CHAPTER ONE

INTRODUCTION

1.1 Background

Globally, the prevalence of all the leading chronic non communicable diseases is increasing, and this is especially so in the developing countries. According to the WHO (World Health Statistics 2008), non communicable diseases now cause more deaths than infectious diseases in many countries. Diabetes mellitus (DM) is one of these non communicable diseases with a rapidly increasing incidence, especially in developing countries.

Fauci *et al* (2008) asserted that the prevalence of DM has increased dramatically around the globe, from an estimated 30 million cases in 1985 to 177 million in 2000. It is estimated that, more than 360 million individuals may develop diabetes by the year 2030

The International Diabetes Federation (IDF) in 2006 reported that 10 million people are affected with diabetes in Africa and this figure is expected to rise to almost 20 million by 2025.

Fauci *et al* (2008) added that although the incidence of both types 1 and 2 DM is on the ascendancy worldwide, type 2 DM is increasingly becoming more common especially as more nations become industrialized and more people adopt the Western lifestyle which encourages less physical activity thus leading to obesity. Thus diabetes is rapidly becoming a daunting public health problem in developing countries.

Diabetes is primarily characterised by ‘hyperglycaemia giving rise to the risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease),

and diminished quality of life' (WHO, 2006). Diabetic nephropathy is the leading known cause of end-stage renal disease (ESRD) in most countries.

Mbanya and Sobngwi (2003) estimated that in Africa, the prevalence of nephropathy varies between 32-57% after a person has had diabetes for 5 to 10 years and 5-28% within the first year following the diagnosis of diabetes. Diabetic nephropathy was reported by Alebiosu *et al* (2006) to be assuming an increasing role as a cause of chronic kidney disease in Nigeria. The incidence of diabetes nephropathy among diabetics in Southern Nigeria has been reported by to be 72.63% (Onovughakpo-Sakpa *et al*., 2009).

Diabetic nephropathy affects patients with both type 1 and type 2 diabetes. However, it is easier to define the natural progression of the condition in type 1 than type 2 diabetes. This is because in type 2 diabetes, the actual time of onset of the disease is usually unknown and mostly the disease is diagnosed with other pathological conditions present in the patient. Unless treatment is aggressively pursued, about 80% of patients with type 1 diabetes, and 20–40% of patients with type 2 diabetes with microalbuminuria will progress to macroalbuminuria (Molitch *et al* 2004) By the time the patient presents with microalbuminuria, most of the pathological effects of diabetic nephropathy are already present (Boon *et al*, 2006), therefore an early detection of increased microalbuminuria helps in the detection of early nephropathy. In Ghana the prevalence of microalbuminuria in diabetics was estimated by Eghan *et al* (2007) to be 43% and the median duration at which patients developed the condition was also found to be 15 years.

According to Morrish *et al* (1990) as nephropathy progresses, the patient presents with proteinuria, with a relentless decline in glomerular filtration rate. Proteinuria as a prognostic marker in type 2 diabetes is less useful, although progressing to proteinuria increases the risk of death by fourfold as compared to those without proteinuria.

1.2 Statement of problem

Diabetic nephropathy has been identified as the leading cause of end stage renal disease leading to haemodialysis. Though this disease causes a broad spectrum of metabolic derangements in the human body, there is paucity of data on the biochemical and haematological profile of such patients in Ghana.

1.3 Objectives of study

The objective of this study is to establish the haematological and biochemical profiles of patients presenting with diabetic nephropathy.

1.3.1 Specific objectives

- To assess the haematological profile of patients with diabetes nephropathy.
- Analyse the various biochemical indices in relation to the progression of nephropathy.
- Analyse the anthropometric parameters of patients presenting with nephropathy.
- To outline the differences, if any, between the biochemical and haematological indices of diabetics with nephropathy and those without nephropathy.

1.4 Hypothesis

Diabetic nephropathy affects the biochemical and haematological profile of patients, and if such data is available, it will enable clinicians efficiently diagnose and track the progression of the disease.

1.5 Justification of study

Diabetes mellitus is one of the leading non-communicable diseases whose prevalence is very much on the ascendancy worldwide. The progression of the disease is associated with several complications including diabetic nephropathy, which is the leading cause of end stage renal disease leading to haemodialysis. When diabetic nephropathy is detected early, there is a possibility of reversing the condition or slowing the progression of the disease with good clinical care. Diabetic nephropathy causes a number of biochemical and haematological derangements in the body. This study therefore seeks to establish the haematological and biochemical profile of such affected patients. The availability of such data will help to provide possible early and sensitive predictors of diabetes nephropathy based on biochemical findings, and also point out other probable causes of anaemia and other haematological disorders. Also, such data could be used to show potential points of early intervention in the progression of diabetes nephropathy.

1.6 Methodology

Data will be obtained for the study through two main ways; primary data will be collected from patients through questionnaires, blood tests and body measurements.

Secondary data will be reviewed from work done by other researchers in this field of study.

1.7 Scope of study

Diabetics who attend the Diabetes Clinic at the Komfo Anokye Teaching Hospital in Kumasi will be recruited for this study

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder which occurs when the pancreas is not able to produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycaemia), which is the main diagnostic feature of diabetes mellitus. The sustained hyperglycaemia in diabetes causes damage, malfunction and sometimes total failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The pathogenesis of diabetes involves several processes. These range from 'autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues' (America Diabetes Association, 2004).

2.1.1 CLASSIFICATION OF DIABETES MELLITUS

Diabetes Mellitus (DM) is classified based on the cause or pathogenesis of the hyperglycaemia. The disease is categorized as follows;

Boon *et al* (2006) explains that Type 1 diabetes, (previously known as insulin-dependent (IDDM) or childhood-onset diabetes), occurs when there is partial or absolute insulin deficiency because of pancreatic β-cell damage. This type of diabetes includes cases of autoimmune or idiopathic pancreatic islet β-cell destruction. Type 1 diabetes progresses slowly and is believed to be mediated by T-cells.

The rate of pancreatic islet β-cell destruction is case-dependant, being rapidly progressive in some individuals and slow in others (Zimet *et al*, 1994).

Humphrey *et al* (1998) also added that the rapidly progressive form is more commonly observed in children; although it may also occur in adults, On the other hand, adults usually manifest the slowly progressive type which is sometimes also referred to as latent autoimmune diabetes. This does not include those conditions such as cystic fibrosis, mitochondrial defects, etc in which there is beta-cell destruction or failure (WHO, 1999).

Type 2 diabetes, previously called non-insulin-dependent (NIDDM) or adult-onset diabetes, is characterized by the body's ineffective use of insulin. This type may range from conditions in which there is usually insulin resistance with or without insulin insufficiency to others in which there is insulin secretory defect with or without resistance (WHO, 1999). Both Mooy *et al* (1995) and Harris (1993) have observed that this form of diabetes is frequently undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke any clinical symptoms of diabetes. However the deleterious effects of the disease will be internally manifesting and many of such patients may have developed macrovascular and microvascular complications at diagnosis.

Type 2 often results from increased body weight and physical inactivity, and affects people with a mainly sedentary lifestyle. The incidence of this type of diabetes is increasing as more nations become industrialized and adopt Western sedentary lifestyles.

Gestational Diabetes is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The affected woman may possibly have had the glucose intolerance before pregnancy though unrecognized. However women who had diagnosed diabetes before pregnancy cannot be said to have gestational diabetes. The definition applies regardless of the type of treatment needed during the course of the pregnancy and whether the patient remains diabetic after delivery. (America Diabetes Association, 2009).

Other specific types of DM have been identified. These are categorized according to the causative agents as follows:

- Genetic defects of beta-cell function
- Genetic defects in insulin action
- Diseases of the exocrine pancreas
- Endocrinopathies
- Drug- or chemical-induced
- Infections
- Uncommon forms of immune-mediated diabetes
- Other genetic syndromes

2.2 COMPLICATIONS OF DIABETES MELLITUS

2.2.1 ACUTE COMPLICATIONS

The usual clinical symptoms of DM include polyuria, polydipsia, weight loss, fatigue, weakness, blurred vision, frequent superficial infections, and poor wound healing. However, patients can occasionally present with acute complications, such as hypoglycaemia, diabetic ketoacidosis, hyperosmolar non-ketotic coma. (Boon *et al*, 2006, Fauci *et al*, 2008).

2.2.2 CHRONIC COMPLICATIONS

The major chronic complications of DM are usually microvascular, neuropathic and macrovascular in nature. The microvascular and neuropathic complications present as retinopathy, nephropathy, peripheral neuropathy, autonomic neuropathy and foot disease. The macrovascular complications present as myocardial infarction/ ischaemia, transient ischaemic attack, stroke and claudication (Boon, 2006). Fauci *et al* (2008) have also indicated that other chronic complications of diabetes may be non-vascular, e.g. gastroparesis, infections and skin changes.

2.2.2.1 MACROVASCULAR COMPLICATIONS

Boyle (2007) stated that in patients with type 2 diabetes, there is an increased risk of macrovascular disease. Factors that may play a linkage role in the development of macrovascular disease in type 2 diabetes include low concentration of the adipocyte specific protein (adiponectin) , increased production of the vascular cell adhesion molecule-1 and the subsequent adhesion of T-lymphocytes to the endothelial walls of the coronary arteries, higher procoagulation with increased expression of plasminogen activator inhibitor-1 (PAI)-1, and an

increased production of matrix metalloproteinases (MMPs) by macrophages which ultimately leads to an instability of atherosclerotic plaques.

Patients with type 1 diabetes have a disproportionately higher burden of coronary heart disease. Studies have shown that these patients have a higher mortality from ischemic heart disease at all ages compared to the general population. In individuals above 40 years of age, women experience a higher mortality from ischemic heart disease than men (Laing *et al*, 2003). Almdal *et al* (2004) also indicated that Type 2 diabetes typically occurs in the setting of the metabolic syndrome, which also includes abdominal obesity, hypertension, hyperlipidaemia, and increased coagulability. These other factors can also act to promote cardiovascular disease. In this setting of multiple risk factors, type 2 diabetes itself acts as an independent risk factor for the development of ischemic disease, stroke, and death.

2.2.2.2.1 RETINOPATHY

Diabetic retinopathy which occurs in all forms of diabetes is the commonest cause of blindness in adults in most developed countries. The development of retinopathy, as with all diabetic complications, depends on the duration of the disease.

The natural history of diabetic retinopathy according to Nathan (1993) has been best defined in IDDM, where it is possible to predict the date of onset of the disease. Course of progression of retinopathy in NIDDM is more difficult to ascertain, since the diabetes may be progressing silently for many years before it is diagnosed. As a result, patients with NIDDM may present with retinopathy and even, rarely, advanced retinopathy at the time of diagnosis. However, the

development of retinopathy is still dependent on how long the patient has had NIDDM, as it is in IDDM.

The clinical features of diabetic retinopathy are; 'microaneurysms, retinal haemorrhages, exudates, cotton wool spots, neovascularisation, fibrosis, pre-retinal and vitreous haemorrhages. These features occur in various combinations in different patients and are used to classify the severity of the disease' (Boon 2006).

2.2.2.3 NEUROPATHY

This is a relatively early and common complication affecting approximately half of all patients diagnosed with both types 1 and 2 diabetes. Fauci (2008) asserts that the condition may manifest as polyneuropathy, mononeuropathy, and/or autonomic neuropathy. As with other complications of DM, the development of neuropathy largely depends on duration of diabetes and how well or otherwise glucose levels are controlled. Also increased body mass index and smoking are considered risk factors for developing the complication.

Many mechanisms lead to diabetic neuropathy. Little *et al* (2007), indicated that main causative agents are 'continued oxidative stress, ischaemia and inflammation leading to axonal dysfunction and loss, associated to some extent with secondary demyelination'.

2.2.2.4 DIABETIC NEPHROPATHY

Diabetic nephropathy, also known as Kimmelstiel-Wilson syndrome, or nodular diabetic glomerulosclerosis, was discovered in 1936 by Clifford Wilson and Paul Kimmelstiel. The disease has been described by Parving *et al* (1996) as a clinical syndrome in which there is a

steady urinary albumin excretion, a progressive fall in glomerular filtration rate (GFR), raised arterial blood pressure and a relatively high incidence of cardiovascular diseases.

2.2.2.4.1 STAGING AND CLINICAL FEATURES OF DIABETIC NEPHROPATHY

Mogensen *et al* (1983) and Schelling *et al* (2005) described five stages of diabetic nephropathy. These are;

1. Normoalbuminuria with elevated GFR (typically occurs within 5 to 10 years after patient becomes diabetic):

This stage is usually associated with enlargement of the tubules and glomeruli. The kidneys may also appear enlarged on ultrasound examination. Characteristically, there may be albuminuria especially after strenuous physical activity. Changes that occur at this stage may be partially treated with good glycaemic control. This stage is also associated with raised blood pressure.

2. Microalbuminuria/incipient diabetic nephropathy (typically occurs within 5 to 15 years after becoming diabetic):

At this stage there is urinary albumin excretion of 30 to 300 mg albumin/g creatinine, which may be measured quantitatively or semi quantitatively with dipsticks. Stage 2 develops silently over many years and there may be lesions on the renal morphology without any clinical symptoms. However, kidney function tests and examinations on biopsy specimens reveal the changes. This stage is characterized by increased GFR. When there is good glycaemic control, the patient tests negative for urine albumin, but strenuous physical activity unmasks the changes in urine albumin levels. With persistent hyperglycemia, urine albumin levels are increased at all times. This stage may be persistent throughout the life time of a number of patients. Retinopathy is common from this stage onwards.

3. Macroalbuminuria/overt proteinuria (typically occurs within 10 to 20 years after becoming diabetic):

This stage is also defined by urinary albumin excretion of >300 mg albumin/g creatinine. This stage precedes overt diabetic nephropathy, and its cardinal feature is the abnormally increased urinary albumin excretion. This is a very decisive phase of renal disease in diabetes as blood pressure is rising. The increased level of albumin excretion is higher in patients with increased blood pressure. The incidence of cardiovascular disease increases at this stage.

4. Decline in glomerular filtration rate (typically occurs within 15 to 25 years after becoming diabetic):

Stage 4 is overt diabetic nephropathy, with the cardinal feature of persistent proteinuria (greater than 0.5 g/24 h). This stage is usually accompanied by high blood pressure which if not aggressively treated will invariably lead to a decline in renal function (GFR). Long-term antihypertensive treatment reduces the fall rate by about 60% and thus postpones uraemia considerably.

5. End-stage renal disease (ESRD) with uraemia within 5 years of developing nephrotic-range proteinuria.

   At this stage, patients appear ill and anaemic. They may not necessarily be edematous, and may even show signs of sodium and water depletion. Metabolic acidosis with an unusually deep respiration (Kussmaul's respiration) may be present together with anorexia and nausea. Later hiccoughs, pruritis, vomiting, muscular twitching, fits, drowsiness and coma ensue (Boon *et al*, 2006).

2.2.2.4.2 PATHOGENESIS OF DIABETIC NEPHROPATHY

It has been postulated by researchers and various schools of thought around the world that diabetic nephropathy occurs as a result of a multifactorial interaction between metabolic and haemodynamic factors in the renal microcirculation.

Raptis *et al* (2001) indicated that clinically diabetic nephropathy followed a well defined course of progression, starting with the excretion of minute amounts of albumin, to macroalbuminuria. Then there is accumulation of nitrogenous waste products in the blood and this eventually ends in end stage renal disease. They further asserted that there are several renal functional changes such as increased renal perfusion and filtration and an increased capillary permeability to macromolecules even before overt proteinuria is manifested. It has long been identified that basement-membrane thickening and mesangial expansion are cardinal pathological features of diabetes.

According to Cao *et al* (2011), glucose-dependent pathways, such as advanced glycation end-products and their receptors as well as various vasoactive hormones, such as components of the renin–angiotensin system are some of the metabolic and haemodynamic factors that play a role in the pathogenesis of diabetic nephropathy. There is believed to be an interaction in the activity of these metabolic and haemodynamic factors through shared molecular and signaling pathways, such as nuclear factor kappa-light-chain-enhancer of activated B cells and protein kinase C with associated reactive oxygen species generation. The pathological damage caused by these factors affects especially the podocytes of the glomerulus as well as the tubular interstitium.

Arya *et al* (2010) further theorized that several intracellular second messengers such as protein kinase C (PKC), mitogen-activated protein (MAP) kinase, nuclear transcription factors such as NF-kB and various growth factors such as the prosclerotic cytokine, TGF-β and the permeability enhancing growth factor, vascular endothelial growth factor, (VEGF) are all activated by various haemodynamic pathways. Hyperglycaemia induces increased glucose dependent pathways within the diabetic kidney which results in enhanced oxidative stress, renal polyol formation and an increased accumulation of advanced glycation end products (AGEs). Together, these processes ultimately lead to increased renal albumin excretion and extracellular matrix accumulation, which results in increased urinary protein excretion, glomerular injury and ultimately fibrosis of the tubular interstitium.

Oxidative stress has been known to play an important role in the development and progression of diabetic nephropathy, but the actual cell-level processes regulated by reactive oxygen species (ROS) are still debatable. Jyoti and Purnima (2010) theorized that, glucose metabolism and the associated formation of excess advanced glycation end-products in the hyperglycaemic state activates reactive oxygen species in the cells, and these eventually up-regulates transforming growth factor-beta 1, plasminogen activator inhibitor-1, and extracellular matrix proteins by glomerular mesangial cells, thus causing mesangial expansion. Further, these reactive oxygen species also induces the activity of other signaling molecules, such as protein kinase C, mitogen-activated protein kinases and transcription factors, such as nuclear factor-kappa B and activator protein-1 leading to transcription of genes encoding cytokines, growth factors, and extracellular matrix proteins.

The action of reactive oxygen species has been found to be ameliorated by various antioxidants.

Jyoti and Purnima (2010) postulates that, antioxidants exert an inhibitory effect on mesangial cell activation which is caused by hyperglycaemia and so reduces the clinical features of diabetic nephropathy. Thus, reactive oxygen species are believed to function as intracellular messengers and integral glucose-signaling molecules in glomerular mesangial cells in diabetic nephropathy. Traditionally diabetic nephropathy is regarded as a non-immune disease, however, recent studies have identified the role of immunologic and inflammatory factors in its development and progression.

For example, Gonzalez and Fernandez (2008) asserts that particular cells in the body including white cells such as leukocytes, monocytes, and macrophages, as well as other molecules, such as chemokines (monocyte chemo-attractant protein-1), adhesion molecules (intercellular adhesion molecule-1 (ICAM-1)), enzymes (cyclooxygenase-2, nitric oxide synthase), growth factors (vascular endothelial growth factor, growth hormone, IGF, TGF-β), and nuclear factors (NF-κB), are believed to be involved in processes that are related to the development of diabetic nephropathy. Furthermore, the inflammatory cytokines, IL-1, IL-6, IL-18 and tissue necrosis factor (TNF) are all believed to play roles in developing diabetic nephropathy as well as its complications. (Gonzalez and Fernandez, 2008).

2.2.2.4.3 CURRENT METHODS OF DETECTION OF DIABETIC NEPHROPATHY

In the early 1980s, studies in Europe by Mogensen *et al* (1984), Parving *et al* (1982) and Viberti *et al* (1982) revealed that small amounts of albumin in the urine, not usually detected by

conventional methods, were predictive of the later development of proteinuria in diabetic patients. This stage of renal involvement was termed microalbuminuria or incipient nephropathy. Chiarelli *et al* (1997) have indicated that although some researchers have debated the prognostic value of microalbuminuria with respect to the development of overt diabetic nephropathy, keeping track of the urinary albumin excretion rate from the microalbuminuric stage is still a considerably reliable marker for prediction of the later development of diabetic nephropathy. Therefore microalbuminuria screening programmes for diabetics is of extreme importance in intervening and possibly reducing the incidence of diabetic nephropathy cases.

Considering the increasing global incidence of diabetic nephropathy, it is of utmost importance to screen and detect the disease early, especially at the reversible stage to avert the economic and mortality implications of the disease. (Larijani *et al*, 2002).

Schelling *et al* (2005) have asserted that glomerular filtration rate (GFR) estimation is useful for assessing the stage and therapeutic progress of nephropathy patients. However the 24-hour collection of urine used to assess creatinine clearance has been found to be inaccurate and cumbersome for patients. Therefore GFR estimate from predictive equations is a currently more preferred alternative, and several researchers recommend that equations based on Modification of Diet in Renal Disease (MDRD) study should be used rather than Cockcroft-Gault equation. Estimation of GFR based on predictive equations has been found to be a more accurate method for assessing renal function among the chronic kidney disease patients (Owiredu *et al*, 2008). However, according to Peralta *et al* (2011), a new creatinine-based equation, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) has reported better accuracy than the

Modification of Diet in Renal Disease study equation, especially when estimating GFR levels above 60 ml/min per 1.73m^2. However, all creatinine-based predictive equations are limited in that serum creatinine levels are affected by factors such as muscle mass, age sex and race. This limitation is especially so among the elderly, non-white populations, and also when GFR is just slightly reduced. This can lead to care-givers misclassifying patients who may actually be at risk of progressing into chronic renal disease.

According to Alsaad and Herzenberg (2007), histopathologically, diabetic nephropathy presents with glomerulosclerosis that may be diffuse or nodular, fibrosis of the tubular interstituim and atrophy, as well as variable degrees of arterial hyalination and arterial sclerosis. Since several other conditions such as; amyloidosis, monoclonal immunoglobulin deposition disease, fibrillar glomerulopathy etc present with similar histological findings as seen in diabetic nephropathy, it is essential that a detailed histopathological examination of renal biopsy specimens using immunofluorescence and electron microscopy, together with the clinical presentations be used to differentiate diabetic nephropathy from other causes of nodular glomerulosclerosis.

Alsaad and Herzenberg (2007) also identified a ‘diffuse or nodular mesangial matrix increase, glomerular basement membrane thickening and arteriolar hyalinosis’ as typical manifestations of diabetic nephropathy. Other findings can include fibrin caps, capsular drops, and capillary microaneurysms. Therefore, it is of outmost importance that there is a close comparison of clinical and pathological evidence before a correct diagnosis of diabetic nephropathy can be made. Also, the patient must be diagnosed as been diabetic or showing evidence of impaired glucose metabolism.

2.2.2.4.4 PREVALENCE AND RISK FACTORS OF DIABETIC NEPHROPATHY

According to the American Diabetes Association (2004), diabetes has become the most common single cause of end-stage renal disease (ESRD) in the U.S. and Europe with diabetic nephropathy accounting for about 40% of new cases of ESRD in the United States. About 20–30% of patients with type 1 or type 2 diabetes develop evidence of nephropathy, and although just a small fraction of type 2 diabetes do progress to ESRD, because of the increased prevalence of the condition, such patients constitute over half of those diabetic patients who need dialysis.

Schieppati and Remuzzi (2005) reported that though the global human population is growing at 1.3%, the population of ESRD patients is estimated to be 8% per annum. Also, of the nearly 1 million people receiving haemodialysis worldwide, 80% of them are treated in Europe, North America, and Japan. The remaining 20% are treated in 100 developing countries where over 50% of world's population live. This implies that a sizeable proportion of people living in the poorest countries die of uraemia because of the unavailability of proper treatment regimen.

Across Asia the prevalence of microalbuminuria was found to be 39.8% whilst the prevalence of macroalbuminuria was 18.8% (Wu *et al*, 2004). In urban Asian Indians, the prevalence of overt nephropathy and microalbuminuria has been reported to be 2.2 and 26.9%, respectively (Unnikrishnan *et al*, 2007).

According to a survey by Lee (2003), diabetic nephropathy is the most common cause of end-stage renal disease in 9 of 10 Asian countries, with an incidence that had increased from 1.2% in 1998 to 14.1% in 2000. In China, the proportion of cases of end-stage renal disease that were

caused by diabetic nephropathy increased from 17% in the 1990s to 30% in 2000. In India, researchers expect diabetic nephropathy to develop in 6.6 million of the 30 million patients with diabetes. These findings imply the emergence of a huge public health problem in developing countries where appropriate treatment regimens are usually unavailable and ESRD could well be a death sentence.

Mbanya and Sobngwi (2003) estimated that in Africa, the prevalence of nephropathy varies between 32-57% after a patient has had diabetes between 5-10 years, and 5-28% within the first year following diagnosis of diabetes. Diabetic nephropathy was found by Alebiosu (2006) to be assuming a leading role as a cause of chronic kidney disease in Nigeria, and the incidence of diabetes nephropathy among diabetics in Southern Nigeria was found by Onovughakpo-Sakpa et al (2009) to be 72.63%.

Gross *et al* (2005), have asserted that approximately 40% of diabetics develop diabetic nephropathy develops even in the presence of sustained hyperglycaemia. This assertion brings to the fore the idea that some patients has an increased tendency to develop diabetic nephropathy. Furthermore, several studies have reported that there is a genetic component in the development of diabetic nephropathy in patients with both type 1 and type 2 diabetes. For such susceptible persons, modifiable factors are sustained hyperglycemia and hypertension. Other assumed risk factors are glomerular hyperfiltration, smoking, dyslipidemia, and dietary factors.

Potentially modifiable risk factors that can affect the development and progression of kidney disease especially in type 2 diabetics include duration of disease, baseline albumin excretion,

age, glycemic control, blood pressure, serum cholesterol levels and use of renin-angiotensin system blockers (Reutens and Atkins (2011), Gall *et al* (1997) and Unnikrishnan *et al* (2007)).

Besides these well known factors anthropometry has also been found to have an effect on the incidence of diabetic nephropathy. Today, more than 1.1 billion adults worldwide are overweight, and 312 million of them are obese. In addition, at least 155 million children worldwide are overweight or obese, according to the International Obesity Task Force. This task force and the World Health Organization (WHO) have considered ethnic differences in their definition (Hosssain *et al*, 2007).

Tomic *et al* (2003) found that the problem of overweight and obesity was quite common among type 2 diabetics. This problem leads to a higher risk of developing macrovascular complications. There was also a correlation between BMI and nephropathy as with increasing obesity, they observed a significant deterioration of HbA1c and increased LDL-cholesterol as well as blood pressure.

In Trinidad, Nayak *et al* (2008) found increased microalbumin levels to be strongly correlated with hypertension and waist to hip ratios in type-2 diabetic patients. In Ghana the prevalence of microalbuminuria in diabetics was estimated by Eghan *et al* (2007) to be 43% and the median duration at which patients developed the condition was also found to be 15 years.

2.2.2.4.5 IMPLICATIONS OF DIABETIC NEPHROPATHY

Diabetic Nephropathy has serious health, economic and social implications.

The kidneys have many functional roles, including fluid and electrolyte balance, waste removal, acid-base balance, bone metabolism, and stimulation of red blood cell production. According to the Center for Disease Control, diabetic nephropathy which eventually progresses to Chronic Kidney Disease (CKD) presents with several biochemical derangements such as fluid overload, electrolyte imbalances, bone and mineral disorders, anaemia, and diminished quality of life. CKD is an identified risk factor for several cardiovascular diseases. Diabetic nephropathy has also been identified as the leading cause of End Stage Renal Disease (ESRD) in several parts of the world.

Low *et al* (2007) added that the number of deaths caused by ESRD is increasing, especially in the Western world, and with it comes an increased number of patients with diabetes and cardiovascular diseases. In older patients, dialysis does not usually improve their condition since most of them present with other confounding health problems. ESRD also greatly affects the economic status of patients and the nation.

Zelmer (2007) reports that less than 0.1% of Canadians have ESRD; however, the economic burden of the disease was about $1.3 billion in the year 2000. A lot more money is spent on a patient with ESRD averagely than for all other health conditions. Cass *et al* (2006) noted that in most high-income countries, Renal Replacement Therapy (RRT) programs exert a sizeable economic burden. For example the UK, Germany and France spent 0.7, 1.3 and 1.5 per cent of their health care budget on dialysis in 1994, despite dialysis patients comprising 0.02, 0.05 and

0.04 per cent of these populations respectively. It is estimated that by year 2010 Medicare expenditures on RRT in the United States will increase, at a rate of 7.7% per annum, from $12 billion in 1998 to approximately $28 billion.

Cass *et al* (2006) further asserted patients with ESRD usually have diminished quality of life, and even RRT may not completely alleviate all symptoms, and may itself bring forth other lifestyle changes that all impact the quality of life. Dialysed patients usually have several complaints such as body aches, insomnia, itchy/dry skin, gastrointestinal upsets, difficulty concentrating, headaches, sexual dysfunction, cramps etc. Although most of these complaints are reduced in patients who receive kidney transplants, the immunosuppressive medications needed may bring on other complaints.

ESRD also exerts emotional and psychological effects on patients. The US Nephrology Journal (2007) reports that depressive disorders are common complaints among ESRD patients. They reported that studies have shown a prevalence of depression of 26–27% and major depressive disorder of 17–19% in dialysed patients. Meanwhile among the general population, the prevalence of major depressive disorder was reported at approximately 5%. In the population of patients with ESRD on dialysis those with depression have a more negative perception and usually presents with a poorer outcome compared to those without depression. Dialysis is quite disruptive to the normal lifestyle of patients, and those with depression report lower quality of life and poorer social support systems.

2.2.2.4.6 BIOCHEMICAL AND HAEMATOLOGICAL EFFECTS OF DIABETIC NEPHROPATHY

Diabetic nephropathy has extensive effects on the biochemical and haematological profile of patients.

Generally, chronic kidney disease patients in stages 1-3 usually show no evidence of metabolic disturbances. These disturbances usually start manifesting in stages 4 and 5.

From the microalbuminuric stage, the patients progresses to eventual overt proteinuria. At this stage, plasma creatinine levels may be normal but the patient may be only a few years away from end-stage renal failure. Patients with overt nephropathy may present with nephrotic syndrome, low serum albumin levels and oedema. They may also show a normochromic normocytic anaemia and a raised erythrocyte sedimentation rate (ESR). Increased blood pressure which is a usual complication of diabetic nephropathy may itself damage the kidney still further. Late in the progression of the disease, there is usually a rise in plasma creatinine, at a rate that is dependent on the particular patient (Kumar and Clark, 2005).

With progression of chronic kidney disease (CKD), disorders of mineral metabolism appear. According to Lorenzo and Torrregrosa (2008), the mineral disorder usually begins with deficient calcitriol synthesis and retention of phosphorus. Consequently, serum calcium is reduced and parathyroid hormone (PTH) is stimulated, eventually leading to osteitis fibrosa while other patients may present with low turnover (LT) bone disease.

Arroyo (2008), elaborated that in advanced chronic kidney disease, urine osmolality becomes equal to plasma osmolality and this presents clinically as symptoms of nocturia and polyuria, especially in tubulo-interstitial kidney diseases. Increasing or reducing water intake will then affect sodium balance. The extracellular fluid volume of a person depends on the total body sodium level so when dietary sodium intake is reduced in advanced CKD, the patients presents with clinical symptoms of fluid imbalances.. Also, progressive reduction of glomerular function decreases the kidneys' ability to excrete potassium. This leads to the body relying on aldosterone and increased intestinal excretion maintain potassium homeostasis. The raised plasma potassium a level in CKD is usually attributed to drugs, reduced glomerular filtration rate, constipation and prolonged fasting.

Sexauer and Matson (1981) also indicated that anaemia is one of the commonest and clinically visible presentations of chronic renal failure. Recent research has shed more light on the etiology of this anemia. There are three main recognized causes: reduced erythropoietin production due to damaged kidneys, presence of inhibitors to red blood cell (RBC) production in uraemic serum; and increased red blood cell haemolysis due to uraemia.

Arora (2011) further explains that in the presence of reduced renal synthesis of erythropoietin, there is usually a normocytic normochromic anaemia, which increases in severity as GFR declines progressively. There is usually no reticulocyte response and the red cells also survive for a shorter period of time. The accompanying uraemia has an effect on platelet function leading to increased bleeding tendencies. There are other causes of anaemia in chronic kidney disease including secondary hyperparathyroidism, inflammation and nutritional deficiency.

Anaemia in these patients presents other complaints that reduces the patient's quality of life, and increases the risk of developing cardiovascular disorders (Arora, 2011).

Anaemia is common in diabetic patients with CKD. According to Mehdi and Toto (2009) it is estimated that a fifth of patients with diabetes and stage 3 CKD have anemia which worsens in severity as the patient advances in CKD. They identified iron and erythropoietin deficiencies and hypo-responsiveness to the actions of erythropoietin as the main causes of anaemia in CKD patients. Furthermore, anaemia exerts physical and mental impairments on diabetic CKD patients.

Patients with diabetic nephropathy commonly presents with anaemia earlier more severely than patients with other causes of renal failure. Anaemia has been identified as a major cause for the need for renal replacement therapy in diabetes; also, a declining haemoglobin level is significantly associated with a more rapid decrease in the glomerular filtration rate (GFR). It is very advantageous to detect and treat anaemia early as this reduces the associated risk of cardiovascular diseases and improves patients' survival. (Craig *et al*, 2005, Ephraim *et al*, 2008).

Furthermore, Hermann *et al* (2004) also asserted that Metformin is generally accepted to be a safe treatment for diabetes, but in the long term may affect the absorption and eventually lead to deficiency of vitamin B12. This deficiency can lead to megaloblastic anaemia, myelopathy and neuropathy as well as raised serum homocysteine and methylmalonic acid levels. Homocysteine has been identified as an independent cardiovascular risk factor. Vitamin B12 deficiency also

presents with such serious health implications as megaloblastic anaemia, impaired nervous system functioning and low cobalamin levels associated with dementia.

2.2.2.4.7 PREVENTION AND TREATMENT OF DIABETIC NEPHROPATHY

Several researchers agree that the basis for the prevention and treatment of diabetic nephropathy is the treatment of its known risk factors: hypertension, hyperglycemia, smoking, and dyslipidemia. These are also risk factors for cardiovascular disease and should be vigorously treated.

According to Gross *et al* (2005), since hyperglycaemia has been linked to the development of almost all the chronic complications of diabetic nephropathy, it is essential that vigorous glycaemic control aiming at HbA1c < 7% should be pursued in order to as much as possible prevent the development of microalbuminuria. Also, there should be an aggressive control of blood pressure levels in diabetics since hypertension is common in most diabetics even in the absence of renal complications. About 40% of type 1 and 70% of type 2 diabetic patients with normoalbuminuria have blood pressure levels > 140/90 mmHg.

In the United Kingdom Prospective Diabetes Study, when systolic blood pressure levels were reduced from 154 to 144 mmHg, there was a reduction in the risk for the development of microalbuminuria by 29%. Pylypchuk and Beaubien (2000) also identified smoking as one factor that can worsen diabetic renal disease, since it raises the plasma levels of the vasoconstrictor endothelin I and reduces renal plasma flow. Also, studies have identified

hypercholesterolemia to decrease renal function, and it is a modifiable risk factor for cardiac disease, and should therefore be controlled in such patients.

Pylypchuk and Beaubien (2000) further stressed that angiotensin-converting enzyme (ACE) inhibitors are useful in diabetic nephropathy aside from their blood pressure lowering effect. The ACE inhibitors positively affect intra-renal haemodynamics and decreases intra-glomerular blood pressure; however, they also have potential side effects such as increasing plasma potassium levels and cough.

Elaborating further, Batuman (2011) stressed that long-term treatment with ACE inhibitors, usually combined with diuretics, reduces blood pressure and albuminuria as well as improving kidney function in patients with hypertension, type 1 DM, and nephropathy. Treatment with ACE inhibitors has been experimentally shown to delay the progression of diabetic nephropathy. ACE inhibitors was reported to exert a long lasting delay on the progression of microalbuminuria to overt diabetic nephropathy and was found to be associated with the preservation of a normal glomerular filtration rate (GFR).

In preventing diabetic nephropathy, the importance of screening for microalbumin cannot be overemphasized. Several researchers agree that this step is of outmost importance in the prevention and treatment of diabetic nephropathy.

Treatment in patients with overt nephropathy is more to delay the onset of ESRD. Once the patient develops microalbuminuria, the likely of a downward progression is significantly

increased, as such it is essential to screen and detect such patients early. Therefore, diabetics should be routinely and regularly screened for microalbuminuria as part of their clinical care (Evans and Capell, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 ETHICAL APPROVAL

Ethical approval for the study was obtained from the Committee on Human Research Publication and Ethics of the College of Health Sciences, Kwame Nkrumah University of Science and Technology before the research was carried out.

3.2 STUDY POPULATION AND SETTING

Type 2 diabetics who were managed at the Diabetes Clinic of Komfo Anokye Teaching Hospital were randomly selected for the study.

3.3 QUESTIONNAIRES

Structured questionnaires were administered to the study participants to find out among other things their age, how long they have had DM and the type of treatment plan they were on.

3.4 EQUIPMENT AND APPARATUS USED

- Ethylene Diamine Tetra-acetic Acid (EDTA) tubes (Dist: Cantoment, Accra)
- Sodium Fluoride tubes (BD Vacutainer, Plymouth, UK).
- Clinical Chemistry tubes with serum separator gel (BD Vacutainer, Plymouth, UK).
- Urine sample collection bottles
- Medical gloves
- Tourniquet

- Alcohol (Unit Pharm. Dept., KATH, Kumasi)
- Cotton wool
- Hypothermic needles
- Microscopic slides
- Measuring Tape
- Wall-mounted measuring tape
- Ruler
- Sphygmomanometer
- Stethoscope (Minnesotta, USA)
- Bathroom scales
- Dirui H11-MA urinalysis reagents strips (Jilin, China).
- Leishman stain
- Dispette ESR tubes (CHAM, Switzerland)
- Sysmex XT 2000i haematology analyzer (Kobe, Japan)
- ELISA Microtitre plate Reader (Shanghai, China)
- Microplate Washer (Bio-Rad, France)
- COBAS Integra 4OO (Manheim, Germany)
- Selectra ProS Analyzer (Vital Scientific B. V., Netherlands)
- Pipettes
- Spectrophotometer (Rochester, USA)

3.5 ANTHROPOMETRIC VARIABLES

Anthropometric variables including height to the nearest meter without shoes, weight to the nearest 0.1kg, arm and wrist circumference were taken. BMI was calculated by dividing the subject's weight (kg) by the height squared (m^2).

3.6 BLOOD PRESSURE

Blood pressure was taken by qualified personnel using an analogue sphygmomanometer and stethoscope. Measurements were taken from the upper arm with the hand at the heart level, after the patient had been sitting for more than 5 minutes.

3.7 URINALYSIS

Early morning urine was collected in plastic containers from the subjects and screened for urine microalbumin and protein using qualitative dipsticks (Dirui Ind, Co., Ltd., Jilin, China).

3.8 SPECIMEN COLLECTION

Blood samples were taken from subjects by venepuncture into sterile EDTA anticoagulated bottles, sodium fluoride tubes and clinical chemistry vacutainer tubes with serum separator gel.

3.8.1 Method

1. A sterile, dry 10ml plastic syringe was selected and attached to an appropriate needle.
2. A tourniquet was tied on the upper arm of the patient and was asked to make a fist
3. Cotton wool soaked in 70% alcohol was used to clean (sterilize) the skin for venepuncture

4. Venepuncture was made with the bevel of the needle appropriately angled. A steady withdrawal of the plunger of the syringe was very necessary to prevent injuring the vein.
5. About 10mls of blood was collected before tourniquet was removed
6. The needle was removed carefully and the puncture site pressed with a piece of cotton wool to stop bleeding.
7. The needle from the syringe was capped and carefully disposed off properly
8. The various tubes were filled with the required volume of blood and mixed slowly
9. The site of venepuncture was inspected for bleeding. A piece of cotton wool was placed on the site and a plaster placed on it

3.9 SAMPLE ANALYSIS

3.9.1 BIOCHEMICAL TESTS

Biochemical tests (with the exception of fasting blood sugar) were run on Selectra ProS analyzer (Vital Scientific B. V., Netherlands), using ELITech Clinical Systems reagents (Sées, France). Electrolytes were run using Dry ISE (Ion Selective Electrode) unit attached to the Selcetra ProS analyzer.

3.9.2 BLOOD FILM COMMENT

Principle of test

A thin film is prepared for the investigation and management of anaemia, infections and other conditions which produce changes in the morphology of blood cells.

Procedure

1. A small drop of blood was placed on one end of a clean dry slide
2. A clean smooth edged spreader was drawn back to touch the drop of blood and allowed to extend along the edge of the spreader.
3. The spreader was held at an angle of about 35^{o} and the blood was spread on the slide to make a thin film with a smooth tail.
4. The end of the spreader was wiped clean, and the slide air dried.
5. The blood film was covered with a few drops of undiluted Leishman stain for about 2-3 minutes.
6. Twice the volume of tap water was added (avoiding overflow).Thorough mixing of the water and the stain was ensured and allowed to stain for 10 minutes.
7. The stain was washed off with more of the water. The back of the slide was cleaned, placed in a draining rack and allowed to dry.

3.9.3 ERYTHROCYTE SEDIMENTATION RATE (ESR)

Principle of test

When citrated blood in a vertically positioned Westergren pipette is left undisturbed, the red cells aggregate, stick together to form rouleaux, and sediment through the plasma. The ESR is the rate at which this sedimentation occurs in 1 hour as indicated by the length of the column of clear plasma above the red cells, measured in mm.

Test Method

1. 1.6 ml of venous blood or EDTA anticoagulated blood was poured into the Dispette 2 ESR tube (to the mark) and mixed well.

2. A Westergren pipette was inserted and ensured that it is positioned vertically.

3. A timer was set for 1 hour. Care was taken to ensure that the ESR stand and pipette will be undisturbed and not exposed to direct sunlight.

4. After exactly 1 hour, the level at which the plasma meets the red cells was read and reported as mm fall per hour.

3.9.4 FULL BLOOD COUNT

Principle of tests (Sysmex XT 2000i Haematology Analyzer)

Sodium Lauryl Sulfate (SLS) Hemoglobin Analysis Method

The SLS-hemoglobin method is an analysis method that makes use of the advantages of two methods; cyanmethaemoglobin and oxyhaemoglobin. In the SLS-Haemoglobin method, surfactants lyse the red blood cell membrane releasing haemoglobin. The globin group of the haemoglobin molecule is altered by the hydrophilic alkyl group of sodium lauryl sulfate. This induces the conversion of haemoglobin from the ferrous (Fe+2) to the ferric (Fe+3) state forming methaemoglobin, which combines with sodium lauryl sulfate to become SLS-Hb haemichrome molecule. Light (of wavelength 555nm) emitted from the diode passes through the sample in the haemoglobin cell of the analyzer. The concentration of SLS- Haemoglobin is analysed as light absorbance.

Test Method for total and differential White cell count (Flow Cytometry Method Using Semiconductor Laser)

A blood sample is aspirated, measured, diluted to the specified ratio, and stained. The sample is then fed into the flow cell. This sheath flow mechanism improves cell count accuracy and reproducibility. Since the blood cell particles pass in a line through the center of the flow cell, the generation of abnormal blood pulses is prevented and flow cell contamination is reduced. A semiconductor laser beam is emitted to the blood cells passing through the flow cell. The forward scattered light is received by the photodiode, and the lateral scattered light and lateral fluorescent light are received by the photo multiplier tube. This light is converted into electrical pulses, thus making it possible to obtain blood cell information. The result obtained is then printed out.

3.9.5 FASTING BLOOD SUGAR

Principle of Test (COBAS Integra 4OO Chemistry Analyzer)

Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by NADP+ to form NADPH. The concentration of the NADPH formed is directly proportional to the glucose concentration. It is determined by measuring the increase in absorbance at 340 nm.

3.9.6 VITAMIN B12

Principle of ELISA test

Purified Human Vitamin B12 (VB12) antibody is coated onto the microtiter plate wells, making a solid-phase antibody (ABO Swiss Co Ltd. No. 24, Dongming Rd, Xiamen, Fujian 361004 China). When the sample is added to the wells, VB12 antigen in the sample combines with the antibodies. Upon addition of horse radish peroxidase (HRP) - labeled goat anti-Human Conjugate reagent (ABO Swiss Co Ltd. No. 24, Dongming Rd, Xiamen, Fujian 361004 China), an antibody - antigen –antibody- enzyme (Ab-Ag-Ab-E) complex is formed. After washing completely, 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution (ABO Swiss Co Ltd. No. 24, Dongming Rd, Xiamen, Fujian 361004 China) is added which cause a colour development. The reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically (Spectronic 21, Bausch and Lomb, Rochester, USA) at a wavelength of 450 nm. The concentration of VB12 in the samples is then determined by comparing the absorbance of the samples to the standard curve.

3.9.7 FOLIC ACID

Principle of ELISA test

Purified human Folic Acid (FA) antibody is coated onto the microtiter plate wells, making a solid-phase antibody (ABO Swiss Co Ltd. No. 24, Dongming Rd, Xiamen, Fujian 361004 China). When the sample is added to the wells, FA antigen in the sample combines with the antibodies. Upon addition of horse radish peroxidase (HRP) - labeled goat anti-Human Conjugate reagent (ABO Swiss Co Ltd. No. 24, Dongming Rd, Xiamen, Fujian 361004 China), an antibody - antigen -antibody - enzyme complex is formed. After washing completely,

3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution is added which cause a colour development. The reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically (Spectronic 21, Bausch and Lomb, Rochester, USA) at a wavelength of 450 nm. The concentration of FA in the samples is then determined by comparing the absorbance of the samples to the standard curve.

Test method for ELISA (Vitamin B12 and Folic Acid)

1. The original density standard was diluted serially 5 times.
2. Blank wells were set (to which we did not add sample and HRP-Conjugate reagent, every other step is same).
3. Sample diluent was pipette (40µl) into the wells, and then 10µl sample was added (sample final dilution is 5-fold). Avoid touching the well wall as far as possible, and mix gently.
4. The plate was covered with the closure membrane and incubated for 30 min at 37°C.
5. The wash solution was constituted by diluting 30-fold with distilled water and reserved.
6. The closure plate membrane was uncovered and washed using the Microplate Washer (PW 40, Bio-Rad, France) and then dried by patting on sterile gauze.
7. 50µl of the horse radish peroxidase (HRP) -conjugate reagent was added to each well, except blank well.
8. Incubation was done as in step 3.
9. Washing was done as in step 6.
10. 50ul of Chromogen Solution A and Chromogen Solution B was added to each well, avoiding light. Incubation was done for 15 minutes at 37°C.

11. The reaction was stopped by adding 50µl Stop Solution to each well (the blue colour changes to yellow).

12. Assaying was done by taking the blank well as zero, and the absorbance read at 450nm within 15 minutes after adding the stop solution.

3.9.8 FERRITIN

Principle of Test

Ferritin calibrator or patient sample is first added to a streptavidin coated well (Fortress Diagnostics, Antrim BT41 1QS, UK). Biotinylated monoclonal antibody (specific for ferritin), (Fortress Diagnostics, Antrim BT41 1QS, UK) is added and the reactants mixed. Reaction results between the biotinylated ferritin antibody and native ferritin to form an immune complex that is deposited on the coated wells. The excess serum proteins are washed away via a wash step. Another ferritin-specific antibody, labelled with an enzyme is added to the wells (Fortress Diagnostics, Antrim BT41 1QS, UK). The enzyme-labelled antibody binds to the ferritin already immobilized on the well. Excess enzyme-labelled antibody is washed off, and a colour is generated by the addition of a substrate.

The intensity of the colour generated is directly proportional to the concentration of ferritin in the sample.

Test Method for ELISA (Ferritin)

1. 25µl of control or serum was pipetted into the assigned wells.
2. 100µl of the biotinylated antibody was added to each well as close to the bottom as possible.

3. The plate was swirled gently for 20-30 seconds to mix and covered.
4. Incubation was done at room temperature for 30 minutes.
5. The wells were decanted and washed three times with the wash solution using Microplate Washer (PW 40, Bio-Rad, France).
6. 100µl of the anti ferritin-enzyme labelled antibody was added to each well.
7. The plate was swirled gently for 20-30 seconds to mix and covered.
8. Incubation was done at room temperature for 30 minutes.
9. The wells were decanted and washed three times with the wash solution.
10. 100µl of working substrate solution was added to all wells
11. Incubation was done at room temperature for 15 minutes.
12. 50µl of stop solution was added to each well and mixed gently for 15-20 seconds.
13. The absorbance in each well was read at 450nm in a microplate reader.

3.9.9 SERUM IRON

Principle of Test for Serum Iron

Iron is dissociated from the transferrin-iron in a weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions react with Nitro PAPS (2-5-nitro-2-pyridyl-azo)-5-n-propyl-n-(3-sulfopropyl-amino-phenol) to give a coloured complex.

Test Method

1. Pipetting was done into cuvettes as follows

	Sample	Standard	Blank
Iron Free Water	-	-	0.5ml
Sample	0.5ml	-	-
Buffer	2.0ml	2.0ml	2.0ml
Reductant	0.1ml	0.1ml	0.1ml
Standard	-	0.5ml	-
The contents of the cuvettes were mixed, and read initial absorbance against blank			
Chromogen	0.1ml	0.1ml	0.1ml

2. The contents of the cuvettes were mixed and incubated for 5 minutes at room temperature
3. The final absorbance was read against the blank.
4. Initial absorbance was subtracted from the final absorbance to give the actual absorbance of sample or standard.
5. Calculation of the concentration was done as:

$$\text{Conc} = \frac{A_{Sample}}{A_{Std}} \times \text{conc. of standard}$$

3.10 GLOMERULAR FILTRATION RATE (GFR)

Glomerular filtration rate was calculated for each patient using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) method.

3.11 STATISTICAL ANALYSIS

Statistical analysis was done using Stata data analysis and statistical software (version 11.1, by College Station, Texas, USA). Results are expressed as means ± standard deviation. Unpaired Students t tests were used for comparisons between the groups. A P value of <0.05 was considered to be statistically significant.

CHAPTER FOUR
RESULTS

4.1 Socio-demographic and Anthropometric parameters

In all, 153 participants were recruited for the study. 52 (33.99%) were normoalbuminuric (controls), and 101 (66.01%) had microalbuminuria or overt nephropathy (patients). The mean age of the control group was 56.9 years while that of the patients group was 57.5 years. The study patients group had 76 (75.2%) females and 25 (24.8%) males. The demographic and anthropometric characteristics of the study population are shown in Table 1.

Table 1. A comparison of demographic and anthropometric characteristics of participants with normoalbuminuria (controls) vs microalbuminuria (study patients)

PARAMETERS	NORMOALBUMINURIC	MICROALBUMINURIC	P- VALUE
n	52	101	
Demographics			
Gender M/F	10/42	25/76	
Age(years)	56.9 (8.85)*	57.5 (9.6)	0.35
Anthropometry			
Weight(kg)	67.0 (12.3)	66.8 (14.2)	0.46
Height(m)	1.60 (0.07)	1.62 (0.08)	0.07
BMI	26.2 (4.5)	25.6 (5.5)	0.23
Arm(cm)	31.5 (4.5)	31.3 (4.5)	0.40
Wrist (cm)	16.7 (1.5)	17.6 (10.3)	0.25
Waist (cm)	93.6 (11.1)	93.0 (11.6)	0.38

Hip (cm)	98.2 (10.4)	97.2 (9.7)	0.29
WHR	1.0 (0.08)	1.0 (0.07)	0.44
Thigh(cm)	52.0 (8.0)	51.8 (6.6)	0.44
Systolic BP	134.5 (17.3)	146.4 (23.7)	**0.0008**
Diastolic BP	81.2 (9.1)	83.2 (11.5)	0.14
Treatment			
OHA	41	79	
Insulin+ OHA	8	21	
Insulin only	3	1	
Duration			
0-5 years	20	29	
6-10 years	20	36	
11-15 years	8	22	
16-20 years	4	8	
> 20 years	0	6	
Mean Duration	7.4 (4.8)	9.2 (5.8)	**0.03**

WHR=waist/hip ratio, BP= blood pressure, OHA= oral hypoglycaemmic agents, *= mean (standard deviation)

The microalbuminuric (patient) group had a mean disease duration of 9.2 years which was significantly higher than that of the control group (7.4 years).

120 (78.43%) of the study participants use oral hypoglycaemic agents (OHA), 29 (18.95%) were on insulin and OHA while 4 (2.61) were on insulin treatment alone. In the anthropometry, only systolic blood pressure was found to be significantly raised in the microalbuminuric group as compared to the control group. 64 (63.37%) of the microalbuminuric patients had systolic hypertension (SBP ≥140) while 74 (73.27%) had diastolic hypertension (DPB ≥80).

4.2 Haematological and Biochemical Indices

The haematological and biochemical indices of the participants are shown in Table 2.

Table 2. A comparison of the haematological and biochemical indices of participants with normoalbuminuria (controls) vs microalbuminuric (patients).

PARAMETERS	NORMOALBUMINURIC	MICROALBUMINURIC	P-VALUE
n	52	101	
Haematology			
Hb (g/dl)	12.5 (1.4)	12.3 (1.6)	0.20
RBC (10^{12}/L)	5.5 (5.4)	4.7 (0.6)	0.08
PCV (%)	37.7 (6.0)	37.5 (4.6)	0.38
MCV (fL)	81.8 (5.7)	80.3 (6.9)	0.09
MCH (pg)	26.7 (1.9)	26.5 (2.5)	0.31
MCHC (gldL)	32.6 (1.0)	32.9 (1.4)	0.05
WBC (10^9/L)	5.7 (1.9)	6.3 (1.7)	**0.04**
Neutroph (10^9/L)	2.5 (1.5)	3.0 (1.1)	**0.006**
Lympho (10^9/L)	2.5 (0.8)	2.5 (0.8)	0.47
Mono (10^9/L)	0.5 (0.2)	0.5 (0.2)	0.20
Eosino (10^9/L)	0.2 (0.2)	0.2 (0.2)	0.43
Basoph (10^9/L)	0.04 (0.04)	0.03(0.02)	0.20
Platelets (10^9/L)	251.8 (96.5)	261.7 (82.8)	0.25
ESR (mmfall/hr)	34.7 (30.3)	41.4 (32.3)	0.11
Serum Chemistry			
FBS (mmol/l)	8.6 (4.7)	10.7 (4.0)	**0.003**
Serum Total Protein (g/L)	76.7(10.7)	86.0 (10.3)	**<0.0001**
Serum Albumin (g/L)	43.3 (3.5)	45.6 (4.4)	**0.0009**

Globulins (g/L)	33.3 (9.1)	40.3 (8.7)	**<0.0001**
Total Choles. (mmol/L)	4.8 (0.9)	5.4 (1.3)	**0.005**
Triglycerides (mmol/L)	1.61 (0.8)	2.1 (3.1)	0.13
LDL (mmol/L)	3.0 (0.8)	3.4 (1.1)	**0.02**
VLDL (mmol/L)	0.74 (0.3)	0.83 (0.3)	0.06
HDL (mmol/L)	1.1 (0.4)	1.1 (0.3)	0.19
Urea (mmol/L)	3.61(1.4)	4.7 (2.4)	**0.002**
Creatinine (umol/L)	111.8 (39.3)	174.7 (208.30	**0.02**
Sodium (mmol/L)	139.3 (10.2)	148.0 (15.2)	**0.0001**
Potassium (mmol/L)	4.3 (0.7)	4.5(0.8)	0.12
Chloride (mmol/L)	105.0 (9.3)	112.7 (15.1)	**0.0005**
Urea:Creatinine	37.8 (31.2)	30.9 (10.3)	**0.02**
GFR	66.5 (33.3)	46.8 (20.6)	**<0.001**
Serum Vitamin B12 (ng/L)	402.0 (194.6)	342.9 (148.2)	**0.02**
Folic Acid (ug/L)	10.6 (6.5)	9.8 (6.8)	0.25
Iron (ng/ml)	9.6 (6.3)	12.8 (7.0)	**0.005**
Ferritin (ng/ml)	113.1 (93.5)	124.1 (137.4)	0.31

In the haematological analysis, total white cell count and neutrophil count were found to be significantly higher in the study patients group. Of the 76 microalbuminuric females, 25 (32.89%) were anaemic (Hb <11.5) and 51 (67.11%) had normal Hb levels. 10 (40%) of the males had anaemia (Hb<12.5) and 15 (60%) had normal Hb levels.

Fasting blood sugar, total protein, albumin, globulins, total cholesterol and LDL cholesterol were all increased significantly in the microalbuminuric group as compared with the control group.

Iron, urea, creatinine, sodium and chloride were also found to be raised in the group. However GFR and serum vitamin B12 were lower in the microalbuminuric group.

4.3 Stratification of study patients by eGFR

When the study group was stratified by eGFR levels, it was observed that 53.85% of controls had GFR below 60 i.e. CKD (stage 3), while 81.19% of the microalbuminuric patients had GFR below 60.

As shown in table 3, it was observed that wrist, waist and hip circumferences were significantly reduced in the microalbuminuric group with GFR < 60, while systolic blood pressure was significantly higher among them.

Table 3. Anthropometric profile of study patients stratified by GFR

PARAMETER	GFR ≥60	GFR <60	P-VALUE
n	19	82	
Gender M/F	15/4	61/21	
Weight (kg)	67.6 (12.1)	66.6 (14.8)	0.40
Height (m)	1.61 (0.07)	1.6 (0.08)	0.39
BMI	26.1 (5.4)	25.4 (5.6)	0.30
Arm (cm)	31.4 (5.7)	31.3 (4.2)	0.47
Wrist (cm)	22.2 (23.5)	16.6 (1.3)	**0.02**
Waist (cm)	97.5 (10.4)	92.0 (11.7)	**0.03**
Hip (cm)	102.4 (9.3)	96.0 (9.5)	**0.004**
Thigh (cm)	51.1 (8.2)	52.0 (6.2)	0.3
Systolic BP	134.1 (22.5)	149.3 (23.2)	**0.006**
Diastolic BP	82.1 (10.8)	83.4 (11.7)	0.33

Table 4 shows that while total red cell count was significantly reduced in those with GFR <60, their mean cell volume, mean cell haemoglobin, neutrophil count and basophile count were significantly increased as compared with those whose GFR was above 60.

All the females with GFR >60 had normal haemoglobin levels, whilst of the females with GFR <60, 25 had anaemia and 36 had normal haemoglobin levels.

3 of the males with GFR >60 had anaemia while 1 had normal Hb. 7 of the males with GFR< 60 had anaemia whilst 14 had normal haemoglobin levels.

Table 4. Haematological profile of patients stratified by GFR

PARAMETER	GFR ≥60	GFR<60	P-VALUE
HB (g/dL)	12.6 (0.92)	12.3 (1.8)	0.23
RBC (10^{12}/L)	5.1 (0.7)	4.6 (0.60	**0.0009**
PCV (%)	38.3 (3.2)	37.3 (4.9)	0.19
MCV (fL)	76.3 (8.9)	81.2 (6.1)	**0.002**
MCH (pg)	25.1 (3.0)	26.8 (2.3)	**0.003**
MCHC (g/dL)	32.9 (1.0)	33.0 (1.5)	0.38
WBC (10^9/L)	5.7 (1.5)	6.4 (1.7)	0.06
Neutro (10^9/L)	2.5 (1.0)	3.1 (1.1)	**0.01**
Lymph (10^9/L)	2.5 (0.8)	2.5 (0.8)	0.50
Mono (10^9/L)	0.5 (0.2)	0.5 (0.2)	0.23
Eosin (10^9/L)	0.2 (0.3)	0.2 (0.2)	0.49
Basophil (10^9/L)	0.02 (0.02)	0.03 (0.02)	**0.03**
Platelet (10^9/L)	245.3 (73.5)	265.5 (84.7)	0.17
ESR (mmfall/hr)	30.4 (26.5)	41.6 (29.7)	0.06

From the biochemical indices (table 5), it was observed that in the group with GFR <60, total protein, albumin and globulin were significantly higher as were urea, creatinine, sodium and chloride. Urea/Creatinine ratio was found to be significantly lower in the group.

Table 5. Biochemical profile of patients stratified by eGFR

PARAMETER	GFR ≥60	GFR <60	P-VALUE
FBS (mmol/l)	11.8 (3.1)	10.4 (4.1)	0.09
Total Protein (g/L)	77.8 (10.6)	87.9 (9.3)	**<0.0001**
Albumin (g/L)	43.8 (3.7)	46.0 (4.5)	**0.03**
Globulins (g/L)	34.1 (11.1)	41.7 (7.4)	**0.0002**
Total Chol (mmol/l)	5.4 (1.5)	5.4 (1.3)	0.47
Trigly (mmol/l)	1.8 (0.6)	2.2 (3.4)	0.29
LDL Chol (mmol/l)	3.4 (1.3)	3.4 (1.0)	0.45
VLDL Chol (mmol/l)	0.8 (0.3)	0.8 (0.4)	0.30
HDL Chol (mmol/l)	1.2 (0.4)	1.1 (0.3)	0.41
Urea (mmol/l)	3.4 (0.9)	5.0 (2.5)	**0.003**
Creatinine (umol/l)	92.4 (22.4)	193.8 (227.2)	**0.02**
Sodium (mmol/l)	142.3 (7.0)	149.3 (16.3)	**0.04**
Potassium (mmol/l)	4.4 (0.5)	4.5 (0.8)	0.35
Chloride (mmol/l)	101.3 (6.6)	115.3 (15.3)	**0.0001**
Urea/Creatinine	38.1 (13.5)	29.2 (8.7)	**0.0003**
Vitamin B12 (ng/L)	376.8 (126.6)	335.0 (152.4)	0.13
Folic acid (ug/L)	10.2 (6.7)	9.7 (6.8)	0.38
Iron (ng/ml)	11.7 (4.0)	13.0 (7.4)	0.25
Ferritin (ng/ml)	112.7 (62.4)	126.3 (148.0)	0.36

An ANOVA comparison of the anthropometric parameters of the various stages of GFR (Table 6) showed that systolic blood pressure consistently increased significantly down the stages. Diastolic blood pressure significantly increased from stages 1 through 3, decreased at stage 4 and increased again at stage 5.

Table 6. Anthropometric characteristics of microalbuminuric patients stratified by stages of GFR

	GFR STAGES						
PARAMETERS	**1**	**2**	**3**	**4**	**5**	**F**	**P-VALUE**
n	2	17	63	16	3		
Weight (kg)	63	68.12	65.41	70.94	69	0.57	N/S
Height (m)	1.51	1.63	1.61	1.65	1.66	2.20	N/S
BMI	27.59	25.98	25.30	25.91	25.19	0.14	N/S
ARM (cm)	33.00	31.21	31.07	32.44	30.17	0.41	N/S
WRIST (cm)	16.25	22.85	16.49	16.91	17.17	1.34	N/S
WAIST (cm)	101	97.12	90.98	97.31	85	2.26	0.06
HIP (cm)	103	102.35	95.36	98.69	95	2.17	0.07
THIGH (cm)	54.5	50.65	51.45	54.25	51	0.82	N/S
Systolic BP	130	134.59	146.70	151.25	193.33	5.12	**0.0009**
Diastolic BP	80	82.35	83.17	80.63	103.33	2.75	**0.03**

In the haematological profile, haemoglobin concentration and packed cell volume (PCV) decreased significantly down the stages of GFR. There were also significant changes in the total red cell count and mean cell volume (MCV) across the stages. Neutrophil count significantly increased from stage 1 to 4 and decreased at stage 5 GFR. This is shown in table 7.

Table 7. Haematological indices of microalbuminuric patients stratified by GFR stages

	GFR STAGES						
PARAMETERS	**1**	**2**	**3**	**4**	**5**	**F**	**P-VALUE**
n	2	17	63	16	3		
Hb (g/dl)	12.55	12.58	12.49	11.84	9.67	2.78	**0.03**
RBC (10^{12}/L)	4.76	5.13	4.66	4.83	3.56	5.61	**0.0004**
PCV (%)	38.65	38.24	37.96	35.83	30.17	2.93	**0.02**
MCV (fL)	82.0	75.64	81.33	80.09	85.73	3.01	**0.02**
MCH (pg)	26.6	24.9	26.86	26.48	27.4	2.24	N/S
MCHC (g/dl)	32.45	32.92	33.03	32.93	32.0	0.46	N/S
WBC (10^9/L)	5.35	5.76	6.25	6.90	6.09	1.10	N/S
Neutro (10^9/L)	1.64	2.61	3.03	3.55	2.88	2.54	**0.04**
Lymph (10^9/L)	2.96	2.48	2.52	2.64	2.206	0.32	N/S
Mono (10^9/L)	0.55	0.47	0.50	0.57	0.50	0.59	N/S
Eosin (10^9/L)	0.18	0.17	0.18	0.11	0.42	1.05	N/S
Baso (10^9/L)	0.03	0.02	0.03	0.04	0.04	1.00	N/S
Platelet (10^9/L)	248.50	244.94	262.06	282.25	248	0.44	N/S
ESR	33	30.06	40.03	48.38	69.33	1.46	N/S

There were significant differences in the mean total protein and globulin levels across the stages. Table 8 shows that mean urea, creatinine, sodium and chloride levels increased significantly down the stages of GFR, while the other biochemical indices did not show any significant changes.

Table 8. Biochemical parameters of microalbuminuric cases stratified by GFR stages

	GFR STAGES						
PARAMETERS	**1**	**2**	**3**	**4**	**5**	**F**	**P-VALUE**
n	2	17	63	16	3		
FBS (mmol/L)	10.85	11.86	10.82	9.58	6.97	1.37	N/S
Total Prot (g/l)	74.5	78.24	87.46	90.5	84.3	4.78	**0.0015**
Albumin (g/l)	41.5	44.12	46.23	45.94	42.33	1.68	N/S
Globulin (g/l)	33.00	34.18	41.02	44.56	42.00	3.95	**0.005**
Tot Chol (mmol/L)	4.75	5.43	5.34	5.55	5.43	0.2	N/S
Trig (mmol/L)	2.50	1.68	2.29	1.93	1.8	0.16	N/S
LDL (mmol/L)	2.75	3.44	3.36	3.52	3.67	0.29	N/S
VLDL (mmol/L)	1.1	0.75	0.83	0.88	0.83	0.57	N/S
HDL (mmol/L)	0.85	1.19	1.14	1.16	0.97	0.78	N/S
Urea (mmol/L)	3.05	3.4	4.17	6.62	13.63	37.82	**<0.0001**
Creat (umol/L)	50.5	97.35	141.86	231.13	1085.33	40.81	**<0.0001**
Sodium (mmol/L)	141.00	142.41	147.71	150.81	174.00	3.28	**0.01**
Potassium(mmol/L)	4.45	4.44	4.45	4.54	5.73	2.21	N/S
Chloride (mmol/L)	100.00	101.47	112.56	124.44	124	6.83	**0.0001**
Vit. B 12 (ng/l)	455	367.65	341.35	310	335	0.6	N/S
Folic acid (ug/l)	6.9	10.58	9.89	8.19	12.87	0.52	N/S
Iron (ng/ml)	9.13	11.88	12.05	16.00	16.40	1.38	N/S
Ferritin (ng/ml)	170	108.87	127.58	123.75	113.67	0.08	N/S

4.4 Stratification of study patients by blood pressure levels

The microalbuminuric patients were further stratified by the presence of systolic hypertension (i.e. SBP ≥ 140). In the anthropometry (table 9), patients with systolic hypertension had significantly higher weight, body mass index, arm, waist and thigh circumferences. However, when the haematological indices were compared (table 10), no significant changes were observed between those with systolic hypertension (SHTN) and those without.

Table 11 shows that in the biochemical parameters, total protein, albumin and globulin were significantly raised in the patients with systolic hypertension as were total cholesterol, LDL and VLDL cholesterol. Urea, urea/creatinine ratio, sodium and potassium were also significantly increased in the subjects with systolic hypertension. However, they had a significantly lower GFR.

Table 9. Anthropometry of microalbuminuric patients stratified by SHTN

PARAMETER	SBP < 140	SBP ≥ 140	P-VALUE
Weight (kg)	63.35	68.80	**0.03**
Height (m)	1.63	1.61	0.07
BMI	23.74	26.60	**0.006**
Arm Circum (cm)	29.65	32.30	**0.002**
Wrist (cm)	19.15	16.77	0.13
Waist (cm)	90.09	94.74	**0.03**
Hip (cm)	95.70	98.07	0.12
Thigh (cm)	49.91	52.93	**0.02**

Table 10. Haematological parameters of microalbuminuric patients stratified by SHTN

PARAMETER	SBP < 140	SBP ≥ 140	P-VALUE
Hb (g/dl)	12.45	12.25	0.28
RBC (10^{12}/L)	4.72	4.66	0.33
PCV (%)	37.78	37.26	0.30
MCV (fL)	79.96	80.53	0.35
MCH (pg)	26.53	26.45	0.44
MCHC (g/dl)	33.18	32.82	0.12
WBC (10^9/L)	6.25	6.25	0.50
Neutro (10^9/L)	3.00	3.02	0.47
Lymph (10^9/L)	2.52	2.54	0.44
Mono (10^9/L)	0.51	0.50	0.39
Eosin (10^9/L)	0.19	0.17	0.33
Baso (10^9/L)	0.03	0.03	0.16
Platelet (10^9/L)	266.92	258.67	0.32
ESR	36.17	41.25	0.20

Table 11. Biochemical parameters of microalbuminuric patients stratified by SHTN

PARAMETERS	SBP < 140	SBP ≥ 140	P-VALUE
FBS (mmol/L)	10.39	10.85	0.29
Total Protein (g/l)	82.11	88.31	**0.002**
Albumin (g/l)	44.28	46.36	**0.01**
Globulin (g/l)	37.38	41.98	**0.005**
Total Chol. (mmol/L)	4.96	5.62	**0.008**
Triglycerides (mmol/L)	2.37	1.97	0.27
LDL Chol (mmol/L)	3.08	3.57	**0.01**

VLDL Chol (mmol/L)	0.70	0.90	**0.002**
HDL Chol (mmol/L)	1.16	1.13	0.30
Urea (mmol/L)	3.83	5.18	**0.003**
Creatinine (umol/L)	141.49	193.94	0.1
Urea /Creatinine Ratio	28.48	32.27	**0.004**
Sodium (mmol/L)	142.89	150.89	**0.005**
Potassium (mmol/L)	4.31	4.61	**0.03**
Chloride (mmol/L)	111.14	113.55	0.22
GFR	51.66	44.03	**0.04**
Vit. B 12 (ng/L)	320.41	355.86	0.12
Folic acid ug/L)	10.24	9.49	0.30
Iron (ng/ml)	12.86	12.79	0.49
Ferritin (ng/ml)	103.94	134.92	0.15

The patients with microalbuminuria were further stratified by diastolic hypertension (i.e. DBP ≥ 80). When the anthropometric parameters of those with diastolic hypertension (DHTN) were compared with those without diastolic hypertension, their mean weight and BMI, as well as arm, waist and hip circumferences were found to be significantly higher (table 12). However, when the haematological parameters were compared (table 13), only ESR was significantly lower in the group with diastolic hypertension. All the other haematological parameters did not show any significant changes.

Table 14 shows a comparison of the biochemical indices of the two groups. Only potassium and vitamin B12 were significantly higher in the group with DHTN, while ferritin was significantly lower in that group.

Table 12. Anthropometry of microalbuminuric patients stratified by DHTN

PARAMETER	DBP <80	SBP ≥ 80	P-VALUE
Weight (kg)	61.74	68.65	**0.02**
Height (m)	1.62	1.62	0.6
BMI	23.53	26.29	**0.01**
Arm (cm)	29.26	32.08	**0.002**
Wrist (cm)	19.78	16.84	0.10
Waist (cm)	87.91	94.91	**0.003**
Hip (cm)	93.48	98.56	**0.01**
Thigh (cm)	50.30	52.37	0.08

Table 13. Haematological parameters of microalbuminuric patients stratified by DHTN

PARAMETER	DBP <80	DBP ≥80	P-VALUE
Hb (g/dl)	12.43	12.28	0.35
RBC (10^{12}/L)	4.72	4.66	0.36
PCV (%)	37.89	37.29	0.29
MCV (fL)	80.06	80.42	0.41
MCH (pg)	26.53	26.46	0.45
MCHC (g/dl)	33.13	32.89	0.23
WBC (10^9/L)	6.48	6.16	0.20
Neutro (10^9/L)	3.18	2.95	0.18
Lymph (10^9/L)	2.58	2.51	0.35
Mono (10^9/L)	0.51	0.50	0.41
Eosin (10^9/L)	0.18	0.17	0.44
Baso (10^9/L)	0.03	0.03	0.49
Platelet (10^9/L)	250	265.96	0.2

ESR	48.46	36.18	**0.03**

Table 14. Biochemical parameters of microalbuminuric patients stratified by DHTN

PARAMETERS	DBP < 80	DBP ≥ 80	P-VALUE
FBS (mmol/L)	10.23	10.85	0.24
Total Prot (g/l)	85.78	86.14	0.44
Albumin (g/l)	45.15	45.78	0.27
Globulin (g/l)	40.63	40.18	0.41
Total Chol. (mmol/L)	5.14	5.46	0.14
Triglycerides (mmol/L)	2.87	1.86	0.08
LDL Chol (mmol/L)	3.13	3.49	0.07
VLDL Chol (mmol/L)	0.77	0.85	0.16
HDL Chol (mmol/L)	1.17	1.13	0.28
Urea (mmol/L)	4.22	4.86	0.12
Creatinine (umol/L)	150.48	183.57	0.24
Urea /Creatinine Ratio	29.12	31.52	0.15
Sodium (mmol/L)	145.04	149.03	0.12
Potassium (mmol/L)	4.27	4.58	**0.03**
Chloride (mmol/L)	113.96	112.19	0.30
GFR	46.07	47.11	0.41
Vit. B 12 (ng/l)	299.07	358.85	**0.04**
Folic acid(ug/l)	11.59	9.10	0.05
Iron (ng/ml)	12.50	12.87	0.41
Ferritin (ng/ml)	181.52	104.11	**0.007**

4.5 Peripheral blood film comment

49 (48.5%) of patients showed a normal peripheral blood film. The commonest abnormality observed was hypochromasia which was found in 25 of the patients as compared to 14 controls. Microcytosis, red cell anisocytosis, poikilocytosis and reactive and atypical white cells were all more frequently observed in the controls than in the study patients. There was no significant correlation between microalbuminuria and blood cell abnormalities. Table 15 shows the logistic regression of the relationship between microalbuminuria and the observed blood cell abnormalities.

Table 15. Logistic regression analysis of relationship between microalbuminuria and blood cell abnormalities.

		Microalbuminuria			
Film characteristic	**N (%)**	**OR(95%CI)**	**P value**	**aOR(95%CI)**	**P value**
Hypochromasia	25(24.8)	0.9(0.4-1.9)	0.770	0.9(0.4-1.9)	0.785
Poikilocytosis	8(7.9)	1.0(0.3-3.6)	0.960	1.0(0.3-3.6)	0.951
Microcytosis	17(16.8)	1.1(0.4-2.8)	0.819	1.1(0.4-2.6)	0.837
RBC anisocytosis	17(16.)	1.3(0.5-3.4)	0.588	1.3(0.5-3.5)	0.559
WBC Reactive changes	16(16)	1.5(0.5-4.0)	0.460	1.5(0.5-4.0)	0.452
Platelate Anisocytosis	6(5.9)	0.4(0.1-1.3)	0.123	0.4(0.1-1.3)	0.126
Atypical white cells	7(7)	0.7(0.2-2.4)	0.572	0.7(0.2-2.3)	0.554

aOR-age adjusted odds ratio

CHAPTER FIVE

DISCUSSION

5.1 Socio-demography

Diabetic nephropathy is one of the commonest chronic complications of diabetes, which has been established as one of the most common causes of end stage renal disease in the developed world. The development of the complications of diabetes has been found to be duration dependent. In this study, the study patients (diabetics with microalbuminuria and overt nephropathy) were found to have had diabetes for a longer period of time compared to the control group (Table 1), a finding that is consistent with studies by Eghan *et al* (2008), Song and Hardisty (2009) and Khudair (2009) who reported a positive correlation between the duration of diabetes mellitus and the appearance of its chronic complications.

5.2 Anthropometry

Several studies have identified high blood pressure as a risk factor for the development of microalbuminuria and overt nephropathy in diabetics.

Unnikrihnan *et al* (2007) reported that both systolic and diastolic blood pressures are significantly high in patients with microalbuminuria and overt nephropathy, and thus proposed that they are risk factors for developing associated complications. This study has also established that systolic blood pressure is significantly higher in the patients with nephropathy, whereas diastolic blood pressure, though high was not significantly raised (Table 1, pg 42). Hypertension is considered to be both a cause and an effect of diabetic nephropathy, since the hyperfiltration that results from loss of functional nephrons can lead to glomerular hyperfiltration and ultimately

increase intrarenal pressure and cause systemic hypertension (Hall, 2006). On the other hand, when a person has systemic hypertension the afferent arteriole of the glomerulus may be come dilated, and this can lead to hyperfiltration and increased pressure in the glomerulus. The kidney in diabetes may not respond very positively to the renin-angiotensin system (Evans and Capell, 2000).

Furthermore, systolic blood pressure was found to be significantly high in patients with GFR < 60 mL/min/1.73 m^2 (Table 3) and both systolic and diastolic blood pressures were significantly increased as GFR worsened or decreased (table 6, pg 49). This finding is consistent with the findings of Klaq *et al* (1996) who found a strong, graded association between both systolic and diastolic blood pressure and end-stage renal disease in men. Tozawa *et al* (2003) also found that both male and females with high blood pressure or even high normal blood pressure had a significant relative risk of developing of end-stage renal disease. High blood pressure was reported to hasten the increase in albumin levels in patients with type 2 diabetes who have initially normal albumin levels and also cause an accelerated loss of renal function in those with overt nephropathy (Remuzzi *et al*, 2002).

Globally, end stage renal disease is rising alarmingly, and several researchers (Schieppati and Remuzzi 2005, Wu *et al* 2004, Lee 2003) have identified diabetic nephropathy as a leading cause of ESRD. Unfortunately, current screening strategies, based on creatinine or albuminuria, do not identify a considerable number of diabetics with CKD. Interestingly, in this study, 53.85% of controls (i.e. diabetics without nephropathy) had GFR below 60 mL/min/1.73 m^2, a finding which is consistent with studies in several parts of the world. MacIsaac *et al* (2004) reported that

some patients with type 2 diabetes do commonly progress to a significant degree of renal impairment whilst remaining normoalbuminuric. Middleton *et al*, 2006 also reported that undiagnosed CKD is common in diabetics and further recommended that incorporating eGFR into screening for CKD be more effective in identifying such patients. Early detection will enable the arrest of the natural progression of the disease as well as provide the necessary treatment.

Patients with GFR < 60 mL/min/1.73 m^2 were found to have significantly reduced waist, wrist and hip circumferences (Table 3), a finding that agrees with the Kidney Disease Outcome Quality Initiative (KDOQI) CKD Guidelines (2000) which states that as GFR falls to <50 mL/min/1.73 m^2, measurements of body mass show a decline in total mass, fat, and muscle. Kopple *et al* (2000) also found that in patients with chronic renal disease, serum and anthropometric measures of protein-energy nutritional status progressively decline as the GFR decreases.

This reduction in body mass is not unexpected because of the deleterious effects of diabetes such as reduced utilization of glucose which eventually leads to increased lipolysis. There is also increased protein catabolism in the diabetics (Boon *et al*, 2006, Ferry, 2012) Also, the renal disease in these subjects causes increased loss of protein which eventually leads to weight loss (Kopple *et al*, 2000)

Diabetic nephropathy patients who had hypertension in addition to diabetes were found to have significantly higher anthropometric indices (Table 9, pg 52). This finding of increased

anthropometric indices in diabetics with hypertension has been observed by other researchers including Tomic *et al* (2003) who established that overweight and obesity were very prevalent among type 2 diabetics. They indicated that obesity increases the risk of developing macrovascular complications and also reduces life expectancy in all age groups. The prevalence of nephropathy was also found to be independently and significantly related to BMI. With increasing obesity, they observed a significant deterioration of HbA1c and increased LDL-cholesterol, systolic and diastolic blood pressure (Tomic *et al*, 2003).

Obesity has been implicated in the development of both hypertension and cardiovascular diseases (El-Atat *et al*, 2003, Rahmouni *et al*, 2005). Salanitro and Roumie (2010) reported that the estimated prevalence of hypertension in adults with diabetes is 20–60%, which is 1.5–3 times higher than that in individuals of the same age bracket without diabetes. The onset of hypertension differs for people with type 1 compared to those with type 2 diabetes. For individuals with type 1 diabetes, hypertension is usually as a result of the increased intraglomerular pressure present in diabetic nephropathy, with 30% eventually being affected. In contrast, hypertension may be a co-morbid condition when type 2 diabetes is diagnosed or may even be present before diagnosis with hyperglycemia. Most type 2 diabetics are usually old or obese, and these already predispose them to hypertension, so it becomes difficult to assume diabetes to be the cause of hypertension in such patients. (Salanitro and Roumie, 2010).

5.3 Haematological Indices

Total white blood cell (WBC) and neutrophils were found to be significantly raised in the patients with microalbuminuria compared to the control group in this study (Table 2, pg 44).

Tong *et al* (2004) reported that an increase in WBC count, even within the normal range, was found to be associated with the development of both macro- and microvascular complications in type 2 diabetes, and further asserted that chronic inflammation, which is indicated by a raised WBC count, may play a significant part in the development of diabetic complications. Chung *et al* (2005) also reported that peripheral total WBC, monocyte, and neutrophil counts increased in parallel with the progression of diabetic nephropathy.

In this study, haemoglobin concentration decreased significantly with declining GFR (Table 7, pg 50) in agreement with findings by other researchers such as (Ijioma *et al*, 2010, LiVecchi *et al*, 2007).

In this study, patients presenting with chronic kidney disease did not show a significantly lower haemoglobin concentration (Table 4, pg 47) as found in other studies (Ezenwaka *et al*, 2008,LiVecchi *et al*, 2007). This difference may be ascribed to the small number of patients who were in stages 4 and 5 CKD in this study. Diabetics have anaemia more frequently and severely at any level of glomerular filtration rate (GFR) compared to other patients. The identified causes of anaemia in diabetics includes deficiency in erythropoietin synthesis and release due to renal damage, systemic inflammation, iron deficiency and probable drug induced factors, such as, angiotensin converting enzyme (ACE) inhibitors (Bonakdaran *et al*, 2011).

Other factors have also been subscribed to as causing anaemia in diabetics with CKD. These include gastrointestinal bleeding due to platelet dysfunction, shortened erythrocyte survival time (30–60% of the normal 120 days), and increased incidence of haemolysis due to uraemia (O'Mara, 2008).

Hermann *et al* (2004) reported that long-term metformin therapy in type 2 diabetics caused lower cobalamin, holotranscobalamin and higher homocysteine serum concentrations than control subjects, and postulated that such changes indicate a potential risk for development of vitamin B12 deficiency. In this study, patients had significantly lower Vitamin B12 levels compared to controls (Table 2, pg 44). Serum folate was also lower in the patients though not significantly (Table 2, pg 44). This corroborates findings by Pflipsen *et al* (2009) who found a 22% prevalence of metabolically confirmed B_{12} deficiency in type 2 diabetic populations. The biguanide metformin is postulated to cause a reduction in folate and vitamin B_{12} absorption and increase homocysteine levels. Increased serum homocysteine level is positively correlated with the incidence of coronary heart disease, cerebrovascular disease, and peripheral vascular disease (O'Connell, 2001).

Emerging scientific evidence has disclosed unsuspected influences between iron metabolism and type 2 diabetes. The relationship between iron and glucose has been reported to be two-edged in that iron affects glucose metabolism, and glucose metabolism impinges on several iron metabolic pathways (Fernández-Real *et al*, 2002). It has long been recognized that in conditions where iron overload is present such as haemochromatosis and conditions requiring recurrent transfusions e.g. thalassemia, the risk of diabetes is increased. Furthermore, recent studies suggest that an increase in dietary iron (as heme, mainly from meat and meat products) is linked to an increased risk of diabetes (Shah and Fonseca, 2011). Furthermore, Fernández-Real *et al*, (2002) noted that patients with β-thalassemia who had iron overload were observed to develop and progress quickly to diabetic nephropathy. Also, increased proximal tubular lysosomal iron concentration has been observed in patients with diabetic nephropathy. These observations give weight to the

assertions that genetic mutations leading to hereditary haemochromatosis appeared to predict the development of diabetic nephropathy.

Shi *et al* (2006) reported that mean serum ferritin levels increased with increasing fasting plasma glucose levels, and thus concluded that iron status and iron intake were independently associated with risk of diabetes in Chinese women. This assertion was confirmed by findings in this study, in that nephropathy patients who also had high fasting blood glucose levels also had significantly high serum iron levels (Table 2, pg 44). Serum ferritin was also higher in these patients, though not significantly (Table 2, pg 44).

These findings calls for a critical review of iron supplementation in patients with diabetic nephropathy without first assessing serum iron and ferritin levels since iron overload also has dire consequences for the patients. Serum ferritin was significantly lower in patients in this study who had diastolic hypertension (Table 14, pg 56), in contrast to findings by Sharifi *et al* (2008) and Kim *et al* (2012). The reason for this difference should be the subject of further scientific enquiry.

Peripheral blood film of study participants however did not show any significant differences between study patients and controls (Table 15, pg 57).

5.4 Biochemical Indices

Hyperglycaemia has long been identified as a risk factor for the development of microvascular complications in diabetics (Klein *et al* 1996, Evans and Capell, 2000). Patients in this study had significantly higher fasting blood sugar as compared to controls (Table 2, pg 44). Scientific evidence has proven that diabetic nephropathy is common in patients with poor metabolic control. Nephropathy is uncommon in patients with HbA_{1c} consistently <7.58.0%. The actual causative action of hyperglycaemia in renal injury is still being debated in the scientific community, but it is generally agreed that hyperglycaemia does have a deleterious effect on renal tissue.

Schena and Gesualdo (2005) stated that even where a person has all the genetic predisposition, diabetic nephropathy does not develop in the absence of sustained increased blood glucose levels. Hyperglycaemia causes several haemodynamic changes that eventually lead to the activation of protein kinase C, increased production of advanced glycosylation end products (AGEs), and diacylglycerol synthesis. Also, hyperglycaemia has been associated with such haemodynamic alterations as glomerular hyperfiltration, shear stress, and microalbuminuria (Schena and Gesualdo, 2005).

In view of current evidence on the deleterious effects of hyperglycaemia, Fioretto *et al* (2006) asserted that the most important preventive and therapeutic approach to diabetic nephropathy is improvement in glucose control. Tight glycaemic control is also very valuable in arresting the progression of diabetic nephropathy and possibly achieving some level of regression in the disease.

Several studies have found low serum albumin levels in patients with microalbuminuria and overt nephropathy. In this study, the patients had significantly higher levels of serum total protein, albumin and globulins compared to the control group (Table 2, pg 44). Cho *et al* (2012) reported that higher serum albumin level was significantly related to higher diastolic blood pressure, total cholesterol, fasting glucose, conditions that were observed in patients involved in this study (Table 2, pg 44). However, this finding should be the subject of further scientific enquiry to establish its relevance to the pathogenesis and progression of diabetic nephropathy

Dyslipidemia has been identified as a risk factor for the development and progression of diabetic renal disease (Gall *et al,* 1997, Gross *et al* 2005 and Reutens and Atkins 2011).
Diabetic nephropathy usually comes with an alteration in the lipid profile of the patient, especially presenting an elevation in triglyceride rich lipoproteins. These conditions are usually present even when the disease is in its early stages (Bonnet and Cooper, 2000).
Patients presenting with microalbuminuria and nephropathy in this study had significantly higher total cholesterol and LDL (Table 2, pg 44). Triglycerides and VLDL were also higher than in controls though not significant (Table 2, pg 44). These findings corroborate findings by Massy and Andrejak, (2006), Jisieike-Onuigbo *et al* (2011) and Shahid *et al* (2012).
Patients who had systolic hypertension in this study also had significantly higher total cholesterol, LDL and VLDL (Table 11). Shahid *et al* (2012) reported that diabetic hypertensive patients showed significantly high levels of fasting blood glucose, HbA1c, serum triglyceride, cholesterol and LDL cholesterol. Halperin *et al* (2006) also reported in a prospective cohort data that, dyslipidemias may lead to the subsequent development of hypertension.

Garg and Grundy (1990) postulated that obesity, insulin resistance and or a decrease in insulin secretion in type 2 diabetes all accelerates the excessive production of triglyceride-rich VLDL, in the presence of increased glucose and free fatty acids. Secondly, the high serum triglyceride level is probably due to the reduced lipolysis of VLDL triglycerides, most likely secondary to reduced lipoprotein lipase activity.

Diabetic nephropathy is associated with accumulation of various waste substances in the blood. Patients with kidney disease usually present with elevated levels of serum urea and creatinine. Idonije *et al* (2011) reported significantly high levels of serum urea and creatinine in type 2 diabetics. Wagle (2010) also demonstrated increased levels of creatinine in type 2 diabetics. Patients in this study had significantly higher serum urea and creatinine levels as compared to controls (Table 2, pg 44). Also serum urea and creatinine levels increased significantly as kidney function deteriorated (Tables 5 & 8, pg 48, 51). Guyton and Hall (2006) explained that many of the waste products of metabolism, such as urea and creatinine which are largely dependent on glomerular filtration for excretion, accumulate almost proportionally to the rate of destruction of the nephron as kidney disease advances. Therefore, as GFR decreases, the rate of excretion of creatinine also decreases, leading to an accumulation of creatinine in the body fluids and plasma.

Sodium retention occurs as a characteristic alteration in type I or type II diabetes. This may possibly be due to an increased glomerular filtration of glucose which causes an enhanced proximal tubular sodium-glucose co-transport and an extravascular shift of fluid with sodium, which once it occurs leads to renal failure (Shahid *et al*, 2005). Patients in this study had significantly high sodium and chloride levels as compared to controls (Table 2, pg 44), with

sodium levels significantly increasing as renal function deteriorates (Tables 5 & 8, pg 48, 51). Potassium though high was not significantly different from the control group (Table 2, pg 44). Patients with systolic hypertension also had significantly higher serum sodium concentrations (Table 11, pg 53). Shahid and Mahboob (2008) explained that in type 1 as well as type 2 diabetes mellitus, there was an average increase in exchangeable body sodium of about 10%. This abnormality develops in the uncomplicated stage of diabetes and is useful in differentiating diabetic from non-diabetic essential hypertensive patients.

Potassium levels were found to be significantly higher in patients with both systolic and diastolic hypertension (Tables 11 and 14, pg 53 & 56). This finding is in contrast with findings by Shahid *et al* (2005), who stated that in patients with type 2 diabetes and essential hypertension, potassium depletion is a common finding. This contradicting finding should be the subject of further scientific enquiry.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Diabetics who develop microalbuminuria and nephropathy have several haematological and biochemical abnormalities, and these should be assessed and analysed periodically to help effectively manage their conditions.

This study has shown that blood pressure is higher in diabetics with microalbuminuria and nephropathy.

Anaemia in patients in this study may probably be attributed to vitamin B12 and folate deficiency due to metformin intake. Iron deficiency was not found to be a problem in this study, as on the contrary patients had rather high iron and ferritin levels compared to subjects. Erythropoietin levels may not necessarily be low in the patients in this study since their haemoglobin levels were not significantly lower than that of the control group. However, haemoglobin levels decreased significantly as GFR decrease, a finding that could be due to falling erythropoietin levels with advancing renal disease. Therefore, it should be the subject of further scientific enquiry to determine erythropoietin levels in diabetics with microalbuminuria.

Total WBC and neutrophil levels may be of significance in raising suspicion of the development of diabetic complications as patients in this study had significantly higher levels compared to controls.

This study has revealed that microalbuminuria is a very sensitive and early predictor of diabetic nephropathy, and should be routinely assayed for diabetics. However, this study has also revealed that some diabetics remain normoalbuminuric whilst progressing into chronic renal disease and ultimately end stage renal disease. Such patients can only be identified if their GFR is estimated routinely.

Microalbuminuria is associated with increased levels of a number of biochemical indices as revealed by this study. Blood glucose, serum total protein, serum lipids, urea, creatinine and sodium all tend to be higher in patients with microalbuminuria. Therefore, clinicians who see these findings in their patients should request further investigations to assess renal function.

6.2 RECOMMENDATIONS

- Most of the complications of diabetes appear with increasing duration of disease, and as such it is of outmost importance that the longer a patient has diabetes, the more comprehensive the care of such patients should be. A conscious effort should be made to detect and manage complications.
- Caregivers should advice and encourage diabetics to comply with their treatment regimen since uncontrolled blood glucose can lead to developing complications.
- Given the worldwide increasing prevalence of both diabetes and hypertension, and the association of renal complications with both disorders, it is important to detect renal disease promptly–through screening for microalbuminuria. It is recommended that as a matter of urgency, screening for microalbuminuria should be made a standard protocol in the management of diabetics in Komfo Anokye Teaching Hospital.

- Iron and its indices have been found to be positively associated with diabetes. We recommend that diabetics who develop nephropathy should be screened for serum levels of iron and its indices before been given iron supplements to prevent iron overload.
- The serum Vitamin B12 and folate concentrations of diabetics should be assessed periodically, since this study has revealed that serum Vitamin B12 and folate levels decrease the longer the patient has been on diabetic drugs, particularly, metformin.
- A large number of diabetics progress to renal failure while remaining normoalbuminuric. It is therefore strongly recommended that estimation of GFR should be done routinely (perhaps bi-annually), to be able to detect declining renal function early.
- Further scientific enquiry should be made into the prevalence and pathogenesis of this finding in the Ghanaian diabetic population.

REFERENCES

Alebiosu CO,. Ayodele OE. The increasing prevalence of diabetic nephropathy as a cause of end-stage renal disease in Nigeria. Trop Doct (2006) 36:218–9.

Almdal T, Scharling H, Jensen JS, Vestergaard H: The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20 years of follow-up. Arch Intern Med 164:1422 -1426, 2004.

Alsaad KO, Herzenberg AM. Distinguishing Diabetic Nephropathy from other causes of Glomerulosclerosis: an update. J Clin Pathol 2007 60: 18-26

America Diabetes Association: Diagnosis and Classification of Diabetes Mellitus. Diabetes Care January 2004 vol. 27 no. suppl 1 s5-s10

America Diabetes Association: Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, Volume 32, Supplement 1, January 2009

American Diabetes Association. Nephropathy in Diabetes. Diabetes Care January 2004 vol. 27 no. suppl 1 s79-s83.

Arora P and Batuman V (Ed) (2011). Chronic Renal Failure Clinical Presentation, 8th August 2011. [Online] available at http://emedicine.medscape.com. [Accessed on 16.8.2011].

Arroyo AR. Electrolyte and Acid-Base Balance Disorders in advanced Chronic Kidney Disease. Nefrologia 2008; 28 Suppl 3:87-93.

Arya A, Sahil A, Harlokesh NY. Pathogenesis of Diabetic Nephropathy. International Journal of Pharmacy and Pharmaceutical Sciences, Vol 2, Suppl 4, 2010.

Batuman V(Ed) (2011). Diabetic Nephropathy Treatment and Management. 25th July, 2011. [Online] available at http://emedicine.medscape.com. [Accessed on 16.10.2011].

Bonakdaran S, Gharebaghi M, Vahedian M. Prevalence of anemia in type 2 diabetes and role of renal involvement. Saudi J Kidney Dis Transpl 2011; 22:286-90.

Bonnet M and Cooper ME. Potential influence of lipids in diabetic nephropathy: insights from experimental data and clinical studies. Diabetes & Metabolism. Sept 2000. Vol 26 no. p 254.

Boon et al (2006). Davidson's Principles and Practice of Medicine. 20th Edition. USA. Churchill Livingstone.

Boyle PJ: Diabetes mellitus and macrovascular disease: mechanisms and mediators. Am J Med 120: S12-S17,2007.

Cao Z, Cooper M E. (2011), Pathogenesis of diabetic nephropathy. Journal of Diabetes Investigation, Volume 2, Issue 4, pages 243–247, August 2011

Cass A., Chadban S., Craig J., Howard H., McDonald S., Salkeld G., & White S. The Economic Impact of End-Stage Kidney Disease in Australia. Kidney Health Australia, Melbourne, 2006.

Centers for Disease Control and Prevention. National Chronic Kidney Disease Fact Sheet: General Information and National Estimates on Chronic Kidney Disease in the United States, 2010. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2010.

Chiarelli F, Verrotti A, Mohn A, Morgese G. The Importance of Microalbuminuria as an Indicator of Incipient Diabetic Nephropathy: Therapeutic Implications. Annals of Medicine 29: 439-445.1997.

Cho HM, Kim HC, Lee J-M, Oh SM, Choi PD and Suh I. The Association between Serum Albumin Levels and Metabolic Syndrome in a Rural Population of Korea. J Prev Med Public Health. 2012 March; 45(2): 98–104.

Chung F-M, Tsai JC-R, Chang D-M, Shin S-J, Lee Y-J. Peripheral Total and Differential Leukocyte Count in Diabetic Nephropathy. Diabetes Care July 2005 vol. 28 no. 7 1710-1717.

Craig KJ, Williams JD, Riley SG, Smith H, Owens DR, Worthings D, Cavill I, Phillips AO. Anaemia and Diabetes in the Absence of Nephropathy. Diabetes Care May 2005 vol. 28 no. 5 1118-1123.

Eghan BA, Frempong MT, Adjei-Poku M. Prevalence and Predictors of Microalbuminuria in Patients with Diabetes Mellitus: A Cross-Sectional Observational Study in Kumasi, Ghana, Ethnicity & Disease, Volume 17, Autumn 2007

El-Atat F, Aneja A, McFarlane S, Sowers J. Obesity and Hypertension. Endocrinology and Metabolism Clinics of North America. 2003, 32(4):823-54.

Ephraim RKD, Owiredu WKBA, Laing EF, Amidu N, Eghan BA, Ahenkorah L. Anaemia as a Risk Factor for Cardiovascular Disease in Patients with Chronic Kidney Disease, J. Med. Sci., 8 (8): 704-714. 2008

Evans TC and Capell P. Diabetic Nephropathy. Clinical Diabetes. 2000; 18(1):7.

Ezenwaka CE, Jones-LeCointe A, Nwagbara E, Seales D and Okali F.Anaemia and Kidney Dysfunction in Carribean Type 2 Diabetic Patients. Cardiovascular Diabetology 2008, **7**:25.

Fauci et al (2008). Harrison's Principles of Internal Medicine. 17th Edition. United States of America. The McGraw Hill Company.

Fernández-Real JM, Lopez-Bermejo A and Ricart W. Cross-Talk between Iron Metabolism and Diabetes. Diabetes August 2002 vol. 51 no. 8 2348-2354.

Ferry R. Diabetes; Causes, Symptoms and Treatment. [Online] available at http://www.emedicinehealth.com/diabetes. [Accessed on 13.06.2012].

Fioretto P, Bruseghin M Berto I, Gallina P, Manzato E and Mussap M. Renal Protection in Diabetes: Role of Glycaemic Control. JASN April 2006 vol. 17 no. 4 suppl 2 S86-S89.

Gall M-A, Hougaard P, Borch-Johnsen K, Parving H-H. Risk Factors for Development of Overt and Incipient Diabetic Nephropathy in Patients with NIDDM; Prospective, Observational Study. BMJ 314 : 783 (Published 15 March 1997)

Garg A and Grundy SM. Management of Dyslipidemia in NIDDM. Diabetes Care February 1990 vol. 13 no. 2 153-169.

Gross LJ, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic Nephropathy; Diagnosis, Prevention and Treatment. Diabetes Care January 2005 vol. 28 no. 1 164-176.

Guyton AC and Hall JE (2006). Textbook of Medical Physiology. 11th Ed. USA. Elsevier Saunders.

Hall PM. Prevention of Progression in Diabetic Nephropathy. Diabetes Spectrum January 2006 vol. 19 no. 1 18-24.

Halperin RO, Sesso HD, Ma J, Buring JE, Stampfer MJ and Gaziano MJ. Dyslipidemia and the Risk of Incident Hypertension in Men. Hypertension. 2006; 47: 45-50.

Harris MI. Undiagnosed NIDDM; clinical and public health issues. Diabetes Care 1993; 16**:** 642-52.

Hermann LS, Nilsson B and Wettre S. Vitamin B12 Status of Patients Treated with Metformin: a Cross-Sectional Cohort Study. Br J Diabetes Vasc Dis 2004; 4:401–06.

Hermann LS, Nilsson B and Wettre S. Vitamin B12 Status of Patients treated with Metformin; a Cross-sectional Cohort Study. British Journal of Diabetes and Vascular Disease. 2004; 4(6):401-406.

Hossain P, Kawar B, El Nahas M, Obesity and Diabetes in the Developing World- A Growing Challenge. N Engl J Med 2007; 356:213-215.

Humphrey ARG, McCarty DJ, Mackay IR, Rowley MJ, Dwyer T, Zimmet P. Autoantibodies to glutamic acid decarboxylase and phenotypic features associated with early insulin treatment in individuals with adult-onset diabetes mellitus. Diabetic Med 1998; 15**:** 113-19.

Idonije BO, Festus O and Oluba OM. Plasma Glucose, Creatinine and Urea Levels in Type 2 Diabetics Attending A Teaching Hospital.

Ijioma C, Ulasi I, Ijioma U and Ifebunandu N. High Prevalence of Anaemia in Predialysis patients in Enugu, Nigeria. Nephrology Reviews 2010; 2:e14.

International Diabetes Federation: Diabetes Declaration and Strategy for Africa: A call to action. Cape Town, 2006.

Jisieike-Onuigbo NN, Unuigbe EI, Kalu OA, Oguejiofor CO, Onuigbo PC Prevalence of dyslipidemia among adult diabetic patients with overt diabetic nephropathy in Anambra state south-east Nigeria. Niger J Clin Pract. 2011 Apr-Jun;14(2):171-5.

Jyoti D and Purnima DS. Oxidative Stress with Homocysteine, Lipoprotein (A) and Lipid Profile In Diabetic Nephropathy. International Journal of Applied Biology and Pharmaceutical Technology, Vol 1(3): 840-846, 2010.

Khudhair SA. Prevalence of Diabetic Complications in Relation to Duration and Control of Diabetes Mellitus. Thi-Qar Medical Journal (TQMJ): Vol(3) No(1):2009(67-70).

Kidney Disease Outcome Quality Initiative. Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. [Online] available at http://www.kidney.org/professionals/kdoqi/guidelines_ckd. [Accessed on 6.6.2012]

Kim MK, Baek KH, Song KH, Kang MI, Choi JH, Bae JC, Park CY, Lee WY, Oh KW. Increased Serum Ferritin Predicts the Development of Hypertension Among Middle Aged Men. Am J Hypertens. 2012 Apr;25(4):492-7.

Klaq MJ, Whelton PK, Randall BL, Neaton JD, Brancati FL, Ford CE, Shulman NB and Stamler J. Blood Pressure and End Stage Renal Disease in Men. N Engl J Med. 1996 Jan 4; 334(1):13-8

Klein R, Klein BE and Moss SE. Relation of Glycaemic Control to Diabetic Microvascular Complications in Diabetes Mellitus. Ann Intern Med. 1996 Jan 1; 124(1 Pt 2):90-6.

Kopple JD, Greene T, Chumlea WC, Hollinger D, Maroni BJ, Merrill D, Scherch LK, Schulman G, Wang SR, Zimmer GS. Relationship between Nutritional Status and Glomerular Filtration Rate: Results from the MDRD Study. Kidney Int. 2000 Apr;57(4):1688-703

Kumar P., Clark M. (eds) (2005). Kumar and Clark's Clinical Medicine. 6th ed. Edinburg: W B Saunders Ltd.

Laing SP, Swerdlow AJ, Slater SD, Burden AC, Morris A, Waugh NR, Gatling W, Bingley PJ, Patterson CC: Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. Diabetologia 46 : 760-765,2003.

Larijani B, Javadi E, Shafaee A, Mahmoudi M, Baradar-Jallili R, Hemati P. Screening For Microalbuminuria In The Early Detection Of Diabetic Nephropathy: A Cheap And Simple Method. Acta Medica Iranica, Vol 40, No 2 (2002)

Lee G. End-stage renal disease in the Asian-Pacific region. Semin Nephrol 2003;23:107-114

Little AA, Edwards LJ, Feldman LE. Diabetic neuropathies. Pract Neurol 2007;7:82-92.

LiVecchi M, Fuiano G, Francesco M, Mancuso D, Faga T, Sponton A, Provenzano R, Andreucci M, Tozzo C: Prevalence and severity of anemia in patients with type 2 diabetic nephropathy and different degrees of chronic renal insufficiency. Nepron Clin Pract 105:c62 –c67, 2007

Lorrenzo SV and Torregrosa V. Changes in Mineral Metabolism in Stages 3, 4 and 5 Chronic Kidney Disease not on Dialysis. Nefrologia 2008; 28 Suppl 3:67-78.

Low J, Smith G, Burns A, Jones L. The Impact of End Stage Kidney Diseases on Close Persons: A Literature Review. NDT Plus (2008) 1 (2): 67-79.

MacIsaac RJ, Tsalamandris C, Panagiotopoulos S, Smith TJ, McNeil KJ, and Jerums G. Nonalbuminuric Renal Insufficiency in Type 2 Diabetics. Diabetes Care January 2004 vol. 27 no. 1 195-200.

Massy ZA and Andréjak M. Management of Dyslipideamia in Diabetic CKD Patients. Nephrol Ther. 2006 Jan;2 Suppl 1:S49-52.

Mbanya J-C, Sobngwi E. Diabetes microvascular and macrovascular disease in Africa: Journal of Cardiovascular Risk: April 2003 - Volume 10 - Issue 2 - pp 97-102

Mehdi U and Toto RD. Anaemia, Diabetes and Chronic Kidney Disease. Diabetes Care, 32(7), 2009.

Middleton RJ, Foley RN, Hegarty J, Cheung CM, McElduff P , Gibson MJ , PKalra PA , O'Donoghue DJ and New JP. The Unrecognized Prevalence of Chronc Kidney Disease in Diabetes. Nephrol. Dial. Transplant. (January 2006) 21 (1): 88-92.

Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease, with emphasis on the stage of incipient diabetic nephropathy. Diabetes. 1983 May;32 Suppl 2:64-78.

Mogensen CE, Christensen CK: Predicting diabetic nephropathy in insulin-dependent patients. N Engl J Med 311:89– 93, 1984.

Molitch ME, DeFronzo RA, Franz MJ, et al. Nephropathy in diabetes. Diabetes Care 2004; 27(suppl 1):S79–S83.

Mooy JM, Grootenhuis PA, de Vries H, Valkenburg HA, Bouter LM, Kostense PJ *et al*. Prevalence and determinants of glucose intolerance in a Dutch population. The Hoorn Study. *Diabetes Care* 1995; 18**:** 1270-73.

Morrish NJ, Stevens LK, Head J, et al. A prospective study of mortality among middle-aged diabetic patients (the London Cohort of the WHO Multinational Study of Vascular Disease in Diabetics) I: causes and death rates. Diabetologia 1990; 33:538–541.

Nathan DM. Long Term Complications of Diabetes Mellitus. N Engl J Med 1993; 328:1676-1685; June 10, 1993.

Navarro-González JF, Mora-Fernández C. The Role of Inflammatory Cytokines in Diabetic Nephropathy. J Am Soc Nephrol 19: 433-442, 2008.

Nayak SB, Duncan H, Lalloo S, Maraj K, Matmungal V, et al. Correlation of microalbumin and sialic acid with anthropometric variables in type 2 diabetic patients with and without nephropathy. Dovepress Journal March 2008, Vol 2008:4(1)**;** 243 – 247.

O'Connell BS. Select Vitamins and Minerals in the Management of Diabetes. Diabetes Spectrum August 2001 vol. 14 no. 3 133-148.

O'Mara NB. Anaemia in Patients with Chronic Kidney Disease. Diabetes Spectrum January 2008 vol. 21 no. 1 12-19.

Onovughakpo-Sakpa OE, Onyeneke EC, Olumese EF. Incidence of diabetic nephropathy in Southern Nigeria. J. Med. Sci., 9 (6): 264-269, 2009.

Owiredu WKBA, Ephraim RKD, Amidu N, Eghan BA, Quaye L. Predictive Performance of Renal Function Equations Among Ghanaians Presenting With Chronic Kidney Disease. J. Med. Sci 8 (5): 491-497.

Parving HH, Oxenboll B, Svendsen PA, Christiansen JS, Andersen AR: Early detection of patients at risk of developing diabetic nephropathy: a longitudinal study of urinary albumin excretion. Acta Endocrinol (Copenh) 100:550–555, 1982.

Parving H-H, Tarnow L, Rossing P. Genetics of Diabetic Nephropathy. J. Am. Soc. Nephrol. 1996; 7:2509-2517.

Peralta CA, Katz R, Ix J, Fried LF, Sarnak MJ, Palmas W, Siscovick D, Levey AS, Shlipak MG. Cystatin C Identifies Chronic Kidney Disease Patients at Higher Risk For Complications. JASN January 1, 2011 vol. 22 no. 1 147-155

Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D and Topolski R. The Prevalence of Vitamin B12 Deficiency in Patients with Type 2 Diabetes: A Cross-Sectional Study. J Am Board Fam Med September-October 2009 vol. 22 no. 5 528-534.

Pylypchuk G, and Beaubien E. Diabetic Nephropathy, Prevention and Early Referral. Canadian Family Physician, March 2000; vol 4: 636-642.

Rahmouni K, Correia MLG., Haynes WG., Mark AL. Obesity-Associated Hypertension. Hypertension. 2005; 45: 9-14.

Raptis AE, Viberti G. Pathogenesis of Diabetic Nephropathy. Exp Clin Endocrinol Diabetes 2001; 109(Suppl 2): S424-S437

Remuzzi G, Schiepatti A, Ruggenenti P. Nephropathy in Patients with Type 2 Diabetes. N Engl J Med 2002; 346:1145-1151.

Reutens AT, Atkins RC. Epidemiology of Diabetic Nephropathy. Contrib Nephrol. 2011; 170:1-7. Epub 2011 Jun 9.

Salanitro AH, Roumie CL. Blood Pressure Management in Patients with Diabetes. Clinical Diabetes July 1, 2010 vol. 28 no. 3 107-114

Schelling RJ, O'Connor SA. Diabetes and the kidney. American Journal of Kidney Diseases. 2005;46 (4):766-773.

Schena FP and Gesualdo L. Pathogenetic Mechanism of Diabetic Nephropathy. JASN March 1, 2005 vol. 16 no. 3 suppl 1 S30-S33.

Schieppati A, Remuzzi G. Chronic renal diseases as a public health problem: Epidemiology, social, and economic implications. Kidney International (2005) 68, S7–S10.

Sexauer CL and Matson JR. Anaemia of Chronic Renal Failure. Ann Clin Lad Sci, 1981; 11(6): 484-487.

Shah VS and Fonseca VA. Iron and Diabetes Revisited. Diabetes Care July 2011 vol. 34 no. 7 1676-1677.

Shahib MS, Jawed M and Mahboob T. Ionic and Allied Variations in Normotensive and Hypertensive Diabetic Patients. JPMA 55: 153; 2005.

Shahid MS, Nawab NS, Shaikh R and Mahboob T. Glycemic Contol, Dyslipidemia and Endothelial Dysfunction in Co-existed Diabetes, Hypertension and Nephropathy. Pak. J. Pharm. Sci., Vol.25, No.1, January 2012, pp.123-129.

Sharifi F, Nasab MN and Zadeh HJ. Elevated Serum Concentrations in Pre-diabetic Subjects. Diabetes and Vascular Disease Research March 2008 vol. 5 no. 1 15-18

Shi Z, Hu X Yuan B, Pan X, Meyer HE and Holmboe-Ottesen G. Association between Serum Ferritin, Hemoglobin, Iron Intake and Diabetes in Adults in Jiangsu, China. Diabetes Care August 2006 vol. 29 no. 8 1878-1883.

Song SH and Hardisty CA. Early Onset Type 2 Diabetes: A Harbinger for Complications in later Years- Clinical Observations from a Secondary Care Cohort. Oxford Journals QJM (2009) 102 (11): 799-806.

Tomić M, Poljičanin T, Pavlić-Renar I, Metelko Ž. Obesity- A Risk Factor for Microvascular and Neuropathic Complications in Diabetes? Diabetologia Croatia 2003: 32-3.

Tong PC, Lee K-F, So W-Y, Ng MH, Chan W-B, Lo MK, Chan NN, Chan JC. White Blood Cell Count Is Associated With Macro- And Microvascular Complications In Chinese Patients With Type 2 Diabetes. Diabetes Care January 2004 vol. 27 no. 1 216-222.

Tozawa M, Iseki K, Iseki C, Kinjo K, Ikemiya Y and Takishita S. Blood Pressure Predicts Risk of Developing End-Stage Renal Disease in Men and Women. Hypertension. 2003; 41: 1341-1345.

Unnikrishnan R, Mohan R, Pradeepa R, Deepa M, Shanthirani CS, Deepa R, Mohan V. Prevalence and Risk Factors of Diabetic Nephropathy in an Urban South Indian Population: The Chennai Urban Rural Epidemiology Study (CURES 45). Diabetes Care August 2007 vol. 30 no. 8 2019-2024.

US Renal Disease. Depression in the End Stage Renal Disease Population on Dialysis. US Nephrology, 2007; 1(1):89-91.

Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H: Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* 1:1430 – 1432, 1982.

Wagle TJ. Genderwise comparison of serum creatinine and blood sugar levels in type 2 diabetics. Bombay Hospital J., 2010, 52: 64-68.

World Health Organization: Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. Geneva, World Health Org., 2006

World Health Organization: Definition, Diagnosis and Classification of Diabetes Mellitus: report of a WHO consultation. Geneva, World Health Org., 1999.

World Health Organization: World Health Statistics 2008, Geneva, World Health Org., 2008.

Wu AYT, Kong NCT, de Leon FA, Pan CY, Tai TY, Yeung VTF, Yoo SJ. An Alarmingly High Prevalence Of Diabetic Nephropathy In Asian Type 2 Diabetic Patients: The Microalbuminuria Prevalence (MAP) Study. Diabetologia (2005) 48: 17–26

Zelmer JL. The Economic Burden of End Stage Renal Disease in Canada. Kidney International 72, 1122-1129 (November (1) 2007.

Zimmet PZ, Tuomi T, Mackay R, Rowley MJ, Knowles W, Cohen M *et al*. Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. Diabetic Med 1994; 11**:** 299-303.

.