived from marrow megakaryocytes that function in coagulation and Hemostasis (Greer *et al.*, 2013).

Plasma which makes up 45-55% of blood volume, plasma is made up of 90% water, 7-8% soluble proteins (albumin maintains bloods osmotic integrity, others clot, etc), 1% electrolytes, and 1% elements in transit. One percent of the plasma is salt, which helps with the pH of the blood. The largest group of solutes in plasma contains three important proteins to be discussed. There are: albumins, globulins, and clotting proteins (Hoffbrand *et al.*, 2006).

1.2.1. Blood Cells:

1.2.1.1: Red Blood Cells:

Erythrocytes (mature red blood cells) are order to carry hemoglobin into close contact with the tissues and for successful gaseous exchange.

The bulk of the cytoplasm (90-95% of dry weight) consists of the iron carrying pigment hemoglobin. very few organelles are present. The measurement of the hemoglobin concentration in whole blood, referred to simply as hemoglobin involves lysing the erythrocyte. The normal range for hemoglobin is age and sex-dependent, the normal range for young female adults is 12-16 g/dL for young male adults it is 14-18 g/dL (Hoffbrand *et al.*, 2006).

- Shape: normal red blood cell is a biconcave disk to achieve a maximum Surface area to cytoplasmic volume ratio. The surface area of an erythrocyte is calculated to be 128 μm .
- Life span: The mature erythrocyte has a life span of approximately 120 days in the circulation. As red blood cells age, the surface area decreases relative to cytoplasmic volume resulting in a sphere form which is more rigid and is ultimately trapped in splenic cords.
- Function : To provide an environment for the iron containing respiratory pigment, heme, which is complexes to two alpha and two beta globin chains comprising the hemoglobin molecule. The major physiologic role of hemoglobin is oxygen and CO₂ transport.

Red blood cell analytical parameter is defined by three quantitative values, the volume of packed red cells or hematocrit (PCV), the amount of hemoglobin (Hb), and the red cell concentration per unit volume. Three additional indices describing average qualitative characteristics of the red cell population are also collected. These are mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) All of these values are routinely collected and calculated by automated hematology analyzers (Greer *et al .*, 2013).

Frequency (number) the erythrocyte count, also referred to as the RBCs simply involves counting the number of RBCs per unit volume of whole blood. Male = $4.5\text{--}6.5 \times 10^{12}$ cells/L, Female = $3.9\text{--}5.6 \times 10^{12}$ cells/L (Hoffbrand *et al.*, 2006)

❖ Red cell indices

- Mean Corpuscular Volume The average volume of the red blood cell is a useful parameter that is used in classification of anemias and may provide insights into path physiology of red cell disorders.

When the MCV is low, the red blood cells are said to be microcytic, when high, macrocytic. Normocytic refers to blood with a normal MCV. the abnormal variation in cell volume is called anisocytosis (Gartner and Hiatt 2006).

- Mean corpuscular hemoglobin (MCH) The MCH represents the mean mass of hemoglobin in the red cell and is expressed in the mass unit picograms.

The MCH is a reflection of hemoglobin mass. In anemias secondary to impaired hemoglobin synthesis, such as iron deficiency anemia, hemoglobin mass per red cell decreases, resulting in a lower MCH value (Greer *et al.*, 2013).

The normal range is 27-34 pg.

- Mean corpuscular hemoglobin concentration (MCHC)

This is the mean concentration of hemoglobin in the red cell The normal range is (31% – 36%) (Hoffbrand *et al.*, 2006).

The MCHC is expressed in grams of hemoglobin per deciliter of packed RBCs, hemoglobin Cells with normal, high, and low MCHC are referred to as normochromic, hyperchromic, and hypochromic, respectively. These terms will be important in anemia classification (Gartner and Hiatt, 2006).

- The red cell distribution width (RDW) is a red cell measurement that quantifies cellular volume heterogeneity reflecting the range of red cell sizes within a sample. RDW has been proposed to be useful in early classification of anemia as it becomes (Hoffbrand *et al.*, 2006).

1.2.1.2 White Blood Cells:

White blood cells include granulocytes (neutrophils, eosinophils and basophils), lymphocytes and monocytes. The normal ranges of WBCs count and different leukocytes count are as follow (Dace and Lewis, 2011).

- White blood cell count $4.0\text{--}10.0 \times 10^9/\text{l}$
- Differential white cell count

Neutrophils $2.0\text{--}7.0 \times 10^9/\text{l}$ (40–80%)

Lymphocytes $1.0\text{--}3.0 \times 10^9/\text{l}$ (20–40%)

Monocytes $0.2\text{--}1.0 \times 10^9/\text{l}$ (2–10%)

Eosinophils $0.02\text{--}0.5 \times 10^9/\text{l}$ (1–6%)

Basophils $0.02\text{--}0.1 \times 10^9/\text{l}$ (<1–2)

The Functions of differential leukocyte cells:

- Neutrophils:

Defend against bacteria or fungus infection, and when increase in number called neutrophilia.

- Eosinophils:

They enter inflammatory exudates and have a special role in allergic responses, defense against parasites and removal of fibrin formed during inflammation (Hoffbrand *et al.*, 2006).

- Basophils:

These are only occasionally seen in normal peripheral blood. They have many dark cytoplasm granules

Which overlie the nucleus and contain heparin and histamine. In the tissues they become mast cells. They have immunoglobulin E (IgE) attachment sites and their degranulation is associated with histamine release (Hoffbrand *et al.*, 2006).

- Lymphocytes:

The functional mammalian immune system consists of three major lymphocyte populations with different antigen recognition systems: thymus derived (T) cells; bursal or marrow derived (B) cells; and natural killer (NK) cells. The three populations mediate complex and distinct immune effector functions. B cells make antibodies that bind the pathogens to enable their destruction, but T cells are important in defense mechanism against intracellular bacteria. Natural killer cells are able to kill cells of the body which are displaying signal to kill them as they have been infected by virus or have become cancerous. Lymphocytes when increase in values called lymphocytosis (Lichtman *et al.*, 2010).

- Monocytes:

They share the vacuum cleaner (phagocytosis) function of neutrophils, but they are much longer lived as they present piece of pathogens to T-cells so that the pathogens may be recognized again and killed. When monocytes increased in number they called monocytosis (Young *et al.*, 2002).

1.2.1.3. Blood Platelets:

Non nucleated flat, biconvex, round or ovoid disks (2-5 μm diameter); derived from bone marrow megakaryocytes, contain pale-blue peripheral hyalomere with a system of invaginating channels (open canalicular system) connecting with the platelet membrane, system of electron-dense tubules, marginal bundle of 10-15 circumferential microtubules to maintain the disk shape, and actin and myosin. When platelets become activated, the microtubular system squeezes the granules to the center of the platelet, the contents are released, and the coagulation system is triggered (Gartner and Hiatt 2006).

Platelets exist in two distinct forms, resting and activated, with the resting state marked by baseline metabolic activity and the activated form resulting from agonist stimulation (e.g. response to thrombin) (Greer *et al.*, 2013).

The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur ". The normal platelet count is approximately $250 \times 10^9/L$ (range $150-400 \times 10^9/L$) and the normal platelet life span is 7-10 days (Hoffbrand *et al.* , 2006) .

1.2.2 Complete Blood Count test:

The CBC is not primarily a set of function tests but essentially tests changes in anatomical structures. It contains data of two types: the concentration (as well as the average sizes of RBC and platelets) of cells suspended in the plasma, and their morphological appearance, Thirdly the concentration of cells (eg. the number counted) reflects the balance between two factors, the number of cells per unit volume of blood, and the volume of plasma per unit volume of blood. Thus the challenge in interpreting the CBC when dealing with a blood disease, whether suspected or identified, is to make clinical decisions of an essentially pathophysiological nature (e.g., to attempt to evaluate underlying functional change) based upon laboratory reports that are essentially anatomical in origin (Beck, 2009).

The medical technologist perform CBC on haematology cell analyzer to determine RBCs count, Hb, PCV, RBCs indices, WBCs count and differential and platelets count (Bernadette, 1997).

Leukocyte and platelet count measurement of these help to distinguish pure anemia from pancytopenia (adrop in red cells , granulocytes , and platelets) which suggest a more general marrow defect (e.g. caused by marrow hypoplasia or infiltration) or general destruction of cells (e.g. hypersplenism). In anemia s caused hemolysis or hemorrhage, the neutrophil and platelet count are often raised , in infections and leukaemias the leukocyte count is also often raised and there me be abnormal leukocytes or neutrophil precursors present (Hoffbrand *et al.*, 2006)

1.2.3 Diabetes Mellitus:

Diabetes mellitus are group of metabolic disease in which a person has high blood glucose because the body does not produce enough insulin or the cell do not respond to insulin that produced (Lawerence *et al.*,2008).

Over time, high blood glucose damages nerves and blood vessels, leading to complications such as heart disease, stroke, kidney disease, blindness, dental disease, and amputations. Other complications of diabetes may include increased susceptibility to other diseases, loss of mobility with aging, depression, and pregnancy problems (NIH, 2014).

1.2.3.2 Classification of Diabetes Mellitus:

There are three main types of diabetes:

- **Type 1 diabetes (T1DM)**

Type 1 diabetes (due to β -cell destruction, usually leading to absolute insulin deficiency) (American Diabetes Association, 2015).

formerly called juvenile diabetes, is usually first diagnosed in children, teenagers, and young adults. In this type of diabetes, the beta cells of the pancreas no longer make insulin because the body's immune system has attacked and destroyed them (NIH, 2014).

The attack is a marked systemic indices of autoimmune process such as circulating islet cell auto antibodies and change in circulating B lymphocyte subset. The process of beta cell destruction usually takes many months and occurs in cycle deterioration and remission (William and Stephen, 1995).

- **Type 2 diabetes (T2DM)**

Formerly called adult onset diabetes, is the most common type of diabetes, can range from predominant insulin resistance with relative insulin deficiency to prevailing defective secretion with insulin resistance, is frequently associated with other problems of the so-called metabolic syndrome (Kerner and Brückel, 2014).

People can develop type 2 diabetes at any age, even during childhood, but this type of diabetes is most often associated with older age.

Type 2 diabetes mellitus (diabetes) is a major public health problem, and accounts for more than 90% of all diabetes cases. The insidious and initially asymptomatic nature of the disease results in patients not seeking early medical attention, so that 30-85% of cases of type 2 diabetes remain undiagnosed. At the time of eventual diagnosis, approximately 20% of patients will already have complications of the disease (Amod *et al.*, 2012).

In all T2DM patients there is insulin resistance and relative insulin deficiency (William and Stephen, 1995).

- **Gestational diabetes**

Gestational diabetes is hyperglycaemia with blood glucose values above normal but below those diagnostic of diabetes, occurring during pregnancy. Women with gestational diabetes are at an increased risk of complications during pregnancy and at delivery. They are also at increased risk of type 2 diabetes in the future (WHO, 2012).

- **Specific types of diabetes** due to other causes, e.g, monogenic diabetes syndromes (such as neonatal diabetes and maturity onset diabetes of the young (MODY), diseases of the exocrine pancreas (such as cystic fibrosis), and drug or chemical induced diabetes (such as in the treatment of HIV or after organ transplantation) (American Diabetes Association, 2015).

1.2.3.3 Signs and symptoms of diabetes mellitus

The classical symptoms of DM are polyurea ,polydispasia ,polyphagia . prolonged high blood glucose may lead to change in shape of lenses of eye resulting in visual change (Cooke and Plotnick, 2008).

1.2.3.4 Diagnosis of diabetes mellitus:

Blood tests are used to diagnosis diabetes and prediabetes because early in the disease type 2 diabetes may have no symptoms. All diabetes blood tests involve

drawing blood at a health care provider's office or commercial facility and sending the sample to a lab for analysis. Lab analysis of blood is needed to ensure test results are accurate. Glucose measuring devices used in a health care provider's office, such as finger stick devices, are not accurate enough for diagnosis but may be used as a quick indicator of high blood glucose (NIH, 2014).

Testing enables health care providers to find and treat diabetes before complications occur and to find and treat prediabetes, which can delay or prevent type 2 diabetes from developing.

Any one of the following tests can be used for diagnosis:

- A1C test, also called the hemoglobin A1c, HbA1c, or glycohemoglobin test, Normal is less than 5.7%, pre-diabetes is 5.7%-6.4% and diabetes is 6.7% or higher.
- fasting plasma glucose (FPG) test, Diabetes is diagnosed if it is higher than 126mg/dl.
- oral glucose tolerance test (OGTT).

Another blood test, the random plasma glucose (RPG) test, is sometimes used to diagnose diabetes during a regular health checkup. If the RPG measures 200 micrograms per deciliter or above, and the individual also shows symptoms of diabetes, then a health care provider may diagnose diabetes (NIH, 2014).

1.2.3.5 Complications of Diabetes Mellitus

Severely elevated blood glucose level due to lack of insulin or relative deficiency of it which lead to glucose urea which results in loss of fluid and electrolyte in urine or cause inability to store fat and protein along with breakdown of existing fats and protein stored which result in ketoacidosis and release keton in blood which turn into acidic which called DKA (diabetesketoacidosis) (Nathan *et al.*, 2005).

Low blood glucose level due to too much insulin or other glucose lowering medication can lead to central nervous system symptoms such as confusion , dizziness, weakness.

Over time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves and may lead to:

1. Diabetes increases the risk of heart disease and stroke.
2. Combined with reduced blood flow, neuropathy (nerve damage) in the feet increases the chance of foot ulcers, infection.
3. Diabetic retinopathy is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina.
4. Diabetes is among the leading causes of kidney failure (Nathan *et al.*, 2005).

1.2.4 Diabetes Mellitus in Sudan

Sudan has, for a long time, suffered economic collapse, drought and civil war. Diabetes mellitus is currently emerging as an important health problem, especially in urban areas. The actual prevalence of diabetes is unknown although one small study showed a prevalence of 3.4%. Diabetes is the commonest cause of hospital admission and morbidity due to a non communicable disease (7 and 10% respectively). The problems of diabetes care in Sudan include the lack of efficient diabetes care centers, lack of specially trained personnel, the high cost of anti-diabetic treatments, poor compliance with therapy or diet, ignorance and wrong beliefs, food and dietary factors and gender related problems. The goal of efficient diabetes care can be achieved through implementing national diabetes programmed. This programmed should be responsible for personnel training, establishing model care centers, patients education, availability and affordability of insulin, scientific and clinical research and primary prevention (Ahmed and Ahmed, 2001).

1.2.5 Nephropathy

Nephropathy is a medical term used to refer to disease or damage in the kidneys, nephropathy is a clinical diagnosis based upon the finding of proteinuria in a patient with no evidence of urinary infection. A number of types of nephropathies can be seen in medical treatment. Nephropathy is a very common complication of diabetes, resulting from damage to the kidneys caused by high blood sugar, and people with high blood pressure can also develop nephropathy.

The kidneys act as one of the filtration systems in the body, expressing undesirable substances and retaining useful ones in addition to maintaining normal blood pressure levels. They also produce urine, a fluid which is used to express substances which are not needed by the body. When the kidneys are damaged, the lack of filtration can make people extremely sick (Warrell *et al.* , 2003).

1.2.5.1 Types of Nephropathy:

The types of Nephropathy include:

- **Toxin nephropathy:** due to toxins damaging the kidneys.
- **Obstructive nephropathy:** A condition which is characterized by obstructive of the urinary tract resulting in kidney disease and dysfunction.
- **Diabetic Nephropathy:** Kidney damage arises mainly from diabetic high blood sugars causing damage to the very small blood vessels (called microangiopathy or microvascular disease) in the kidney's filtration .
- **Reflux nephropathy:** A condition which is characterized by reflux of urine from the bladder back up the ureters.
- **IgA nephropathy:** IgA nephropathy is a kidney disorder caused by deposits of the protein immunoglobulin A (IgA) inside the glomeruli (filters) within the kidney
- **Analgesic nephropathy:** one causes of nephropathy is the long term usage Of analgesics (Warrell *et al.* , 2003).

1.2.6 Diabetic Nephropathy:

1.2.6.1 Definition and Causes of Diabetic Nephropathy:

Diabetic nephropathy is defined by a raised urinary albumin excretion of >300 mg/day (indicating clinical proteinuria) in a patient with or without a raised serum creatinine level and usually with coexisting diabetic retinopathy (American Diabetes Association, 2010).

The kidney can be affected in up to 30% of patients with diabetes and can occur whether the patient has type 1 or type 2 diabetes mellitus. It is not yet known why some people develop kidney disease while others do not, but diabetic kidney disease (diabetic nephropathy) is more likely to occur if blood glucose control has been poor for a prolonged period of time, it is also more likely in patients with high blood pressure, a family history of high blood pressure and vascular disease, and genetic components also predispose to this disease (Watkins, 2003).

Diabetic nephropathy is the commonest single cause of end stage renal failure(ESRF) requiring renal replacement therapy in the United States, and the second most common in Europe and Japan. The incidence is increasing, largely because the incidence of diabetes itself is reaching what some have termed epidemic proportions, this growth being greatest in the developing world (Warrell *et al .*, 2003).

1.2.6.2 Symptoms of Diabetes Nephropathy:

Diabetic nephropathy is not typically characterized by symptom onset, meaning that most individuals who develop it are unaware of the condition until it has already caused considerable damage. Screening diabetic patients for kidney damage is therefore important in reducing the risk of long-term kidney damage and its associated problems (Mandal, 2013).

Some of the features of diabetic kidney disease that may eventually manifest include:

- Edema or swelling of the ankles, feet, lower legs or hands due to water retention.
- Urine that is foamy or frothy in appearance due to excessive protein being excreted in the urine. This is most commonly seen in the first urine of the day.
- Weight gain due to fluid retention and edema
- Nausea and vomiting , Loss of appetite
- Generalized itching
- High blood pressure and Headaches (Mandal, 2013).

1.2.6.3 Early detection and Diagnosis of Diabetic Nephropathy

Testing urine samples for the presence of protein or microalbuminuria should be routine practice at every clinic visit and is obligatory at the annual review. If proteinuria develops it is important to distinguish the onset of nephropathy from other causes of renal disease. Microalbuminuria is detected by measurement of the albumin/creatinine ratio or urinary albumin concentration. The test is best performed on the first morning urine sample. If albuminuria is discovered for the first time, confirmation is required within one month if proteinuria is present, and if microalbuminuria is detected it should be confirmed twice within the ensuing months. All laboratory and near patient commercial tests specifically designed for microalbuminuria have satisfactory sensitivity (80%) and specificity (90%). If proteinuria has evolved gradually over several years in the presence of retinopathy, and there are no unusual features such as haematuria, unequal size kidneys, or a history of urinary tract complaints, then extensive investigation is not necessary. In Type 2 diabetes, however, there is a greater chance of non diabetic renal disease being present, and renal biopsy may be needed, especially if there are atypical features. In both Type 1 diabetes and Type 2 diabetes the absence of retinopathy should make one suspect other causes of renal failure. Indeed, rapid onset of proteinuria in any patient is never due to diabetes and should always be fully investigated, including a biopsy (Watkins, 2003).

- **Diagnostic categories of diabetic nephropathy**

- **Microalbuminuria**

- Albumin creatinine ratio, 2.5mg/mmol/l (men), 3.5mg/mmol/l (women)
- Urinary albumin concentration 20mg/l
- Urinary albumin excretion rate 30-300mg/24h, or 20-200g/min in an overnight specimen (Watkins, 2003).

- **Proteinuria**

- Urinary albumin excretion rate >300mg/24h
- Urinary protein excretion >500mg/24h (albumin creatinine ratio >30mg/min; albumin concentration >200mg/l) (Watkins, 2003).

- **Investigations in a patient with proteinuria**

Midstream urine, 24 hour urine protein, Renal ultrasonography, Blood count, Erythrocyte sedimentation rate, Antinuclear factor Serum complement, Serum lipids, Renal biopsy only as indicated (Watkins, 2003).

1.2.7 Statistical and Epidemiology of Diabetes Mellitus and Nephropathy

The incidence of diabetes is increasing worldwide, most rapidly in developing countries. It is estimated that by 2010 there will be a near doubling of people with the condition to 221 million (5 million with type 1 and 216 million with type 2 disease) (Warrell, *et al*, 2003).

In 2003, the International Diabetes Federation (IDF) estimated that there would be 285 million people with diabetes by 2010. This was a gross underestimate, as the fifth edition of the IDF Atlas shows that there were 366 million diabetes sufferers worldwide in 2011 (183 million of these were undiagnosed) (Amod *et al.*, 2012).

Some of the wide variation in the reported prevalence of incipient and clinical nephropathy can be explained by different selection of the population under study. However, selecting only population based cohorts with good patient ascertainment, gives prevalence rates for incipient nephropathy of between 5 and 21 per cent for

type 1 and 11 to 42 per cent for type 2 disease. Annual incidence rates are similar at around 2 per cent for both type 1 and type 2 patients.

Many countries now have registers of patients entering renal replacement therapy and all have shown a dramatic increase in the numbers with diabetic nephropathy. It is not clear, however, whether this is a true increase in the numbers of diabetic patients developing ESRF or a reflection of a change in acceptance policy. Either way, there is going to be a continuing increase in the number of patients with diabetes presenting for renal replacement, particularly from ethnic minorities (Afro Caribbean and South African), who will make up around 50 per cent of such patients in the United Kingdom by 2001. Although patients with type 2 disease have always been thought to develop ESRF less frequently than those with type 1, this may have been because such patients were not referred, or that they died of cardiovascular disease before entering renal failure. In 1997, 71 per cent of all diabetic patients on dialysis in the United States were classified as having type 2 disease (Warrell *et al.* , 2003).

1.2.8. Previous studies

A study in Iraq by Mohammad and Almuhammadi (2011) to estimate some possible risk factors of diabetic nephropathy and assessed clinically, haematologically the patients with diabetic nephropathy. Measurement of RBCs counts, blood Hb and PVC and RBCs indices), hormonal study (serum erythropoietin level). The study revealed that diabetic nephropathy prevalence more in female with long duration of DM. In regard to hematological parameters the results of study showed that there a highly significant decreased ($p < 0.01$) in RBCs counts, blood Hb, PCV and RBCs indices, as well as serum erythropoietin in diabetic nephropathy patients in compare to control group. In conclusion, diabetic nephropathy associated with increased and decreased in some hematological parameters may be attributed to anemia that accompanies diabetic nephropathy (Muhammad and Almuhammadi, 2011).

Chung *et al.*, (2005) in Tiwan reported that the peripheral total WBC, monocytes, and neutrophil counts increased in parallel with the advancement of diabetic nephropathy. In contrast, the lymphocyte count decreased. When WBCs counts were analyzed WBC, monocyte, neutrophil, and lymphocyte counts were independently and significantly associated with diabetic nephropathy (Chung *et al.*, 2005).

Bosman *et al.*, (2001) in United Kingdom found that anemia was associated with erythropoietin deficiency are occur early in DN before the onset of advanced renal failure, but does not normally occur in non diabetic renal disease of similar severity (Bosman *et al.*, 2001).

1.3 Rationale:

Diabetic nephropathy is a specific form of renal disease. A major cause of death and disability among diabetics, it account 25% - 40% of all cases with end stage of renal failure (ESRF) and the process that underlies the progression of the disease has poorly understood, and because of increasing evidence that blood play role in the development and progression of diabetes complications.

So specific prospective researches are needed to assess reflect of complete haemogram on Sudanese type 2 diabetic with nephropathy.

Few published study has investigated the association between blood cell count and diabetic nephropathy.

Few published data are available in Sudan, so this study could be addition to the existing data. Also the results of the study may show the effect of nephropathy on complete blood count of type 2 diabetic patients.

1.4 Objective:

General objectives:

- To measure complete blood count of Sudanese type 2 Diabetes Nephropathy patients.

Specific objectives:

- To determine Hb, PCV, red blood cells count and its indices (MCV, MCH, MCHC), white blood cells count, all differential leukocyte count and platelets count in test and control groups.
- To compare between Hb, PCV, red blood cells count and its indices (MCV, MCH, MCHC), white blood cells count, all differential leukocyte count and platelets count in test and control groups.
- To determine the gender distribution of test group.

Chapter two

Materials and Methods

2.1 Study design:

This is a case control study to determine complete blood count in Diabetes type 2 Nephropathy patients from May to October 2015.

2.2 Study area:

The study was carried out in Jabir abualiz center, Alsafa specialized hospital in Khartoum State, Sudan.

2.3 Study populations:

The study subject divided in to three groups, the first group was include type 2 diabetic with nephropathy patients, the second group was include type 2 diabetic patients as a control group and third group were appearance normally healthy individual as a control group.

2.4 Inclusion criteria:

All patients already diagnosed as type 2 diabetes with nephropathy in Jabir abualiz center , Alsafa specialized hospital, both males and females were included.

2.5 Exclusion criteria:

The patient with major illness like cancer, HIV was excluded and also patients with hypertension, liver diseases, anemia, leukemia or has recently received blood transfusion also was excluded.

2.6 Ethical consideration:

An informed consent from selected individuals to be study was taken after being informed with all detailed objective of the study.

2.7 Collection of blood sample:

Three ml venous blood was collected from individual under study and dispensed in EDTA container for CBC (Dacie and Lewis, 2011).

2.8 Procedure of complete blood count (CBC):

Fully automated multichannel instruments require only that an appropriate blood sample is presented to the instrument and usually measure from 8 to 20 components for the basic CBC and white blood cell differential. Impedance counting systems depends on the fact that red cells are poor conductors of electricity, whereas certain diluents are good conductors (Dacie and Lewis, 2011)

2.8.1. Hemoglobin concentration (HGB or Hb)

Automated counter used nonhazardous chemical, such as sodium lauryl sulphate, imidazole, and sodium dodecyl sulphate or dimethyl laurylamineoxide. Modifications include alterations in the concentration of reagents and in the temperature and pH of the reaction. A detergent is included to ensure rapid cell lysis and to reduce turbidity. Measurements of absorbance are made for hemoglobin measurement at various wavelengths depending on the final stable haemochromogen, cyanmethaemoglobin, oxyhaemoglobin, methaemoglobin or monohydroxyferri-porphyrin. Hemoglobin concentration values, in normal male 13 – 17 g/L female 12 - 15 g/L, (Dacie and Lewis, 2011).

2.8.2. Red blood cell count (RBC) and Platelet count (PLTS):

Red cells and other blood cells were counted in systems based on aperture impedance technology. Platelets can be counted in whole blood using the same techniques of electrical detection as is used for counting red cells. An upper threshold is needed to separate platelets from red cells and a lower threshold is needed to separate platelets from debris and electronic noise. RBC normal range in male $4.5\text{--}5.5 \times 10^{12}/\text{L}$ female $3.8\text{--}4.8 \times 10^{12}/\text{L}$, and platelet normal range in $150\text{--}410 \times 10^9/\text{L}$ (Dacie and Lewis, 2011).

2.8.3. Packed cell volume (PCV)

Automated blood cell counter was estimated PCV/haematocrit by technology that has little connection with packing red cells by centrifugation. The passage of a cell through the aperture of an impedance counter leads to the generation of an

electrical pulse, the of which is proportional to cell volume. The number of pulses generated allows the RBC to be determined. Male normal range 0.45 ± 0.05 L/L female 0.41 ± 0.05 L/L (Dacie and Lewis, 2011).

2.8.4. Red cell indices:

2.8.4.1. Mean cell volume (MCV)

MCV is measured directly. normal range 92 ± 9 fl (Dacie and Lewis, 2011).

2.8.4.2. Mean corpuscular hemoglobin (MCH)

The mean amount of hemoglobin per red cell (MCH) is reliably estimated by automated electronic counting devices by dividing the total amount of hemoglobin by the number of red cells in a sample of blood. Men and women normal range 29.5 ± 2.5 pg (Dacie and Lewis, 2011).

2.8.4.3. Mean cell hemoglobin concentration (MCHC)

The MCHC is derived in the traditional manner from the Hb and the Hct with instruments that measure the Hct and calculate the MCV. $MCHC = Hb / PCV \times 100$. Men and women normal range 330 ± 15 g/L (Dacie and Lewis, 2011).

2.8.5. Total white blood cell count (WBC)

The agent is required to destroy the red cells and reduce the red cell stroma to a residue that causes no detectable response in the counting system. The following fluid is satisfactory: Cetrimide 20 WBC is determined in whole blood in which red cells have been lysed. The lytic g, 10% formaldehyde (in 9 g/l NaCl) 2 ml, Glacial acetic acid 16 ml, NaCl 6 g, and water to 1 liter. Residual particles in a diluted blood sample are counted after red cell lysis. Normal range $4 - 10 \times 10^9$ L (Dacie and Lewis, 2011).

2.8.6. Automated differential count

Automated blood cell counter have a differential counting capacity, providing a three-part differential count. Counts are performed on diluted whole blood in which red cells are either lysed or are rendered transparent. A three-part

differential count was categorized leucocytes as WBC-small cell ratio (equivalent to lymphocytes), WBC-middle cell ratio (equivalent to monocytes, eosinophils and basophils) and WBC-large cell ratio (equivalent to neutrophils). Normal differential count neutrophils $2.0-7.0 \times 10^9/L$, lymphocytes $1.0-3.0 \times 10^9/L$, monocytes $0.2-1.0 \times 10^9/L$, eosinophils $0.02-0.5 \times 10^9/L$, basophils $0.02-0.1 \times 10^9/L$ (Dacie and Lewis, 2011)

2.9. Statistical analysis:

Data were entered into computer and analyzed using SPSS version 16, P.Value ≤ 0.05 is considered as significant.

Chapter Three

Results

100 type 2 diabetes with nephropathy patients (group **A**) compared with 50 control groups. the control groups divided into two groups, 25 type 2 diabetes mellitus (group **B**) and 25 control healthy subject (group **C**).

The results showed the ages of group A (Diabetes type 2 nephropathy) was (62 ± 5.62) years, group B (52 ± 4.8) years, group C (45 ± 6.3) years (table 3.1).

The results showed the frequency of case group (A) according to gender, the males was 79 (79%) and females was 21 (21%) and group B, the males was 16 (64%) and females was 9 (36%) and group C, the males was 18 (72%) and females was 7 (28%) (table 3.2).

The results of group A we showed very highly Significant decreased in many parameters when compare with group B and C. Hb g/dl = ($11.9 \text{ g/dl} \pm 1.27$, $13.4 \text{ g/dl} \pm 1.07$, $14.1 \text{ g/dl} \pm 1.08$) respectively. RBCs count = ($4.5 \times 10^{12}/\text{l} \pm 0.50$, $5.03 \times 10^{12}/\text{l} \pm 0.38$, $4.9 \times 10^{12}/\text{l} \pm 0.44$) respectively. PCV = ($35.8\% \pm 4.08$, $40.2\% \pm 3.44$, $41.9\% \pm 3.37$) respectively and MCV = ($82.2 \text{ fl} \pm 3.66$, $84.1 \text{ fl} \pm 4.57$, $84.6 \text{ fl} \pm 3.91$) respectively. MCH = ($27.1 \text{ pg} \pm 1.75$, $27.6 \text{ pg} \pm 2.2$, $28.6 \text{ pg} \pm 1.71$) respectively. MCHC = ($32.5\% \pm 1.26$, 32.9 ± 0.9 , $33.6\% \pm 1.42$) respectively (table 3.3).

The results showed highly significant decrease in Hb, MCH, and MCHC of group B when compared with group C (table 3.3).

When Compared between the results of WBCs count and neutrophil count in group A with the results of group B and control group C. WBCs count = ($6.9 \times 10^9/\text{l} \pm 1.99$, 6.4 ± 1.45 , $5.9 \times 10^9/\text{l} \pm 1.16$) respectively. Neutrophil count ($4.1 \times 10^9/\text{l} \pm 1.8$, 3.6 ± 1.02 , $3.2 \times 10^9/\text{l} \pm 1.16$)) respectively showed significant increase in TWBCs and neutrophile count of group A (table 3.4).

Table 3.1 : The (Mean \pm SD) participators ages / year of study groups.

Group	Age (Mean \pm SD)
Type 2 diabetes nephropathy	62 \pm 5.62
Type 2 diabetes mellitus	52 \pm 4.8
Control	45 \pm 6.3

Table 3.2 : The gender frequencies of study groups.

Group	Males	Percentage %	Females	Percentage %
Type 2 diabetes nephropathy	79	79%	21	21%
Type 2 diabetes mellitus	16	64%	9	36%
Control	18	72%	7	28%
Total (150)	113		37	

Table 3.3: Hb, RBCs count, PCV and RBCs indices of study groups.

Group Parameter	Group A (Mean \pm SD)	Group B (Mean \pm SD)	Group C (Mean \pm SD)	Significant
Hb g/dl	(11.9 \pm 1.27)	(13.4 \pm 1.07)	(14.1 \pm 1.08)	0.000**
RBCs count X 10 ¹² /l	(4.5 \pm 0.5)	(5.03 \pm 0.38)	(4.9 \pm 0.44)	0.000**
PCV %	(35.8 \pm 4.08)	(40.2 \pm 3.44)	(41.9 \pm 3.37)	0.000**
MCV fl	(82.2 \pm 3.66)	(84.1 \pm 4.57)	(84.6 \pm 3.91)	0.000**
MCH pg	(27.1 \pm 1.71)	(27.6 \pm 2.2)	(28.6 \pm 1.71)	0.002*
MCHC %	(32.5 \pm 1.26)	(32.9 \pm 0.9)	(33.6 \pm 1.42)	0.001*

■ ** = very highly significant , * = highly significant

Table 3.4: WBCs count, differential count and Platelets count of study groups.

Group Parameter	Group A (Mean \pm SD)	Group B (Mean \pm SD)	Group C (Mean \pm SD)	Significant
TWBCs count X 10 ⁹ /l	(6.9 \pm 1.99)	(6.4 \pm 1.45)	(5.9 \pm 1.16)	0.050
Neutrophil X 10 ⁹ /l	(4.1 \pm 1.8)	(3.6 \pm 1.02)	(3.2 \pm 1.16)	0.036
Lymphocyte X 10 ⁹ /l	(2.1 \pm 0.57)	(2.2 \pm 0.69)	(2.1 \pm 0.49)	0.845
Monocyte X 10 ⁹ /l	(0.43 \pm 0.18)	(0.4 \pm 0.15)	(0.39 \pm 0.14)	0.562
Eosinophil X 10 ⁹ /l	(0.24 \pm 0.14)	(0.2 \pm 0.14)	(0.23 \pm 0.09)	0.501
Platelets X 10 ⁹ /l	(302 \pm 100)	(289 \pm 115)	(281 \pm 81.5)	0.585

Chapter four

Discussion, Conclusion and Recommendation

4.1 Discussion:

In Sudan diabetes mellitus is considered as a serious problem either in Khartoum and rural area,

There was clear evidence that the diabetes type 2 nephropathy occurrences around age 62 ± 5 years old and occur among male with high rate rather than female with ratio 3:2 matches with the result of study by Rahamtalla *et al.*, (2012) in Elmusbah center in Omdurman and contrast with result of study performed by Muhamed and AlMuhammadi (2011) in that incidence of diabetic nephropathy is more in females than males. This results because of pregnancy and uses of oral contraceptive pills which is associated with worsening of diabetic complication as nephropathy (Rathmann and Giani, 2004).

The results of present study showed significant decreased in Hb, RBCs count, PCV and MCV when compared group A and B, group A and C (table 3.3) with This finding matches with the results obtained by Muhammad AlMuhammadi (2011) in Iraq the study showed highly significant decreased in RBCs counts, blood Hb, PCV and RBCs indices. The result explained as anemia first appears because in renal diseases erythropoietin production usually is insufficient to stimulate adequate red blood cell production by the bone marrow.

It is revealed in this study, there are significant decreased in MCH and MCHC concentration in diabetic type 2 nephropathy patients compared to control group C, (table 3.3). This contrast with other results obtained by Bosman *et al.*, (2001). These results may related to decrease erythropoietin production in CKD, and iron deficient as a result of anorexia and dietary restrictions that limit intake, impaired erythropoietin secondary to inhibitors or toxic metabolites.

Regarding white blood cells count and differential, it is shown in this study there was significant increased in TWBCs and neutrophil count in group A compared to group C (table 3.4). These results matched with results obtained by Chung *et al.*, (2005) which revealed at peripheral WBCs count and neutrophil count increased in diabetic nephropathy. The mechanism responsible for the increased total and differential leukocytes in diabetic patients with nephropathy is a matter of speculation. One of the hypothesis is that leptin (A hormone produced mainly by adipocytes (fat cells) that is involved in the regulation of body fat, with activities on many peripheral cell types) might be involved in increased leukocyte counts. Plasma leptin concentrations are increased in patients with nephropathy. Leptin has been reported to stimulate myeloid differentiation from human bone marrow CD34+ progenitors, and can induce proliferation, differentiation, and functional activation of hemopoietic cells (Laharrague *et al.*, 2000).

Other mechanisms contributing to leukocytosis in patients with nephropathy may be related to changing insulin levels in renal disease. This factor was known to increase WBC counts by increasing neutrophil influx from marrow storage and decreasing efflux from the blood stream (Collier *et al.*, 1990).

Lymphocyte, monocyte, eosinophil case group (A) compared with group B and C showed insignificant difference ($P \leq 0.93$) (table 3.4). In contrast leucocytes count increased but lymphocyte count decreased in diabetic patients a study obtained by Otton *et al.*, (2004). This might play an important role for the impaired immune function and high incidence of infections in poorly controlled diabetic patients (Pallavicini and Willia, 1976).

The result showed insignificant difference in Platelets count in group A compared to group B and C. this finding are contrast to results of study obtained by Sterner *et al.*, (1998) who found that elevated platelet count commonly occurs in diabetes mellitus complicated with nephropathy, both in terminal renal insufficiency and before renal impairment is obvious and the author reported that there was a week

correlation between platelet count and the degree of metabolic control. It seems likely that a high platelet count in diabetic patients is associated with some factor related to the pathogenesis of vascular disease (Sauvage *et al.*, 1994).

4.2 Conclusion

- The type 2 diabetes with nephropathy is common the age 62 year old in this study.
- Type 2 Diabetic with Nephropathy is common in males compare to females in this study.
- Hb, PCV, RBCs count, MCV in type 2 diabetic patients with nephropathy were significantly decreased when compared with type 2 diabetic patients (control group B) and healthy subject (control group C).
- Significant decreased in MCH and MCHC in type 2 diabetic patients with nephropathy when compared with healthy subject.
- Significant decrease in Hb, MCH and MCHC in type 2 diabetic patients compared with healthy subject.
- Significant increase in TWBCs count and neutrophil count in type 2 diabetic patients with nephropathy compared with healthy subject.
- No significant difference in platelets count between three groups.

4.3 Recommendation

- Follow up of diabetic patients are recommend to detect early onset of diabetic complications.
- Continuous monitoring of hematological parameter in type 2 diabetes with nephropathy patients should be induced.
- The sample size should be increased in related subsequent researches and done further hematological parameter.
- Further studies with large sample size are needed to include the duration of nephropathy and diabetes mellitus.

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Appendix(1)

Sudan University of Science and Technology College of Graduate Studies

The estimation of complete hemogram among type 2 diabetes nephropathy Patients in Sudan

Questionnaire

1. General Information

Name:.....No.....

Age:.....Years Sex:.....

Address:.....Tel.

No:.....

2. Statement of present health condition

Type of DM.....

○ Family history of the disease: Yes ☐ No ☐

3. Statement of other health condition

○ Inflammation Yes ☐ No ☐

○ Hypertension Yes ☐ No ☐

○ Pregnancy Yes ☐ No ☐

○ Cancer Yes ☐ No ☐

○ Anemia Yes ☐ No ☐

○ Leukemia: Yes ☐ No ☐

Others.....

Results

Result: WBC.....RBC.....HGB.....

HCT.....MCV.....MCH.....

MCHC.....PLT.....LYM.....

NEUT.....EOSIN.....BASO

Appendix (2)

Informed consent

بسم الله الرحمن الرحيم
جامعة السودان للعلوم والتكنولوجيا
كلية الدراسات العليا
ماجستير مختبرات طبية
تخصص علم امراض الدم ومبحث المناعة الدموية
براءة اخلاقية

الإسم:

سوف يتم أخذ عينة من الدم (3مل) من الوريد بواسطة حقنة طعن وذلك بعد مسح منطقة العينة بواسطة مطهر.

كل الأدوات المستخدمة لأخذ العينة معقمة و متبع فيها وسائل السلامة المعملية

أوافق أنا المذكور اعلاه أخذ عينة لإجراء الدراسة

الإمضاء.....

التاريخ:.....