PREVALENCE OF MICROALBUMINURIA AMONG DIABETIC PATIENTS IN ABUTH, SHIKA ZARIA

BY

MOHAMMAD AHMAD BELLO

DEPARTMENT OF CHEMICAL PATHOLOGY FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

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BY

MOHAMMAD AHMAD BELLO
MSc/MED/43057/2004-05

BSc (ABU 1994), AMLSCN (2000),
FIMLS (2007)

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DEPARTMENT OF CHEMICAL PATHOLOGY FACULTY OF MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA

OCTOBER, 2009

DECLARATION

I solemnly declare that the work herein reported in this dissertation is original and was carried out by me. The works of other investigators are hereby gratefully acknowledged and referred to accordingly. I also solemnly declare that neither this work nor any part of it has ever been submitted elsewhere for a higher degree.

Mohammad Ahmad Bello

Date

CERTIFICATION BY THE HEAD OF DEPARTMENT

The work herein was submitted to the Department of Chemical Pathology, Faculty of Medicine, Ahmadu Bello University, Zaria and was accepted as a partial fulfilment of the requirement for the award of MASTERS DEGREE in Chemical Pathology.

_____

DR. I S ALIYU

(MBBS, FMCPath)

HEAD OF DEPARTMENT

DEPARTMENT OF CHEMICAL PATHOLOGY

AHMADU BELLO UNIVERSITY, ZARIA

CERTIFICATION

This dissertation entitled "Prevalence of Microalbuminuria among Diabetic Patients in ABUTH, Shika, Zaria" by MOHAMMAD AHMAD BELLO meets the regulations governing the award of MASTERS DEGREE of Ahmadu Bello University, Zaria and is approved for its scholarly contribution to knowledge and literary presentation.

_____ _____

EXTERNAL EXAMINER **Date**
Prof OA Afonja (MB, MD, ChB, MSc, FMCPath, FWACP)
Department of Chemical Pathology,
Lagos University Teaching Hospital,
Lagos, Nigeria.

_____ _____

Internal Examiner **Date**
Dr. PO Anaja (BSc, MSc, PhD)
Department Chemical pathology,
Ahmadu Bello University,
Zaria, Nigeria.

_____ _____

Internal Examiner **Date**
Prof. HS Isah (BSc, MSc, PhD, CChem, FRSC)
Department Chemical pathology,
Ahmadu Bello University,
Zaria, Nigeria.

_____ _____

Internal Examiner **Date**
Dr. AG Bakari (MBBS, FWACP)
Department of Medicine,
Ahmadu Bello University,
Zaria, Nigeria.

_____ _____

Dean Post Graduate School, **Date**
Ahmadu Bello University, Zaria

DEDICATION

This work is dedicated to the Family of Alhaji Mohammad Bello Sarkin Kima, and to my wife and Children. There is nothing more comforting than having a lovely and caring FAMILY.

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ABSTRACT

Diabetic nephropathy accounts for a significant reduction in life expectancy of diabetic patients. Diabetic patients with microalbuminuria have increased risk of progression to overt proteinuria and later progress to renal failure which is usually irreversible and eventually leads to death. The prevalence and risk factors of microalbuminuria are not fully described among black African diabetic patients.

The objective of the present study was to determine the prevalence of micro-albuminuria and associated factors among diabetic patients in ABUTH, Shika, Zaria.

This was a cross-sectional study of diabetic patients attending ABUTH, Zaria. Concentrations of Fasting blood glucose (FBG), serum creatinine (SCr), Urinary creatinine (UCr), and glycated haemoglobin (GHbA1c) were measured in 170 diabetic patients and 100 apparently healthy individuals. Microalbuminuria was measured using immunoturbidimetric method and was defined as Albumin-creatinine ratio ACR between 3–30 mg/mmol. Duration of diagnosis (DOD), Body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also measured, where applicable, in both patients and controls.

The prevalence of microalbuminuria among the diabetic patients in this study was 23%. Mean values in patients versus controls of BMI (26.8±0.43 vs 23.4±0.43kg/m^2, $p<0.001$), DBP (80±0.56 vs 72±0.69mmHg, $p<0.001$), FBG (6.3±0.22 vs 3.5±0.07mmol/L, $p<0.001$), UAlb (20.2±2.17 vs 8.0±0.31mg/L, $p<0.001$), GHbA1c (7.2±0.16 vs 4.9±0.07%, $p<0.001$) and ACR (2.7±0.29 vs 1.0±0.04 mg/mmol, $p<0.001$) were observed to be higher with statistically significant differences in patients than controls ($p<0.05$). Similar higher and statistically significant values were also observed in mean values of

FBG, GHbA1c, UCr, UAlb and ACR in microalbuminuric patients than normoalbuminuric patients ($p<0.05$).

Sixty one (35.9%) had good glycaemic control with 11.5% having abnormal ACR, 42 (24.7%) at borderline had 14.3% with abnormal ACR and 67(39.4%) those with poorly controlled glycaemia had 28.4% with abnormal ACR. One hundred and eighteen (69.4%) were within 0-5years of duration having 25(21%) with abnormal ACR, those within 6-10yr of duration were 37(21.8%) had 10(27%) with abnormal ACR while those with duration of ≥11years were 15(8.8%) had 6(40%) indicating linear relation with both GHbA1c and DOD. There was a positive and significant correlation between serum creatinine and age in both patients ($p=0.031$, $r=0.216$) and controls ($p=0.014$, $r=0.244$). Positive correlation was also observed between duration of diagnosis and serum creatinine among patients ($p=0.047$, $r=0.152$). ACR was found to correlate positively with GHbA1c in patients ($p=0.013$, $r=0.189$) and with BMI among controls ($p=0.031$, $r=0.216$). A reference range of 0.14-1.78 mg/mmol and clinical decision limit of 2.19 mg/mmol for microalbuminuria and of 3.43-6.39 % and a clinical decision limit of 7.13 % for glycated haemoglobin were established.

The only modifiable predictor identified in this study was poor glycaemic control for the development of microalbuminuria. Other non modifiable risk factors related to progression of microalbuminuria were age and duration of diagnosis. Aggressive glycaemic control is hereby recommended for better management and preventive measures to halt or slow the progression rate of renal involvement in diabetic patients.

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ABBREVIATIONS AND SYMBOLS USED

A	Absorbance
ABUTH	Ahmadu Bello University Teaching Hospital
ACR	Albumin-creatinine ratio
AGEs	Advance glycation end products
BMI	Body mass index
Bp	Blood pressure
^{o}C	Degree Celsius
DCCT	Diabetic control and complication trial
DM	Diabetes mellitus
DBP	Diastolic blood pressure
DKA	Diabetic ketoacidosis
ESRD	End stage renal disease
FBG	Fasting blood glucose
FDA	Food and drug agency
GHbA1c	Glycated haemoglobin A1c
GFR	Glomerulus filtration rate
GDM	Gestational diabetes mellitus
Hb	Haemoglobin
HNS	Hyperosmolar non-ketotic state
HPLC	High performance liquid chromatography
IDDM	Insulin dependent diabetes mellitus
IAA	Insulin auto-antibodies

ICA	Islet cell cytoplasmic antibodies
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
kg/m^2	Kilogram per metre square
mL	Millilitre
mmol	Millimol
mmHg	Millimetre mercury
mg/dl	Milligram per decilitre
mEq/L	Milliequivalent per litre
mg/mmol	Milligram per millimol
µmol/L	Micromole per litre
NIDDM	Non insulin dependent diabetes mellitus
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
nm	Nanometre
%	Per cent
pH	Negative log of hydrogen ion concentration
SCr	Serum creatinine
SLE	Systemic lupus erythematous
SBP	Systolic blood pressure
UKPDs	United kingdom prospective diabetes study
UCr	Urinary creatinine
UAE	Urinary albumin excretion
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Diabetes mellitus is the commonest endocrine disorder encountered in clinical practice. It is a syndrome characterised by hyperglycaemia due to an absolute or relative lack of insulin and or insulin resistance (Bakari *et al*, 2003). The term diabetes (Greek: diabētēs) was coined by Aretaeus of Cappadocia. It was derived from the Greek verb, diabaínein, meaning "to stride asunder"; hence, its derivative diabētēs meant "one that straddles," or specifically “siphon." The sense "siphon" gave rise to the use of diabētēs as the name for a disease involving the discharge of excessive amounts of urine (Mann *et al*, 1983). Diabetes was first recorded in English, in the form diabetes, in a medical text written around 1425. In 1675, Thomas Willis added the word mellitus, from the Latin meaning "honey", a reference to the sweet taste of the urine. In 1776, Matthew Dobson confirmed that the sweet taste was because of an excess of a kind of sugar in the urine and blood of people with diabetes (Mann *et al*, 1983).

The ancient Indians tested for diabetes by observing whether ants were attracted to a person's urine, and called the ailment "sweet urine disease" (Madhumeha). In medieval Persia, Avicenna (980-1037) provided a detailed account on diabetes mellitus in The Canon of Medicine, "describing the abnormal appetite and the collapse of sexual functions and he documented the sweet taste of diabetic urine." Like Aretaeus before him, Avicenna recognized primary and secondary diabetes. He also described diabetic gangrene, and treated diabetes using a mixture of lupine, trigonella (fenugreek), and

zedoary seed, which produces a considerable reduction in the excretion of sugar, a treatment which is still prescribed in modern times. Johann Peter Frank (1745-1821) first differentiated between diabetes mellitus and diabetes insipidus. The first description of the disease as an epidemic was by John in 1921, representing the beginning of a serious scientific attempt to explore the aetiology of the disease (Mann *et al*, 1983).

In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients are likely to be found by 2030. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet (Wild *et al*, 2004).

Diabetes mellitus is the commonest endocrinological disorder in Nigeria with a national prevalence rate of 2.7%. In the northern part of Nigeria; that occupies the savannah region, the prevalence of 1.6% has been reported in a suburban community (Bakari *et al*, 1999). Type II diabetes mellitus formally known as Non-insulin dependent diabetes mellitus (NIDDM) has been found to be commonest with 77% among all cases of diabetes mellitus (Onyemelukwe and Bakari 1998).

Tight glycaemic control has been shown in several studies to decrease the risk of micro-vascular disease in both type 1 and type 2 diabetes. In the DCCT, intensive glucose control in patients with type 1 diabetes decreased the incidence of microalbuminuria by

39% in the primary prevention group and decreased the progression from microalbuminuria to macroalbuminuria by 54% in the secondary prevention group (DCCT, 1993). In the United Kingdom Prospective Diabetes Study (UKPDS), there was a 34% decrease in the risk of microalbuminuria in patients with type 2 diabetes treated more intensively for glycaemic control. The benefit in type 1 diabetes was accomplished with an average 20% reduction in HbA_{1c} (9.0-7.1%), while the benefit was seen with an 11% reduction in HbA_{1c} (7.9-7.0%) in the UKPDS study group (UKPDS, 1998).

This suggests that there is benefit to be gained by decreasing hyperglycaemia at any level of starting glucose control. Furthermore, it suggests that this benefit can be attained in patients with either type 1 or type 2 diabetes mellitus.

Glycaemic control remains critical in stabilizing and slowing nephropathy even with established renal damage. As noted above, not only was primary prevention of microalbuminuria achieved by tight glycaemic control in the DCCT, but also secondary progression from microalbuminuria to macroalbuminuria was slowed (Timothy *et al*, 2000).

The kidney plays a central role in the homeostatic mechanism of the human body and reduced renal function strongly correlates with increasing morbidity and mortality. Biochemical investigations, both routine and specialised form an important part of the clinicians' diagnostic tools (David *et al*, 2007).

Diabetic nephropathy is an insidious and lethal complication of diabetes mellitus leading to renal failure in a substantial fraction of patients with diabetes mellitus (Araki *et al*, 2005). Epidemiological studies in humans suggest that the lifetime risk for development

of diabetic nephropathy is 35% (Timothy *et al*, 2000). Significant racial differences have been reported including increased incidence in Native Americans and Asian populations, indicating that differential susceptibility to diabetic nephropathy exists in humans. In patients susceptible to diabetic nephropathy, patients progressively increase urinary albumin excretion (UAE) followed by a decline of glomerular filtration rate (GFR) (Qi *et al*, 2005). These functional changes are accompanied by characteristic renal pathological features including mesangial expansion, glomerulo-sclerosis, and tubulo-interstitial fibrosis (Peter 1996).

Genetic factors have been suggested to contribute to this differential susceptibility to diabetic nephropathy in humans (Qi *et al*, 2005). Despite substantial effort, mapping of susceptibility genes in people has been hindered because of the genetic heterogeneity in human populations as well as the diversity of environmental factors including treatment (Timothy *et al*, 2000). Diabetic nephropathy accounts for end-stage renal disease (ESRD) in 40% of patients who need dialysis or renal transplantation in the U.S. and in 15–20% of patients in Italy (Romano *et al*, 2006). The early changes include kidney enlargement due to hypertrophy of all the elements of the glomerulus and this causes an increase in capillary filtration area. GFR is found to increase and also increase in tubular cell glycogen content leading to vacuolisation, the Armani-Ebstein lesion. At this time microalbuminuria is noticed (Frank 1985).

Renal disease is a relatively common micro-vascular complication of both type I and type II diabetes. Diabetic nephropathy could be defined as a term referring to any deleterious effect on kidney structure and/or function caused by diabetes mellitus. More specifically, diabetic nephropathy is thought of in stages, the first being that characterised by

microalbuminuria (30-300 mg urinary albumin per 24 hours or as albumin/creatinine ratio in men of > or = 2.5-29.9 mg/mmol, in women > or = 3.5-29.9 mg/mmol) (Farkas *et al*, 1999, Lutale *et al*, 2007). This may progress to macroalbuminuria, or overt nephropathy (>300 mg urinary albumin per 24 hours). Later still, progressive renal functional decline characterised by decreased GFR results in clinical renal insufficiency and ESRD (Timothy *et al*, 2000).

The glomerular basement membrane shows early changes such as loss of anionic charge that are associated with increased glomerular permeability and increased excretion of proteins including albumin. The combination of a metabolic derangement and the development of advanced glycated end products that cause basement membrane damage, activation of many renal cell types such as endothelial, mesangial, tubule-epithelial and increased intraglomerular blood pressure results in increased protein excretion and a progressive nephropathy (David, 1996).

Increased urinary protein excretion is the earliest clinical finding of diabetic nephropathy. The urine dipstick, however, is an insensitive marker for initial increases in protein excretion, not becoming positive until protein excretion exceeds 300 to 500 mg/day.

The demonstration that microalbuminuria is predictive of overt diabetic nephropathy has created a demand for the routine measurement of urinary albumin in diabetic patients (Rowe *et al,* 1990 and Isah *et al,* 2001) and an elevated risk of cardiovascular disease (Thorkild *et al,* 1994). It is considered to be the best predictor of high risk for development of diabetic nephropathy in both type I and II diabetes and have been documented and demonstrated in number of trials of its usefulness in intervention studies

(Parving *et al,* 2002 and Comper *et al,* 2004). Nephropathy is a common cause of morbidity and mortality in patients with diabetes mellitus (Knowles *et al,* 1974 and Maloney *et al,* 1983). Early detection therefore allows intervention aimed at preventing diabetic nephropathy (Isah *et al*, 2001 and Tobe *et al,* 2002).

In Nigeria, there is paucity of data on microalbuminuria in diabetic patients. In a comparative work done by Isah *et al* in 2001, a considerably higher value of microalbuminuria (23.3±0.6mg/L) was observed in diabetic patients than in normal individuals with a value of 14.3±0.2mg/L. In a prevalence study, Moore and Colleague in 2000, reveals 9.7% for abnormal urine albumin/creatinine ratio (UAC) in a cohort study of 1007 children and adolescents aged 10-20 years. A prevalence of 22% was reported by Parving *et al* (2002) and found an association with an increase prevalence of arterial hypertension, proliferative retinopathy, blindness, and peripheral neuropathy. In a more recent study, a prevalence of 10.6% was documented among African diabetic patients in Dar Es salaam, Tanzania (Latule *et al,* 2007). Thus, urinary excretion of albumin should be monitored routinely in patients with IDDM (Parving *et al*, 1998, Isah 2001). Microalbuminuric patients had a significantly higher risk of developing not only clinical proteinuria, but also of dying from cardiovascular mortality because atherogenesis is accelerated in diabetes and is associated with coexistence of hyperglycaemia, dyslipidaemia and hypertension. The hyperglycaemia and dyslipidaemia adversely affect the endothelium resulting to its dysfunction and injury. This may lead to release of growth factors that stimulate the proliferation of smooth muscles cells and the deposition of lipid laden foam cells (Messent *et al,* 1992). Diabetic patients with microalbuminuria

should be carefully controlled in order to prevent or to decrease deterioration of renal function due to diabetic nephropathy (Farkas *et al,* 1997).

Establishing the diagnosis of microalbuminuria requires the demonstration of a persistent elevation in albumin excretion. Fever, exercise, heart failure, and poor glycaemic control are among the factors that can cause transient microalbuminuria (David *et al,* 2007). Other factors that also cause microalbuminuria include lead poisoning, hepatitis, Kimmelsteil-Wilson- syndrome, multiple myeloma, polynephritis, polyarteritis nodosa, trichinosis, systemic lupus- erythematous (SLE), scleroderma, toxaemia of pregnancy, and psittacosis (Jacques 1990). Although the 24-hour urine collection was previously the gold standard for the detection of microalbuminuria, it has been suggested that screening can be more simply achieved by a timed urine collection or an early morning specimen to minimize changes in urine volume that occur during the day. Microalbuminuria is unlikely if the albumin excretion rate is below 20µg/min in a timed collection or if the urine albumin concentration is less than 20 to 30 mg/L in a random specimen. Higher values (particularly those just above this range) may represent false positive results, and should be confirmed by repeated measurements (David *et al*, 2007).

1.2 STATEMENT OF THE PROBLEM

Diabetes mellitus is the most common cause of end-stage renal disease (ESRD) in developed nations and an emerging cause even in developing countries today. Approximately 40% of patients with type I diabetes and 5 - 15% of patients with type II diabetes eventually develop ESRD, although the incidence is substantially higher in certain ethnic groups (Timothy *et al,* 2000).

The prevalence and risk factors of microalbuminuria are not fully described among black African diabetic patients (Lutale *et al,* 2007). Therefore, there is a need to establish these for proper diagnosis and management of our patients. Diabetic nephropathy accounts for a significant reduction in life expectancy of diabetic patients. It is the leading cause of end-stage renal disease in the Western world. In the Caucasians, the cumulative incidence of diabetic nephropathy in Type 1 and Type 2 diabetic patients has been estimated to range from 30% to 40% and is currently the leading cause of ESRD. Diabetic patients with renal damage are at higher risk of fatal and nonfatal cardiovascular events and have also become an economic issue (Ferdinando *et al,* 2006).

Extensive studies in the Western world have demonstrated that diabetic patients with microalbuminuria have increased risk of progression to overt proteinuria, and later develop progressively to renal failure which is usually irreversible and eventually leads to death. Without any intervention, approximately 80% of type1 patients with persistent microalbuminuria develop overt nephropathy after 10–15 years. Eventually 50% of these develop end stage renal failure within 10 years and 75% by 20 years. In Type 2 diabetic patients, 20–40% with microalbuminuria progress to overt nephropathy and 20 years later, approximately 20% develop end stage renal failure (Timothy *et al,* 2000).

There are racial difference in the prevalence of diabetic nephropathy and end stage renal failure. African American patients for instance have been reported to suffer greater diabetic nephropathy and kidney damage than their Caucasian counterparts (Timothy *et al,* 2000, ADA 2009).

Information on nephropathy in African diabetic populations is scarce. Available data between 1971 and 2002 shows the prevalence of microalbuminuria in Africa to vary between 26% and 57% in diabetic patients, with variable duration of the disease (Lutale *et al,* 2007). Early medical treatment and lifestyle adjustments have been shown to halt the progression from micro- to macroalbuminuria and eventually end stage renal failure (Isah *et al,* 2001 and Lutale *,* 2007). Early detection of microalbuminuria in the course of the disease is very important in the management of diabetic nephropathy. In the developing countries, this is even more important because of the financial constraints and kidney replacement therapy is seldom an option. It is therefore imperative that those who care for patients with diabetes mellitus be knowledgeable about diabetic nephropathy and attentive to its prevention, onset, progression, and treatment in their patients (Timothy *et al,* 2000).

1.3 AIMS AND OBJECTIVES

1.3.1 Main Objective:

The aim of this study is to determine the prevalence of microalbuminuria among diabetic patients in ABUTH, Zaria.

1.3.2 Specific Objectives:

The specific objectives will include:

1. To measure microalbuminuria in the diabetic patients and control subjects.

2. To establish reference ranges for microalbuminuria and glycated haemoglobin.

3. To establish the prevalence of microalbuminuria among diabetic patients in ABUTH, Shika, Zaria.
4. To measure Glycated haemoglobin A1c, Blood Glucose, Creatinine and Urinalysis in patients and controls.
5. To compare the clinical findings and biochemical results obtained in diabetic patients and controls.
6. To compare the microalbuminuria (ACR) and other analytes employed in diabetic monitoring.

CHAPTER TWO

2.0 **LITERATURE REVIEW**

2.1 **DIABETES MELLITUS**

Diabetes mellitus is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in hyperglycaemia. This leads to an acute signs of excessive urine production, resulting compensatory thirst and increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism. It is the most common endocrine disorder seen (Anochie *et al,* 2004)

The principal two idiopathic forms of diabetes mellitus are types 1 and 2 as shown in table 2.1. The term "type1 diabetes" has universally replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes (IDDM). Likewise, the term "type2 diabetes" has replaced several former terms, including adult-onset diabetes, obesity-related diabetes, and non-insulin-dependent diabetes (NIDDM) (WHO 2000).

2.2.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is characterised by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to absolute insulin deficiency. The main cause of this beta cell loss is a T-cell mediated autoimmune attack (Rother 2007). There is no known preventive measure which can be taken against type 1 diabetes(ADA 2009); it is about 5-10% of diabetes mellitus cases in North America, Europe and Nigeria (Bello-Sani *et al,* 2007). Most affected people are otherwise healthy and of a healthy

Table 2.1 **Classification of Diabetes Mellitus** (WHO 2000, ADA 2009)

I. type 1 diabetes	a. Immune mediated b. Idiopathic
II . Type 2 diabetes.	
III. Other specific types.	a. Genetic defects of b-cell function b. Genetic defects of insulin action c. Diseases of the exocrine pancreas d. Endocrinopathies e. Drug or chemical induced f. Infections g. Uncommon fruits of immune mediated diabetes h. other genetic syndromes sometimes associated with diabetes.
IV. Gestational Diabetes Mellitus	

weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children (Sacks *et al,* 2002).

2.2.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is characterised differently due to insulin resistance or reduced insulin sensitivity, combined with reduced insulin secretion. The defective responsiveness of body tissues to insulin almost certainly involves the insulin receptor in cell membranes (Gall *et al,* 1991, Sacks *et al,* 2002). In the early stage the predominant abnormality is reduced insulin sensitivity, characterised by elevated levels of insulin in the blood. At this stage hyperglycaemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. As the disease progresses the impairment of insulin secretion worsens, and therapeutic replacement of insulin often becomes necessary (Barette *et al,* 2002). A recommended targets for metabolic control in diabetes were shown in table 2.2.

Central obesity is known to predispose individuals for insulin resistance. Abdominal fat is especially active hormonally (Bakari *et al,* 2007), secreting a group of hormones called adipokines that may impair glucose tolerance. Obesity is found in approximately 55% of patients diagnosed with type 2 diabetes. Other factors include aging with about 20% of elderly patients in North America having diabetes and family history; type 2 is much more common in those with close relatives who have had it (Timothy 2000).

Table 2.2: **Recommended targets for metabolic control in diabetes (WHO 2000)**

	Good	Borderline	poor
Fasting plasma glucose (mmol/L)	4.4 - 6.1	6.2 – 7.8	>7.8
Post-prandial plasma glucose (mmol/L)	4.4 – 8.0	8.1 -10.0	>10.0
HbA1c (%)	<6.5	6.5- 7.5	>7.5
Urine glucose (%)	0	0 -0.5	>0.5
Total cholesterol (mmol/L)	<5.2	5.2 – 6.5	>6.5
HDL cholesterol (mmol/L)	>1.1	0.9 – 1.1	<0.9
Fasting triglyceride (mmol/L)	<1.7	1.7 – 2.2	>2.2
Body mass index (Kg/m^2) – Females	19 – 24	25 – 26	>26
Body mass index (Kg/m^2) – Males	20 – 25	26 – 27	>27
Blood pressure (mm Hg)	<140/90	140/90 – 160/90	>160/90
Smoking	non-smoker	pipe	cigarette

Type 2 diabetes may go unnoticed for years because visible symptoms are typically mild, non-existent or sporadic, and usually there are no keto-acidotic episodes. However, severe long-term complications can result from unnoticed type 2 diabetes, including renal failure due to diabetic nephropathy, vascular disease such as coronary artery disease, vision damage due to diabetic retinopathy, loss of sensation or pain due to diabetic neuropathy, and liver damage from non-alcoholic steato-hepatitis (Barrett *et al,* 2002).

2.2.3 Gestational Diabetes

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy (WHO 2000).

Gestational diabetes may be transient. However, untreated gestational diabetes can damage the health of the foetus or mother (Lawrence *et al*, 2008). Risks to the baby include macrosomia, congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased foetal insulin may inhibit foetal surfactant production and cause respiratory distress syndrome (David *et al,* 1996).

Hyper-bilirubinaemia may result from red blood cell destruction. In severe cases, peri-natal death may occur, most commonly as a result of poor placental profusion due to vascular impairment. Induction may be indicated with decreased placental function. A

caesarean section may be performed if there is marked foetal distress or an increased risk of injury associated with macro-somia, such as shoulder dystocia (Wikipedia 2007).

This is particularly problematic as diabetes raises the risk of complications during pregnancy, as well as increasing the potential that the children of diabetic mothers will also become diabetic in the future (David *et al,* 1996).

2.2.4 Other Types

There are several rare causes of diabetes mellitus that do not fit into type 1, type 2, or gestational diabetes as in table 2.1. Some cases of diabetes are caused by the body's tissue receptors not responding to insulin although insulin levels are normal, which is very uncommon (Sacks *et al,* 2002). Genetic mutations either auto-somal or mitochondrial can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes such as chronic pancreatitis and cystic fibrosis. Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes which is typically resolved once the hormone excess is removed. Many drugs impair insulin secretion and some toxins damage pancreatic beta cells (Sacks *et al,* 2002, Walley *et al,* 2006).

2.3 SIGNS AND SYMPTOMS

The classical triad of diabetes symptoms is polyuria, polydipsia and polyphagia, which are, respectively, frequent urination; increased thirst and consequent increased fluid intake; and increased appetite (WHO 2000). Symptoms may develop quite rapidly in

type 1 diabetes, particularly in children. However, in type 2 diabetes the symptoms develop much more slowly and may be subtle or completely absent. Type1 diabetes may also cause a rapid yet significant weight loss despite normal or increased eating habit and irreducible fatigue. All of these symptoms except weight loss can also manifest in type 2 diabetes in those patients with poorly controlled diabetes (Barrett *et al,* 2002).

When the glucose concentration in the blood is raised beyond the renal threshold, re-absorption of glucose in the proximal renal tubules is incomplete, leading to glycosuria that increases osmotic pressure of the urine and resulting in increased urine production as polyuria and increased fluid loss. This if not arrested will cause dehydration and increased thirst (David *et al,* 1996).

2.4 PATHOPHYSIOLOGY

Type I diabetes mellitus results from a cellular mediated auto immune destruction of the insulin secreting cells of the pancreatic β-cells while α, δ and other islet cells remain intact. The autoimmune process resulting into type I diabetes begins many years back before the clinical manifestation. About 80 – 90% reduction in the volume of the β-cells is required to induce symptomatic type I diabetes mellitus. The rate of the islet cell destruction is found to vary and is usually more rapid in children than adult. Many factors were found to play role in triggering type 1 diabetes mellitus. Antibodies such as antibodies like islet cell cytoplasmic antibodies (ICA), Insulin auto-antibodies (IAA), and Glutamic acid decarboxylase antibodies (anti-GAD) were found in higher percentage among diabetic than normal individuals. Other risk factors include genetic and environmental factors.

Type II has two major identifiable pathological defects. The primary underlying pathological cause is insulin resistance due to decreased ability of insulin to act on peripheral tissues. The other cause is the β-cells dysfunction, which is an inability of the pancreas to produce enough insulin to compensate for the insulin resistance. If the insulin available is insufficient, or cells shows insulin insensitivity, resistance or defective, then glucose will not be properly absorbed by those body cells that require it nor will it be stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements (David *et al,* 1996).

2.5 LABORATORY INVESTIGATIONS

This is aimed at the Screening, Diagnosis, Prognosis and or monitoring in proper management of the patients (Sacks *et al,* 2002).

2.5.1 Screening

Diabetes screening is recommended for many people at various stages of life, and for those with any of several risk factors. The screening test varies according to circumstances and local policy, and may be a random blood glucose test, a fasting blood glucose test, a blood glucose test two hours after 75 g of glucose (Mshelia *et al*, 2003), or an even more formal glucose tolerance test. Many healthcare providers recommend universal screening for adults at age 40 or 50, and often periodically thereafter. Earlier screening is typically recommended for those with risk factors such as obesity, family history of diabetes, high-risk ethnicity (Sacks *et al,* 2002).

This is recommended for those at risk, and for those asymptomatic people with age 45 yr and above. If the FPG is < 6.1mmol/L, test should be repeated at 3yr interval. At younger age also, it is quite recommended especially those with increased risk from the age of 10yr every 2yr in overweight with family history, race/ethnicity and signs of insulin resistance. The onset of type 2 is over 4 – 7 yr before clinical diagnosis. People with a confirmed diagnosis of diabetes are tested routinely for complications which includes urine testing for microalbuminuria (ADA 2009)

2.5.2.1 Genetic Markers

Genetic markers are currently of limited clinical value in the evaluation and management of patients with diabetes. However, they hold promise for the future. For immune-mediated (type 1A) diabetes (IMD), HLA typing can be useful to indicate absolute risk of diabetes, as extended by insulin (*INS*) gene typing, and can assist in assigning a probability of the diagnosis of IMD to diabetes of uncertain aetiology (Sacks *et al,* 2002).

2.5.1.2 Autoimmune Markers

In newly diagnosed patients with type 1 diabetes, ICA is found in ~75-85%, GAD65A in ~ 60%, IA-2A in ~ 40% and IA-2A in ~ 20%. IAA are positive in more than 90% of children who develop type 1 diabetes before age 5 years, but in fewer than 40% in individuals developing diabetes after age 12.

In some laboratories ICA is considered to be the most sensitive and specific single test for the detection of type 1 diabetes (Sacks *et al,* 2002).

If one autoantibody is found, the others should be assayed because the risk of IMD increases if two or more auto-antibodies are positive (David 1998).

2.5.2 Diagnosis

It is used to establish the presence of DM using an established fact from the analysis conducted. In DM, the diagnosis is achieved by demonstration of hyperglycaemia in a suspected individual by diagnostic criteria enlisted in the clinical diagnosis. The diagnosis of diabetes mellitus depends ultimately on the demonstration of hyperglycaemia as the hallmark (WHO 2000).

2.5.2.1 Glucose

The diagnosis of diabetes is established exclusively by the documentation of hyperglycaemia. In 1997, the diagnostic criteria were modified to better identify subjects at risk of retinopathy and nephropathy. The revised criteria include: (a) symptoms of diabetes and casual plasma glucose of 11.1 mmol/L (200 mg/dL), (b) fasting plasma glucose (FPG) of 7.0 mmol/L (126 mg/dL) or (c) 2-h post-load glucose of 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT). If any one of these three criteria is met, confirmation by repeat testing on a subsequent day is necessary to establish the diagnosis (WHO 2000). Although included as a criterion, the OGTT is not recommended for routine clinical use in non-pregnant individuals (Sacks *et al,* 2002, Wikipedia 2007).

With no symptoms, diagnosis should not be based on a single plasma glucose determination. At least another plasma glucose on another day with a value in the diabetic range is essential, either fasting or at 120 min after a glucose load (Sacks *et al,* 2002).

2.5.2.2 Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)

1. IFG: fasting plasma glucose > 6.0 mmol/L but <7.0 mmol/L.
2. IGT: fasting plasma glucose < 7.0 mmol/L and 2 hour plasma glucose during an OGTT > 7.8 but < 11.1 mmol/L. (WHO 2000)

2.5.3 Monitoring

Intensive plasma control in DM patients proves helpful in reducing micro vascular complication but not much significant in macro vascular reduction as in stroke and infarction. In this respect HbA_{1c} is more helpful than plasma glucose concentration in outcome result (Sacks *et al,* 2002).

2.5.3.1 Glycated Haemoglobin

Glucose combines with many proteins in circulation and in tissues via a non-enzymatic, irreversible process to form advanced glycosylation end products (AGEs). The best known of these is glycosylated haemoglobin, a family of glucose-haemoglobin adducts. Haemoglobin A_{1C} (HbA_{1c}) is a specific member of this group and is useful as an indicator of average glycaemia during 2-3 months before measurement. For haemoglobin, the rate of synthesis of GHb is principally a function of the concentration of glucose to which the erythrocytes are exposed (Miedema *et al,* 1984). The assay of HbA_{1c} was standardized for the Diabetes Control and Complications Trial (DCCT) and is now widely accepted as the standard measurement of glycosylated haemoglobin. Other AGEs are presumed to contribute to the complications of diabetes, such as glycosylated proteins of the basement membrane of the renal glomerulus (Awojobi *et al,* 1991, Timothy *et al,* 2000).

Concentrations of other blood-based glycated proteins such as glycated serum/plasma proteins, fructosamine also reflect mean glycaemia, but over a much shorter time than GHb of 15-30 days and 60-120 days, respectively (Yahaya *et al,* 2001).

The current recommended goal for HbA1c in patients with diabetes is <7.0%, which is considered good glycaemic control, although some guidelines are stricter (<6.5%). People with diabetes, who have HbA1c levels within this range, have a significantly lower incidence of complications, including retinopathy and diabetic nephropathy (Awojobi *et al,* 1991).

2.5.4 Prognosis

Patient education, understanding, and participation are vital since the complications of diabetes are far less common and less severe in people who have well-controlled blood glucose levels (ADA 2009). Wider health problems accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. According to a study, women with high blood pressure have a threefold risk of developing diabetes (Sacks *et al,* 2002).

Strong evidence was established that, there is a correlation between high glucose concentration and poor prognosis. It was documented that the risk of death was significantly increased for patients with persistent high FPG (Manaviat *et al,* 2004)

2.5.4.1 Microalbuminuria

Diabetes is the leading cause of end-stage renal disease in the US and Europe. Early detection of diabetic nephropathy relies upon tests for urinary excretion of albumin. Conventional qualitative tests (chemical strips or “dipsticks”) for protein (>300mg) do

not detect the small increases of urinary albumin excretion seen in early stages of nephropathy (Modobe *et al,* 2000).

Microalbuminuria is defined as 3-30 mg/mmol of creatinine. Early detection of microalbuminuria allows early intervention with a goal of delaying the onset of overt diabetic nephropathy (Wayne *et al,* 2004). As stated earlier, microalbuminuria is a marker of increased risk of cardiovascular morbidity and mortality in both type 1 and type2 diabetes. Thus, it is a signal for more intensive efforts to reduce cardiovascular risk factors (Manaviat *et al,* 2004, Newman *et al,* 2005).

Microalbuminuria rarely occurs with short duration of type 1 diabetes or before puberty. Thus testing is less urgent in these situations. Although the difficulty in precisely dating the onset of type 2 diabetes warrants initiation of annual testing early after diagnosis of diabetes, older patients (age > 75 years or life expectancy < 20 years) may never be at risk for clinically significant nephropathy in view of a projected life-span that is too brief for renal dysfunction to develop. In such patients, the role of treating microalbuminuria is far from clear, and the need to screen for it is, thus, uncertain at best (Rowe *et al,* 1990, Sacks *et al,* 2002)

2.5.4.2 Lipid Profile

Lipid such as total cholesterol, low density lipoprotein, very low density lipoprotein, high density lipoprotein and triglycerides are used generally in the assessment of patients with cardiovascular disease, alcoholics, people with high social status, malnourished children and monitoring of patients with diabetes mellitus (Akuyam et al, 2009). Presence of hyperlipidemia predicts poor prognosis in patients which increases the risk of

cardiovascular morbidity and mortality in those with hypertension and or diabetes mellitus. Low levels of HDL cholesterol, often associated with elevated triglyceride levels, are the most prevalent pattern of dyslipidaemia in persons with type 2 diabetes. Secondarily diabetes mellitus is known to have features of hyper-lipoproteinaemia with usual dominant lipid abnormality of increased triglyceride (Gaw *et al,* 1995). The hyperglycaemia and dyslipidaemia adversely affect the endothelium resulting to its dysfunction and injury. This may lead to release of growth factors that stimulate the proliferation of smooth muscle cells and the deposition of lipid laden foam cells (Messent *et al,* 1992).

For most patients with diabetes, the first priority of dyslipidemia therapy, unless severe hyper-triglyceridaemia is the immediate issue, is to lower LDL cholesterol to a target goal of 2.60 mmol/l. Lifestyle intervention, increased physical activity, weight loss, and smoking cessation, may allow some patients to reach lipid goals. Nutrition intervention should be tailored according to each patient's age, type of diabetes, pharmacological treatment, lipid levels, and other medical conditions and should focus on the reduction of saturated fat, cholesterol, and trans-unsaturated fat intake. Glycaemic control can also beneficially modify plasma lipid levels, particularly in patients with very high triglyceride and poor glycaemic control (ADA 2009).

2.5.4.3 Sorbitol Metabolism

Aldose reductase is present in both the glomerulus and the renal tubule. It uses NADPH as a co-factor and catalyses the reduction of glucose to sorbitol. The enzyme has a low affinity for glucose. Flux through the polyol pathway beginning with the conversion of glucose to sorbitol by aldose reductase is enhanced in hyperglycaemia. The resultant

increase in tissue sorbitol has been postulated to contribute to the micro vascular complications of diabetes. Clinical trials of aldose reductase inhibitors have shown beneficial effects in reducing microalbuminuria in humans. The analyte is useful as an index of metabolic control in diabetics and as a risk factor for evaluating and monitoring the development of some chronic complications that may benefit from aldose reductase inhibitor (Anaja *et al,* 1997). A reference range of 4.1-14.9 nmol/gHb and clinical decision limit of 17.0 nmol/gHb were also established locally because of its clinical utility (Anaja *et al,* 2001). Sorbitol is known to cause osmotic swelling, altered ion gradients, Schwann cell injury and neuronal degeneration.

2.6 TREATMENT AND MANAGEMENT

Diabetes mellitus is currently a chronic disease, without a cure, and medical emphasis must necessarily be on managing and or avoiding possible short-term as well as long-term diabetes-related problems. There is an exceptionally important role for patient's education, dietetic support, sensible exercise, self glucose monitoring, with the goal of keeping both short-term blood glucose levels, and long term levels as well, within acceptable bounds (WHO 2000, ADA 2009). Careful control is needed to reduce the risk of long term complications. This is theoretically achievable with combinations of diet, exercise and weight loss in type 2, various oral diabetic drugs (type 2 only), and insulin use (type 1 and increasingly for type 2 not responding to oral medications). In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications should be undertaken to control blood pressure and cholesterol by exercising more, smoking cessation, consuming an appropriate diet, wearing diabetic socks, and if necessary,

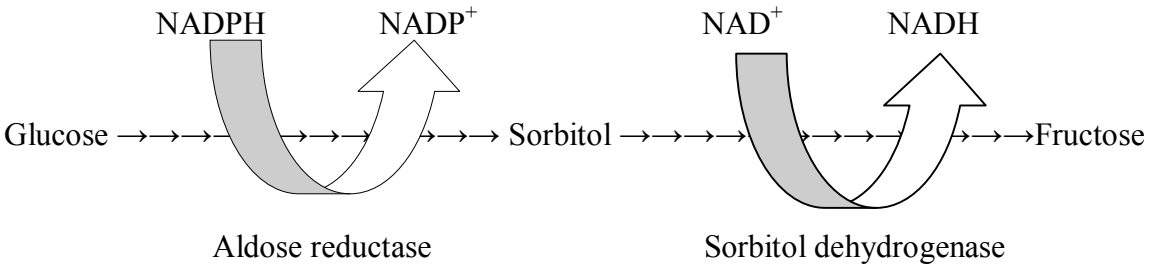


Fig: 2.1 Sorbitol metabolism (Collier and Small, 1991)

taking any of several drugs to reduce pressure (Wilmer *et al,* 2003, Nadler *et al*, 2002).

The principal treatment of type 1 diabetes, even from its earliest stages, is replacement of insulin combined with careful monitoring of blood glucose levels using blood testing monitors. Without insulin, diabetic ketoacidosis often develops which may result in coma or death. Treatment emphasis is now also placed on lifestyle adjustments through diet and exercise though these cannot reverse the progress of the disease (David 1996). Apart from the common subcutaneous injections, it is also possible to deliver insulin by a pump, which allows continuous infusion of insulin 24 hours a day at present levels, and the ability to program doses (a bolus) of insulin as needed at meal times. An inhaled form of insulin, Exubera, was approved by the FDA in January 2006, although Pfizer discontinued the product for business reasons in October 2007 (FDA, 2006). Non-insulin treatments, such as monoclonal antibodies and stem-cell based therapies, are effective in animal models but have not yet completed clinical trials in humans (Ballies 2008).

Oral medication may eventually fail due to further impairment of beta cell insulin secretion. At this point, insulin therapy is necessary to maintain normal or near normal glucose levels in type 2 (Barrett *et al,* 2002).

2.7 DIABETIC COMPLICATIONS

2.7.1 Acute Complications

2.7.1.1 Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is an acute and dangerous complication that is always a medical emergency. The liver converts fat into ketone bodies for use as fuel. This is

normal when periodic, but can become a serious problem if sustained leading to decrease in the blood pH, resulting to DKA. They are typically dehydrated, and breathing rapidly and deeply. Abdominal pain is common and may be severe. The level of consciousness is typically normal until late in the process, when lethargy may progress to coma (Sacks *et al,* 2002). Ketoacidosis can become severe enough to cause hypotension, shock, and death. Urine analysis reveals significant levels of ketone well before overt symptoms. Prompt, proper treatment usually results in full recovery, though death can result from inadequate or delayed treatment, or from complications. Ketoacidosis is much more common in type 1 diabetes than type 2 (David 1996). General diabetic complication could be controlled by keeping the recommended targets for metabolic control in diabetic patients sighted by WHO in 2000 as in table 2.2.

2.7.1.2 Nonketotic Hyperosmolar Coma

Hyper-osmolar non-ketotic state (HNS) is an acute complication sharing many symptoms with DKA, but an entirely different cause and different treatment. In a person with very high blood glucose levels ~16 mmol/l, water is drawn out of cells into the blood by osmosis and the kidneys dump glucose into the urine. This results in loss of water and an increase in blood osmolarity. If fluid is not replaced, the osmotic effect of high glucose levels combined with the loss of water will eventually lead to dehydration. The body's cells become progressively dehydrated as water is taken from them and excreted. Electrolyte imbalances are also common and dangerous. In DKA, urgent medical treatment is necessary especially rehydration therapy (Wikipedia 2007).

2.7.1.3 Hypoglycaemia

Hypoglycaemia, or abnormally low blood glucose, is an acute complication of several diabetes treatments. The patient may become agitated, sweaty, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings similar to dread and immobilized panic. Consciousness can be altered or even lost in extreme cases, leading to coma, seizures, or even brain damage and death. Hypoglycaemia may be caused by incorrectly timed and or insulin and other hypoglycaemic agents overdose. Strenuous or incorrectly timed exercise which decreases insulin requirements may as well be able to stimulate hypoglycaemia. An inadequate food intake especially glucose-producing carbohydrate is also implicated (Gama *et al,* 2003).

Iatrogenic hypoglycaemia is the result of the interplay of absolute or relative insulin excess and compromised glucose counter-regulation in type 1 and advanced type 2 diabetes mellitus. In insulin-deficient diabetes exogenous insulin levels do not decrease as glucose levels fall, and the combination of deficient glucagon and epinephrine responses causes defective glucose counter-regulation (Simon *et al,* 2002).

2.7.1.4 Infection

Infections of the urinary tract are a common health problem worldwide which can either be complicated and uncomplicated such as cystitis seen much often in young women (Gaw *et al,* 1995). Developing countries exhibit a different pattern of urinary tract infection. The disease causes lesion in the bladder and predisposes those with the condition to secondary infection, bladder cancer and chronic pyelonephritis. Genitourinary TB is a common form of extra-pulmonary TB and is always secondary to

the primary lesion. Lesions referred to as ulcero-cavevous or miliary affect the kidney, if unattended, may progress to kidney destruction (Wikkipedia 2007).

2.7.2 Chronic Complications

2.7.2.1 Vascular Disease

Chronic elevation of blood glucose level leads to damage of blood vessels known as angiopathy. The endothelial cells lining the blood vessels take in more glucose than normal, since they don't depend on insulin. They then form more surface glycoproteins than normal, and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under micro-vascular disease because of damage to small blood vessels and macro-vascular disease resulting from damage to the arteries (Wikipedia 2007).

The damage to small blood vessels leads to a micro-angiopathy, which can cause one or more of the following:

- Diabetic retinopathy; growth of friable and poor-quality new blood vessels in the retina as well as macular oedema, which can lead to severe vision loss or blindness. Retinal damage (from micro-angiopathy) makes it the most common cause of blindness among non-elderly adults (Rowe *et al* 1990, Gall *et al,* 1991).
- Diabetic neuropathy; abnormal and decreased sensation, usually in a 'glove and stocking' distribution starting with the feet but potentially in other nerves, later often fingers and hands. When combined with damaged blood vessels this can lead to diabetic foot (see below). Other forms of diabetic neuropathy may present

as mononeuritis or autonomic neuropathy. Diabetic amyotrophy is muscle weakness due to neuropathy (Parving *et al*, 1998, Barrett *et al,* 2002).

- Diabetic nephropathy; damage to the kidney which can lead to chronic renal failure, eventually requiring dialysis. Diabetes mellitus is the most common cause of adult kidney failure worldwide in the developed world (Rowe *et al,* 1990, Peter 1996, Timothy *et al,* 2000, Wilmer *et al,* 2003, Wikipedia 2007,).

Diabetic foot; often due to a combination of neuropathy and arterial disease, may cause skin ulcer and infection and, in serious cases, necrosis and gangrene. This is why diabetics are prone to leg and foot infections and why it takes longer for them to heal from leg and foot wounds. It is the most common cause of adult amputation, usually of toes and or feet, in the developed world (Barrett *et al,* 2002).

2.8 KIDNEY

The kidneys are a pair of fist-sized organs located outside the peritoneal cavity on each side of the spine. Together with the skin and the respiratory system, the kidneys are the body's primary excretory organs. The kidney is a highly specialised organ that maintains the internal environment of the body by selectively excreting or retaining various substances according to specific body needs (Cameron *et al,* 1998). The kidney has several functions including the excretion of water, soluble waste e.g. urea and creatinine, and foreign materials such as drugs. It is responsible for the composition and volume of circulating fluids with respect to water, electrolyte and acid/base status. It has an endocrine function playing a part in the production of Vitamin D and erythropoietin, and as part of the rennin-angiotensin-aldosterone axis. Measurements of renal function rely

on measuring, in various ways the degree to which the kidney is successful in these roles. The importance of urine formation and excretion as a life-sustaining function is highlighted in situations in which kidney function is suddenly lost (Fagazzi *et al,* 1998).

Urine is a very complex fluid comprising 95% water and 5% solids. The approximately 1-2 litres of urine excreted each day are the end product of metabolism carried out by billions of cells in the renal and urinary systems. The kidney dilutes or concentrates urine, according to an individual's changing physiological needs, and regulates electrolyte excretion. Impaired renal function has adverse effects on blood chemistry, blood pressure, fluid balance, nutrient intake, and the person's general state of health (Cameron *et al,* 1998). When kidney function becomes compromised by disease, the processes of glomerular filtration and renal tubular re-absorption and secretion become affected. Blood and urine biochemical tests reflect the extent of this dysfunction (Cameron 1998). Acute renal failure is rapid in onset and can often be reversed. Acute renal failure is classified as pre-renal, renal, and post-renal failure (Edelstein *et al,* 1998, Wikipedia 2008,). Pre-renal failure is caused by decreased blood flow to the kidneys, as occurs with congestive heart failure. Renal failure results from injury to the kidney's glomeruli and tubules. The most common causes of renal failure are glomerulonephritis, pyelonephritis, and tubular damage caused by drugs, heavy metals, and viral infection (Cameron 1998). Post renal failure is caused by obstruction in the urinary tract below the kidneys. This can occur from urinary tract stones, tumours, and anatomic obstruction due to benign prostatic hypertrophy (Relly 1999). Chronic renal failure has a slow onset, may follow episodes of acute renal failure, and is not reversible. Chronic renal failure often results from acute glomerulonephritis or pyelonephritis (Fisch 1999, Wikipedia 2007). Other

reasons for chronic renal failure include diabetes mellitus, atherosclerosis of the renal blood vessels (MacFarlene *et al,* 2003), hypertension (Kaiser *et al,* 2003), polycystic kidney disease, and kidney stones (Watts 1998, Wikipedia 2007).

2.9 DIABETIC NEPHROPATHY

Diabetic nephropathy is a deleterious effect on kidney structure and/or function caused by diabetes mellitus. Diabetic nephropathy simply defined clinically as the presence of microalbuminuria or overt nephropathy in patients with diabetes who lack indicators of other renal diseases (McFarlane *et al,* 2003). It is also defined as a small elevation of urinary albumin excretion not detected by conventional tests. It is associated with elevated arterial pressure, proliferative retinopathy, lipoprotein abnormalities and left ventricular hypertrophy and is predictive of later diabetic nephropathy.(Walker *et al,* 1991). More specifically, diabetic nephropathy is thought of in stages, the first being that characterised by microalbuminuria (30-300 mg/24hr AER or 3-30mg/mmol ACR). This may progress to macroalbuminuria, or overt nephropathy (>300 mg urinary albumin per 24 hours). Later still, progressive renal functional decline characterised by decreased GFR results in clinical renal insufficiency and ESRD (Timothy *et al,* 2000).

Dialysis and renal transplantation are costly, and can have a devastating effect on quality and length of life. Renal dysfunction is typically identified in the macroalbuminuria stage, and can progress over time to end stage renal disease (Goldschmid *et al,* 1995). Detection of microalbuminuria identifies individuals at high risk of progression to later stages of renal disease, cardiovascular events and death. The diagnosis of nephropathy

only requires a kidney biopsy when clinical indicators leave doubt as to the diagnosis (David *et al,* 1998, MarcFarlene *et al,* 2003).

It has become clear over time that once overt nephropathy has developed, treatment is a delaying rather than a preventive tactic. Consequently, a high priority in the care of patients with diabetes is screening for early signs of micro-vascular disease so that measures can be taken to delay or prevent loss of renal function through early detection and initiation of effective therapies, and to manage complications in those identified with renal disease. For this reason, regular screening for microalbuminuria should be part of routine diabetic preventive care (McFarlane *et al,* 2003, Wayne *et al,* 2004).

Among the earliest changes demonstrable in diabetic nephropathy is glomerular hyperperfusion. This is accompanied by microalbuminuria, which serves as a sensitive early indicator of adverse effects of diabetes on the kidney and is a powerful predictor of the subsequent course. Eighty percent of type 1 diabetes patients with microalbuminuria will progress to overt nephropathy within 10-15 years. Of these, 50% will develop ESRD within 10 years and 75% within 20 years in the absence of specific intervention. Among patients with type 2 diabetes, 20-40% of patients with microalbuminuria will progress to overt nephropathy, though only 20% of those patients will go on to ESRD within the next 20 years. Microalbuminuria is also a powerful predictor of cardiovascular disease in both type 1 and type 2 diabetes (Timothy *et al,* 2000).

In type 1 diabetes, annual screening should begin after puberty and 5 years after initial diagnosis. In type 2, there is likelihood that diabetes has been present for several years at

the time of diagnosis, annual microalbuminuria screening should begin at the time of diagnosis (Farkas *et al,* 1992).

2.10 MICROALBUMINURIA

2.10.1 Definition

Microalbuminuria is an increased excretion of albumin above the reference range for healthy non-diabetic subjects, but undetectable by the Albustix dipstick test or as modified by expert group as a urinary albumin excretion rate between 20 - 200µg/min (3-30 mg/mmol ACR) in an overnight or 24 hours sample on at least 2 of three occasions within a period 6 months (Rowe *et al,* 1990, Ibadin *et al,* 2004). Microalbuminuria is also defined as the urinary excretion of 30-300 mg of albumin per day (Timothy *et al,* 2000, Olatunde *et al,* 2002). Screening procedure was illustrated in appendix iv.

2.10.2 History

In 1963 a sensitive RIA method was developed in view of the assessment and to examine the prevalence of diabetes mellitus and associated systemic complication in Bedford, UK with a reported finding 3-4 fold increase in urinary albumin above the reference range. Mogenson, in 1971 observed a significant increase in urinary albumin among all newly diagnosed IDD patients and revert to normal on insulin therapy. In 1979, Viberti *et al,* demonstrated that 30-40% of Albustix negative IDD patients especially those with a duration of diabetes between 10-20yr , had a raised albumin excretion rate, and 3yr later that microalbuminuria was a powerful predictor of overt nephropathy in IDD (Rowe *et al,* 1990).

2.10.3 Prognostic Value

Greater proteinuria is found associated with faster kidney disease progression. It has been recently reported that proteinuria particularly is heavy and non selective can be nephrotoxic through variety of mechanisms. Its reduction is associated with slower subsequent GFR decline while if allowed unattended or worsened is associated with faster GFR decline (Wilmer *et al,* 2003). In both types of diabetic patients microalbuminuria is a powerful predictor of clinically overt diabetic renal disease as well as cardiovascular mortality (Messent *et al,* 1992, Perkins *et al, 2003*).

2.10.4 P**revalence of Microalbuminuria**

Microalbuminuria possibly reflects a state of increased renal endothelial permeability and is considered an early marker of diffuse endothelial dysfunction (Raman 1980, Cottone *et al,* 2007). Cross-sectional studies revealed prevalence of microalbuminuria ranging between 6-40% which is believed to depend on urine collection method, cut-off level of albumin excretion and the clinical characteristics of the population (Mathiensen *et al*, 1986, Rowe *et al,* 1990). The patients with longer duration of diabetes mellitus, hypertension, older age, higher BMI and adverse lipid profile are more prone to develop microalbuminuria (Hitha *et al,* 2008). Available data between 1971 and 2002 show the prevalence of microalbuminuria in Africa to vary between 26% and 57% in diabetic patients, with variable duration of the disease (Lutale *et al,* 2007). Screening tests for microalbuminuria are recommended annually for patients with type 1 diabetes of greater than 5 years duration and for all patients with type 2 diabetes from the time of diagnosis. It appears that the prevalence of microalbuminuria in childhood increases with the age of the population (Moore *et al,* 2000).

2.10.5 Urine Sampling for Microalbuminuria

Several urine collection methods are available for screening and give comparable results. The gold standard is the 24-hour urine collection, which, if accompanied by serum and urine creatinine, also allows calculation of creatinine clearance rate and serves as a reference for future comparison. Somewhat more convenient for the patient is the albumin/creatinine ratio on a spot urine sample or albumin excretion rate in a timed specimen measuring albumin without relating it to duration of collection or creatinine concentration is less sensitive and specific because of dilution variability (Timothy *et al,* 2000).

Screening for microalbuminuria should be performed using the random urine test for albumin to creatinine ratio (ACR). A urine dipstick test should also be performed on the urine specimen, either in the laboratory or at the point of care, as a screen for non-diabetic renal disease.

While 24-hour or timed overnight urine collections have been the gold standard for clinical trials, these tests are difficult to perform correctly in routine practice and may yield false results. The random urine ACR accurately predicts the urinary protein level detected by 24-hour collections, and is easier to perform and more agreeable to patients than timed collections (Moore *et al,* 2000).

2.10.6 Screening and Monitoring

In type 1 diabetes, annual screening should begin after puberty and 5 years after initial diagnosis. In type 2 diabetes; because of the likelihood that diabetes has been present for several years by the time it is diagnosed, annual microalbuminuria screening should begin at the time of diagnosis. Several methods are available for screening and give

comparable results. Though the 24-hour urine collection (normal albumin excretion <30 mg per 24 hours) was considered as gold standard (Rowe *et al*, 1990), if accompanied by serum and urine creatinine, it allows the estimation of creatinine clearance rate and serves as a reference for future comparison (Timothy *et al,* 2000). Somewhat more convenient for the patient is the albumin/creatinine ratio on a spot urine sample (normal <30 mg albumin per g creatinine) or albumin excretion rate in a timed specimen (4 hours or overnight, normal <20 mg albumin per min) which must be related to a duration of collection or creatinine concentration because of dilution variability (Farkas *et al,* 1992, ADA 2009).

2.10.7 Therapeutic Intervention on Microalbuminuria

Knowledge of the pooled magnitude of risk associated with current definitions of microalbuminuria in addition to an improved understanding of the risk relationship of varying levels of baseline albuminuria with cardiovascular and renal outcomes could have important implications in screening and treatment recommendations for persons with diabetes (Rockville, 2003). Prior to initiating treatment, persistence and/or progression of microalbuminuria must be demonstrated by repeat sampling conducted every 3 to 4 months over a 12-month period. Antihypertensive therapy slows the development of microalbuminuria (Hitha *et al,* 2008) and the progression of nephropathy in hypertensive patients, which is evident from several controlled intervention trials (Kaiser *et al,* 2003). Rigorous glycaemic control with continuous subcutaneous insulin infusion has consistently been shown to retard and or reverse the progression of microalbuminuria (Rowe *et al,* 1990).

2.10.8 Microalbuminuria Qualitative/ Quantitative tests

There is growing evidence that slight increases in urinary albumin below the concentration detectable by routine semi quantitative strip are considered to be predictive of clinical nephropathy among diabetic patients. It has also been shown that strict glycaemic control can reverse the progression of microalbuminuria and hence slow the development of nephropathy. In view of this, rapid and accurate measurement of such subclinical increases of microalbuminuria is of paramount importance. Among these methods include Radioimmunoassay (measures immunoreactive), HPLC (measures total albumin i.e. immunoreactive and immune-unreactive) (Comper *et al,* 2004). Other methods include Immunoturbidimetric method, immunonephlometric assay (Moore *et al,* 2000) and enzyme immunoassay (McBain *et al,* 1987). Qualitative tests were based on turbidity, the protein error of indicators' principle and latex bead immunoagglutination of which their lower limit of detection is at best 15mg/L. They were found to fail in albumin measurement in dilute urine (Rowe *et al,* 1990).

2.10.9 Sample Storage and Stability

Sample stored in aliquots are stable at -20˚C for at least six months without preservative and for at least 1 week at 4˚C on thawing. Urine albumin is sufficiently stable at room temperature for 4 days for posting and can be kept for 2 weeks at 4˚C without preservative. Sodium Azide at 0.03mol/L concentration may be used for longer storage if required. Neither centrifugation nor sedimentation affects urinary albumin estimation (Rowe *et al,* 1990).

2.10.10 Effect of pH, Freezing, Centrifugation on Microalbuminuria

The frequency and severity of the effect of centrifugation, storage time and pH on urinary albumin in frozen sample are highly variable from one study to another. This may be partly accounted for by analytical variability during the course of the studies. Samples should be vortexes immediately prior to analyses (Rowe *et al,* 1990).

2.10.11 Effect of Bacterial Contamination on Microalbuminuria

Urinary albumin may be affected by causing false-negative which resulted largely due to bacterial hydrolysis of albumin by its proteases. In a study conducted by Rowe *et al,* in (1980), it was observed that gross contamination of urine over 14 days at room temperature does not materially affect the albumin concentration.

2.10.12 Microalbuminuria as a Predictor of Systemic Diabetic Complication

Among the earliest changes demonstrable in diabetic nephropathy is glomerular hyper-perfusion. This is accompanied by microalbuminuria, which serves as a sensitive early indicator of adverse effects of diabetes on the kidney and is a powerful predictor of the subsequent course (Timothy *et al,* 2000). Microalbuminuria is the best documented predictor of high risk for development of diabetic nephropathy in both type 1 and type 2 diabetes, and numerous trials in both types have documented and demonstrated the usefulness of microalbuminuria in intervention studies (Parving *et al,* 2002). Earlier detection allows treatment to prevent progression to persistent albuminuria and renal failure (Comper *et al*, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SUBJECTS

3.1.1 Patients and Controls

A total of 170 diabetic patients and 100 controls were recruited for the present study. Patients attending ABUTH outpatient diabetic clinic for their regular visits and who voluntarily agreed to participate in the study including the controls were included. Informed consent verbally and in writing was obtained from all subjects and was interviewed using an agreed questionnaire.

Information obtained from the subjects include age of the patient, diabetic duration, sex, body mass index, blood pressure. Other analytes assessed include serum creatinine (Jaffe method, 1919), and glycated haemoglobin A_{1c} (Quimica Clinica Aplicada S.A.). Urinalysis was also done to check for proteinuria, leukocyturia, glycosuria, haematuria, and nitrite to assess for urinary tract infection.

Patients were classified into Type 1 and Type 2 according to the WHO clinical stage criteria based on the information obtained from patient's diabetes sheet and data collected on enrolment.

Weight was measured without shoes or heavy clothing with a hospital standard weighing scale and was recorded to the nearest kilogram. Height was also determined to the nearest centimetre against a ruled vertical wall and the body mass index (BMI) was calculated using the ratio of weight and the square of height (Kg/m^2).

Blood pressure was measured with a suitable mercury sphygmomanometer by a clinician. After a 10 minutes rest with the subjects in the sitting position, blood pressure was measured two times at 5 minutes interval. The second blood pressure measurement was used as the blood pressure for the individual. Hypertension was considered as SBP ≥140 and/or DBP of ≥90 mmHg or use of hypertensive medication.

3.1.2 Inclusion Criteria

Adult volunteers attending ABUTH, Zaria with established diabetes mellitus (type I and II), who agreed to participate, normotensive (≤140/90mmHg) and free from kidney infection were included in the study. Apparently healthy individuals of same age group who were normotensive and neither diabetic nor having kidney infection were included as controls.

3.1.3 Exclusion Criteria

Patients who were found to have disease condition that will cause positive microalbuminuria measurement were not included, such as fever, hypertension, menstruation, end-stage renal disease, haematuria, leukocyturia, or heart failure.

3.1.4 Sample Size

The sample size for the study was determined from a standard formula for the calculation of minimum sample size (Oyejide 1992; Singha, 1996). Sample size n was given by the formula

$$n = \frac{(Z_1 - a)^2 \quad (P) \quad (1-P)}{d^2}$$

Where n= minimum sample size; Z_1-a= value of standard normal deviate which at 95% confidence level has been found to be 1.96.

P = the best estimate of the population prevalence obtained from literature review (1.6%) (Bakari and Onyemelukwe, 1999) and d = difference between the true population rate and sample that can be tolerated , that is the absolute precision required (in percentage point) on either side of the population .

$n = \frac{(1.96)^2 (0.016) (1-0.016)}{(0.02)^2}$ = 151 as the minimal number of samples for the study.

Therefore a total of 151 with 10% of these subjects will be added to the research for attrition, making a total of 167 total subjects.

3.1.5 Ethical Approval

The study was approved by the hospital Ethical Committee of Ahmadu Bello Teaching Hospital, Shika- Zaria.

3.2 SAMPLING TECHNIQUES

Arrangement was made with the clinicians where those that satisfied the study inclusion criteria were selected. The nature of the study and reasons for the research were explained fully to the subjects in an appropriate language. Subject consent was a priority and was obtained (Appendix i and ii). Specimen collection of both urine and blood samples were made. Their clinical history and biochemical findings were fully documented in the proforma (Appendix iii).

3.2.1 Blood Sample Collection

Using aseptic technique, a morning fasting sample of 10 ml venous blood was drawn from each subject and centrifuged within one hour of collection using Hettich Universal 32 Centrifuge (Germany). The sera samples were analysed immediately. Few volume of the whole blood was dispensed in to EDTA bottle for GHbA1c, which was also analysed on same day of collection.

3.2.2 Urine Sample Collection

Spot urine specimen was collected on visit. Patients with microalbuminuria were asked to produce two more urine samples at three week interval. Patient/control subjects were given a clear oral instruction on how to collect the urine sample into a container with no preservatives. Collected urine was screened for signs of infection using a dipstick (Unipath, multistrip). Samples found to be infected were discarded. Urine samples were analysed immediately for albuminuria and creatinine. Urinary albumin was measured by immunoturbidimetric assay (Elvin *et al,* 1989) and the urinary creatinine using Jaffe method . Albumin-Creatinine ratio (ACR) was adapted to categorize subjects as having normo-albuminuria (ACR < 3 mg/mmol), microalbuminuria (ACR 3–30 mg/mmol), and macroalbuminuria (ACR > 30 mg/mmol).

3.3 EQUIPMENT

A centrifuging machine of Hettich Universal 32 model was used for spinning the blood samples. A Beckman Coulter DU-20 general purpose UV/ Vis Spectrophotometer was used for the measurement of microalbuminuria, glucose, urea, glycated haemoglobin and

creatinine. Flame photometer of model CONING 410 was used for the estimation of sodium and potassium.

3.4 ANALYTICAL METHODS

3.4.1 Albuminuria

Albuminuria (Immunoturbidimetric Assay for Urinary Albumin kit manufactured by FORTRESS DIAGNOSTICS LIMITED (Bakker 1988, Elving, 1989)

3.4.1.1 Principle

This test is used for quantitative determination of albumin in urine. Undiluted sample is added to a buffer containing antibody specific for human albumin. The absorbance at 340nm of the resulting turbid solution is proportional to the concentration of albumin in the urine.

3.4.1.2 Procedure

Into 3 test tubes labelled blank, test and standard; 1000µl of assay buffer (R1) was added in to each tube. A volume of 20µl of sample and standard was then added to the test and standard tubes respectively. The tubes were then mixed and were left for 5 minutes at 37ºC. Absorbance was read at 340nm and recorded as A_1. A volume of 200µl antibody (R2) was added to each tube and mixed gently and kept for further 10 minutes at 37ºC and the absorbance was measured at 340nm as A_2. A difference between absorbencies (A_1-A_2) was calculated and the concentrations of the test samples were extrapolated from the calibration curve.

3.4.2 Serum Glucose

Serum glucose was measured by glucose oxidase method (Trinder, 1969)

3.4.2.1 Principle

Glucose oxidase is a specific enzyme, which promotes the oxidation of glucose to acid with the production of an equivalent amount of hydrogen peroxide, in the presence of a second enzyme (peroxidase). The oxygen from peroxide can be transferred to a suitable acceptor (4-amino phenazone) with the production of a coloured compound, the intensity of which is proportional to the concentration of glucose present in the sample.

$2\text{Glucose} \xrightarrow{\text{glucose oxidase}} 2\text{Glucoronic acid} + 2H_2O_2 \xrightarrow{\text{peroxidase}} 2H_2O + 2O^-$

$2O^- + \text{4-amino phenazone} \rightarrow \text{coloured compound}$

3.4.2.2 Procedure

Three test tubes were prepared and labeled as blank, test and standard. Phenol reagent (4 ml) was added into each tube. Distilled water, serum and standard (50 µl) were then added into tubes 1 (blank), 2 (test) and 3 (standard) respectively. Glucose colour reagent, (1 ml) was then added to all tubes. The contents were then mixed, incubated at 37^0C for 20 minutes and absorbance read at 510 nm using the blank to set the instrument to zero.

3.4.2.3 Calculation

Serum glucose (mmol/L) = Ab test / Ab std x Conc of std (10mmol/L)

Where Ab = Absorbance.

3.4.3 Glycated Haemoglobin

(QUIMICA CLINICA APLICADA S.A. Kit) (Triveli *et al*, 1971).

3.4.3.1 Principle

After preparing the hemolysate, where the labile fraction is eliminated, the GHbA1c is specifically eluted after washing away the HbA1a+b fraction and is quantitatively estimated by direct photometric reading at 415 nm.

3.4.3.2 Procedure

A. Haemolysate preparation and labile fraction elimination

A volume of 200 µl of reagent1 was dispensed into a tube,

and 50µl of the blood sample, the content was mixed and incubated for 15min at room temperature (≤ 25ºC).

B. Column preparation

The upper cap of the column was removed and the column bottom tip snaped

A pipette flat end was used to push the upper disc down enough to have contact with the resin and liquid content was drained completely to waste.

C. **HbA1 separation**

From the haemolysate prepared, 50µl was carefully added on the upper filter of the column

Reagent 2 of 200µl volume was added on the same upper filter of the column which was then followed with 2ml of same reagent 2 and the column was allowed to drain to waste.

The column was then placed on to a test tube and 4ml of reagent 3 was added and the elute was collected as HbA1c fraction. The content was then shaken and the absorbance (A) was read at 415 nm as $A_{HbA1c.}$

D. **Total haemoglobin (HbA1c)**

From the haemolysate, 50 µl was dispensed into a test tube and followed with 12ml of reagent 3.

The mixture was thoroughly mixed and the absorbance (A) was read at 415 nm as $A_{Hb\text{-}TOTAL.}$

Reading:

Wavelength: 415 nm.

Blank: water

Stability: 1 hour.

3.4.3.3 Calculations

Then, % HbA1c = A_{Hb1c} x V_{Hb1c} / $A_{Hb\text{-}TOTAL}$ x $V_{Hb\text{-}TOTAL}$ x 100

Or % HbA1c = A_{HbA1c} / $A_{Hb\text{-}Total}$ x 100 /3

A_{HbA1c} = Absorbance of HA1c

$A_{Hb\text{-}TOTAL}$= Absobance of Hb-$_{TOTAL}$

V_{HbA1c}= volume of HbA1c = (4ml)

$V_{Hb\text{-}TOTAL}$= volume of Hb-$_{TOTAL}$ = (12ml)

3.4.4 Creatinine

Creatinine was estimated using Jaffe method, 1919

3.4.4.1 Principle

Creatinine gives a red colour with alkaline solutions of picric acid (Jaffes reaction). The red colour is measured in the presence of high background (yellow colour) and the solutions appear orange at low creatinine concentrations.

3.4.4.2 Procedure

Into 3 set of test tubes, labelled test, standard and control; 1.5 ml of distilled water was added to test and to blank while the standard tube was left empty. Half (0.5) ml each of 10% sodium tungstate and 2⁄3N sulphuric acid was added to test and blank tubes. The content was then mixed and centrifuged for 10 min. Volumes of 1.5 ml from the supernatants above were transferred to the new glass tubes and the standard solution. An addition of 0.5ml each of picric acid and 0.75N sodium hydroxide were followed with incubation for 15 min at room temperature. The absorbance readings were made at 520 nm.

Urine sample was diluted to 1 in 100 and then treated as supernatant.

3.4.4.3 Calculation

Creatinine (µmol⁄l) = test⁄std x 530 (serum)

Creatinine (µmol⁄l) = test⁄std x 8830 (urine)

3.4.5 Urinalysis

Uripath (multistrip) series (Reagent strip by plasmatec laboratory)

3.4.5.1 Principles

Urobilinogen

Modified Ehrlichs reaction. Urobilinogen present react with Erhlichs reagent to form a red coloured compound. Colour changes from light orange–pink to dark pink.

Glucose

Glucose oxidase is a specific enzyme, which promotes the oxidation of glucose to acid with the production of an equivalent amount of hydrogen peroxide, in the presence of a second enzyme (peroxidase). The oxygen from peroxide can be transferred to a suitable acceptor (4-amino phenazone) with the production of a coloured compound, the intensity of which is proportional to the concentration of glucose present in the sample.

$$2\text{Glucose} \xrightarrow{\text{glucose oxidase}} 2\text{Glucoronic acid} + 2H_2O_2 \xrightarrow{\text{peroxidase}} 2H_2O + 2O^-$$

$$2O^- + \text{4-amino phenazone} \rightarrow \text{coloured compound}$$

Bilirubin

Azo-coupling reaction of bilirubin with a diazonium salt in an acid medium to form an azo-dye. Colour changes from light tan to beige or light pink.

Ketones

Legals test nitroprusside reaction. Acetoacetic acid in an alkaline medium reacts with nitroferricyanide to produce a colour change from beige to purple.

Specific Gravity

Ionic solutes present in the urine cause protons to be released from a polyelectrolyte. As the protons are released the pH decreases and produces a colour change of bromo-thymol blue from blue-green to yellow-green.

Occult blood

The test is based on the pseudo-peroxidase activity of the haem-moiety of haemoglobin. The chromogen is oxidized by a hydrogen peroxide in the presence of haem and changes colour from yellow to blue.

pH

Double indicator system; Indicators of methyl red and bromo-thymol blue are used to give distinct colour changes from orange to green to blue (pH 5.0 to 9.0).

Protein

The test is based on the "protein error" principle of indicators. When pH is held constant by a buffer, indicator dyes release H ions because of the protein present and change colour from yellow to blue green.

Nitrite

The test is based on the diazotisation reaction of nitrite with an aromatic amine to produce a diazonium salt. It is followed by an azo-coupling reaction of this diazonium salt with an aromatic compound on the reaction pad. The azo-dye produced causes a colour change from white to pink.

Leucocytes

This test pad contains an indoxyl ester and diazonium salt. It is followed by an azo-coupling reaction of the aromatic amine formed by leucocyte estrase with a diazonium salt on the reaction pad. The azo-dye produced causes a colour change from beige to violet.

3.4.5.2 Procedure

The test strip was dipped into the urine sample briefly not more than 1 sec. Excess urine was removed by drawing the strip along the brim of the vessel avoiding contact with the test area and onto an absorbent to remove the remaining urine. After an exactly 60 seconds, the strip was semi-quantitatively read by comparing it against the provided colour chart under a good light illumination.

3.4.6 Quality Control

All analytical tests were done according to the standard operating procedures. It involved the use of inter and intra-run of control sera along with the samples. Pre-analytical, analytical and post analytical precautions were observed.

3.5 STATISTICAL ANALYSIS

The analysis of the data obtained was treated accordingly using Statistical Program for the Social Sciences (SPSS 15.0) for windows (SPSS Inc., Chicago, 1L). The data obtained for microalbuminuria were compared against age groups, sex, ethnic group of the subjects and were related between diabetes and control subjects using student's t-test.

ANOVA was used to compare between more than two groups. Other analytes estimated along with the microalbuminuria were correlated to find relation between them and their effect in controlling diabetic nephropathy using Pearson's correlation. A p-value equal to or less than 0.05 ($p \leq 0.05$) was considered as statistically significant value.

CHAPTER 4

4.0 RESULTS

4.1 CHARACTERISTICS OF THE STUDY POPULATION

The characteristics of the studied population are shown in table 4.1. A total of 270 subjects were recruited in the present study. It consisted of 170 normotensive diabetic patients (male 79, Female 91). The patients and controls were also stratified into 3 major age groups of 20-39, 40-59 and 60-79. Hausa/Fulani being the predominant ethnic group has the highest percentage of 51.8% (88) followed by the combined minor ethnic groups with 34.7% (59), Yoruba 7% (12) and Igbo 6.5% (11).

4.2 MEAN VALUES OF CLINICAL PARAMETERS AMONG PATIENTS AND CONTROLS

The mean age of the patients group was 48 years while that of the control subject was 34 years as shown in table 4.2. The two categories were having a similar age range of 21-79 yr. The mean BMI and DBP results in patients group were (26.8±0.4) and (80.0±0.6) mmHg. In controls the mean BMI and DBP were (23.4±0.4) and (72.7±0.7) mmHg respectively. Comparing these values, patients had a statistically higher BMI and DBP than their control counterpart ($p<0.05$).

4.3 MEAN VALUES OF SERUM AND URINARY BIOCHEMICAL ANALYTES IN PATIENTS AND CONTROLS

Table 4.3 shows the mean results of serum FBG, GHbA1c, SCr, and urinary UCr, UAlb and ACR among diabetic patients and controls. The results of FBG, GHbA1c, UAlb and ACR were significantly higher in patients than the controls group ($P<0.05$) while UCr

Table 4.1 CHARACTERISTICS OF THE STUDIED POPULATION

Subjects	n	(%)	Mean Age ±SEM(yr)
Patients	170	-	48.2 ±0.94
Male	79	46	49.5±1.40
Female	91	54	47.1±1.30
DOD			
1-5yr	118	69.8	46.4±0.42
6-10yr	37	21.8	52.3±2.11
11yr above	15	8.8	53.3±2.89

n =number in the population group, % = percentage, SEM =standard error of mean, DOD = duration of diagnosis, yr = year

Table 4.2 MEAN VALUES OF CLINICAL PARAMETRES (MEAN±SEM) AMONG PATIENTS AND CONTROLS

Subjects	n	Age(yr)	BMI (Kg/m^2)	DOD (yr)	SBP (mmHg)	DBP (mmHg)
Patients	170	48.2±0.94	26.8±0.43	5.3±0.35	125±0.58	80±0.56
Control	100	33.7±1.25	23.4±0.43	-	123±0.91	72±0.69
P – Values		< 0.001	< 0.001	-	> 0.05	<0.001

n =sample size, BMI=body mass index, DOD=duration of diagnosis, SBP=systolic blood pressure, DBP=diastolic blood pressure, SEM=standard error of the mean, p-value is between patients and control

Table 4.3 FASTING BLOOD GLUCOSE, GLYCATED HAEMOGLOBIN, SERUM AND URINE CREATININE, URINARY ALBUMIN AND ALBUMIN - CREATININE RATIO (MEAN±SEM) IN PATIENTS AND CONTROLS

Subjects	n	FBG(mmol/L)	GHbA1c(%)	UCr(μmol/L)	SCr(μmol/L)	UAlb(mg/L)	ACRmg/m
Patients	170	6.3±0.22	7.2±0.16	9442±4784	80.6±1.60	20.2±2.17	2.7±0.29
Controls	100	3.5±0.07	4.9±0.07	8495±1913	78.9±1.40	8.0±0.31	1.0±0.04
P-Value		<0.001	<0.001	>0.05	>0.05	<0.001	<0.001

FBG, Fasting blood glucose; GHbA1c, glycated haemoglobin; UCr, Urine creatinine; SCr, serum creatinine; UAlb, urine albumin; ACR, albumin creatinine ratio; SEM, standard error of mean

and SCr were also high in patients but observed to have no significant difference (P>0.05).

4.4 AGE, BMI, DURATION OF DIABETES, SYSTOLIC BLOOD PRESSURE AND DIASTOLIC BLOOD PRESSURE (MEAN±SEM) AMONG DIABETIC NORMO- AND MICRO-ALBUMINURIC PATIENTS.

Table 4.4 Show the results of Age, BMI, duration of diabetes, systolic blood pressure and diastolic blood pressure. There were no statistical differences found with Age, BMI, DOD SBP and DBP among the Normo- and microalbuminuric diabetic patients (P>0.05).

4.5 SERUM FASTING BLOOD GLUCOSE, GLYCATED HAEMOGLOBIN, SERUM AND URINARY CREATININE, URINARY ALBUMIN AND ALBUMIN-CREATININE RATIO (MEAN±SEM) AMONG DIABETIC NORMO- AND MICRO-ALBUMINURIC PATIENTS.

Table 4.5 Shows the results of FBG, GHbA1c, urinary and serum creatinine, urinary albumin and albumin-creatinine ratio. A statistically significant differences were found with FBG, GHbA1c, UCr, UAlb and ACR (P<0.05). Exceptionally, SCr was seen to be statistically insignicant between the normo- and microalbuminuric patients.

4.6 DISTRIBUTION OF MICROALBUMINURIA IN DIFFERENT DURATION OF DIABETES DIAGNOSIS

Out of the total 170 patients in the present study, 118 (69.4%) fell within 0-5years of diagnosis with 25(21%) having abnormal ACR. Thirty seven (21.8%) have a DOD of 6-11 years with 10(27%) having abnormal ACR while 15(8.8%) of those with equals to or greater than 11years of diagnosis were observed to have 6(40%) of ACR abnormality.

Table 4.4 AGE, BMI, DURATION OF DIABETES, SYSTOLIC BLOOD PRESSURE AND DIASTOLIC BLOOD PRESSURE (MEAN±SEM) AMONG DIABETIC NORMO- AND MICROALBUMINURIC PATIENTS.

Subjects	n	Age (yr)	BMI (Kg/m^2)	SBP (mmHg)	DBP (mmHg)	DOD (yr)
N-Albuminuria	131	47.9±1.00	26.0±0.46	125±1.00	79.8±0.64	5.1±0.34
M-Albuminuria	39	49.5±2.3	27.0±0.72	126±1.90	80.8±1.42	5.9±1.16
P-Value		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

n =sample size, BMI=body mass index, DOD=duration of diabetes, SBP=systolic blood pressure, DBP=diastolic blood pressure, SEM=standard error of the mean, N-Albuminuria= normo-albuminuria, M-Albuminuria=microalbuminuria.

Table 4.5 MEAN FASTING BLOOD GLUCOSE, GLYCATED HAEMOGLOBIN, SERUM AND URINE CREATININE, URINARY ALBUMIN AND ALBUMIN - CREATININE RATION (MEAN±SEM) AMONG NORMO- AND MICROALBUMINURIC DIABETIC PATIENTS

Subjects	n	FBG (mmol/L)	GHbA1c (%)	UCr (µmol/L)	SCr (µmol/L)	UAlb (mg/L)	ACR (mg/mmol)
N-Alb	131	5.9±0.22	7.0±0.15	5916±503	81.0±1.70	13.4±0.45	1.46±0.06
M-Alb	39	8.1±0.59	8.3±0.53	10312±409	78.0±4.1	41.8±7.92	7.17±0.89
P-Value		< 0.001	< 0.05	< 0.001	> 0.05	< 0.001	<0.001

n, sample size; FBG, Fasting blood glucose; GHbA1c, glycated haemoglobin; UCr, Urine creatinine; SCr, serum creatinine; UAlb, urine albumin; ACR, albumin creatinine ratio; SEM, standard error of mean; N-Alb, normo-albuminuria; M-Alb, microalbuminuria

TABLE 4.6 DISTRIBUTION OF MICROALBUMINURIA IN DIFFERENT DURATION OF DIABETES DIAGNOSIS

DOD (yr)	n (%)	Microalbuminuria(%)	
0 - 5	118(69.4)	25	(21)
6 -10	37(21.8)	10	(27)
>10	15(8.8)	6	(40)

DOD=duration of diagnosis, n = number

4.7 DISTRIBUTION OF MICROALBUMINURIA IN GLYCAEMIC CONTROL AMONG DIABETIC PATIENTS

Out of the total 170 patients in the present study, 35.9% fell within good glycaemic control (≤6.39%) with 11.5% having abnormal ACR. Those at borderline (6.39-7.13%) were 24.7% having abnormal ACR while 39.4% had a poor glycaemic control (≥7.13%) and were observed to have 28.4% of ACR abnormality as shown in table 4.7.

4.8 CALIBRATION OF THE METHOD FOR URINARY ALBUMIN

A typical plot of absorbance versus concentration of albumin standard (0.00 – 400 mg/L) measured in duplicate is shown in fig 4.1 on a Beckman coulter DU-520 spectrophotometer. Table 4.8 shows the absorbance measurement for series of albumin calibrators.

4.9 RELATIONSHIP BETWEEN AGE AND BIOCHEMICAL ANALYTES IN DIABETIC PATIENTS AND CONTROLS

There was no statistically significance between Age and FBG in both patients (r =0.082, p=0.287) and control (r=0.025, p=0.807). There was also no correlation between age and urine creatinine in patients (r=0.114, p=0.137) but a negative and statistically significant correlation was observed in control (r=0.263, p=0.008). A positive and statistically significant correlation was seen between age and serum creatinine in both patient and control groups, (r=0.216, p=0.005 and r= 0.244, p=0.014 respectively) as shown in fig. 4.2 and fig 4.3. A negative but statistically insignificant relation was found between age and urinary albumin in patients (r=0.006, p=0.933) and controls (r=0.072, p=0.476). There was no statistical difference between age and glycated haemoglobin in patients (r=0.080, p=0.303) but a positive and statistically significant relation was observed in

Table 4.7 DISTRIBUTION OF MICROALBUMINURIA IN GLYCAEMIC CONTROL AMONG DIABETIC PATIENTS

GHbA1c (%)		n (%)	Microalbuminuria (%)
≤6.39	(good control)	35.9	11.5
6.40-7.13	(Borderline)	24.7	14.3
≥7.13	(poor control)	39.4	28.4

GHbA1c, *Glycated* haemoglobin; n, sample size; %, per cent

Table 4.8 Calibration of the Method for Measurement of Urinary albumin on Beckman Coulter DU-520 spectrophotometer at 340nm.

Calibrators	A	B	C	D	E
Concentration (mg/L)	0	20	32	160	400
Absorbance (340nm)	7	16	20	38	52

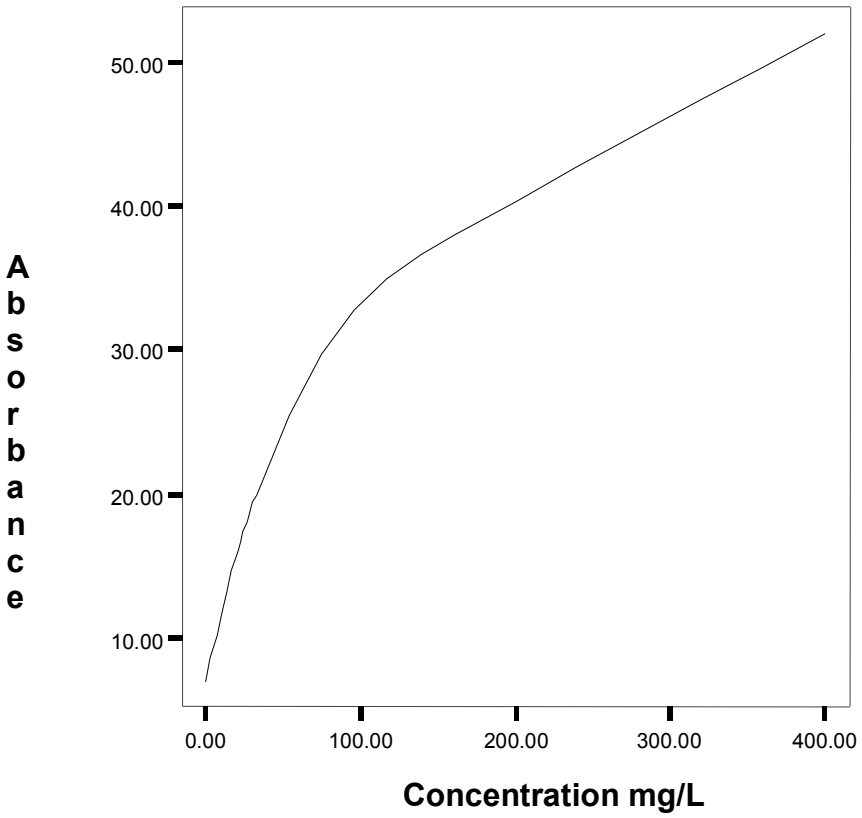


Fig. 4.1 Standard Calibration Curve for Urinary Albumin Estimation on Beckman Coulter DU-520 at 340 nm.

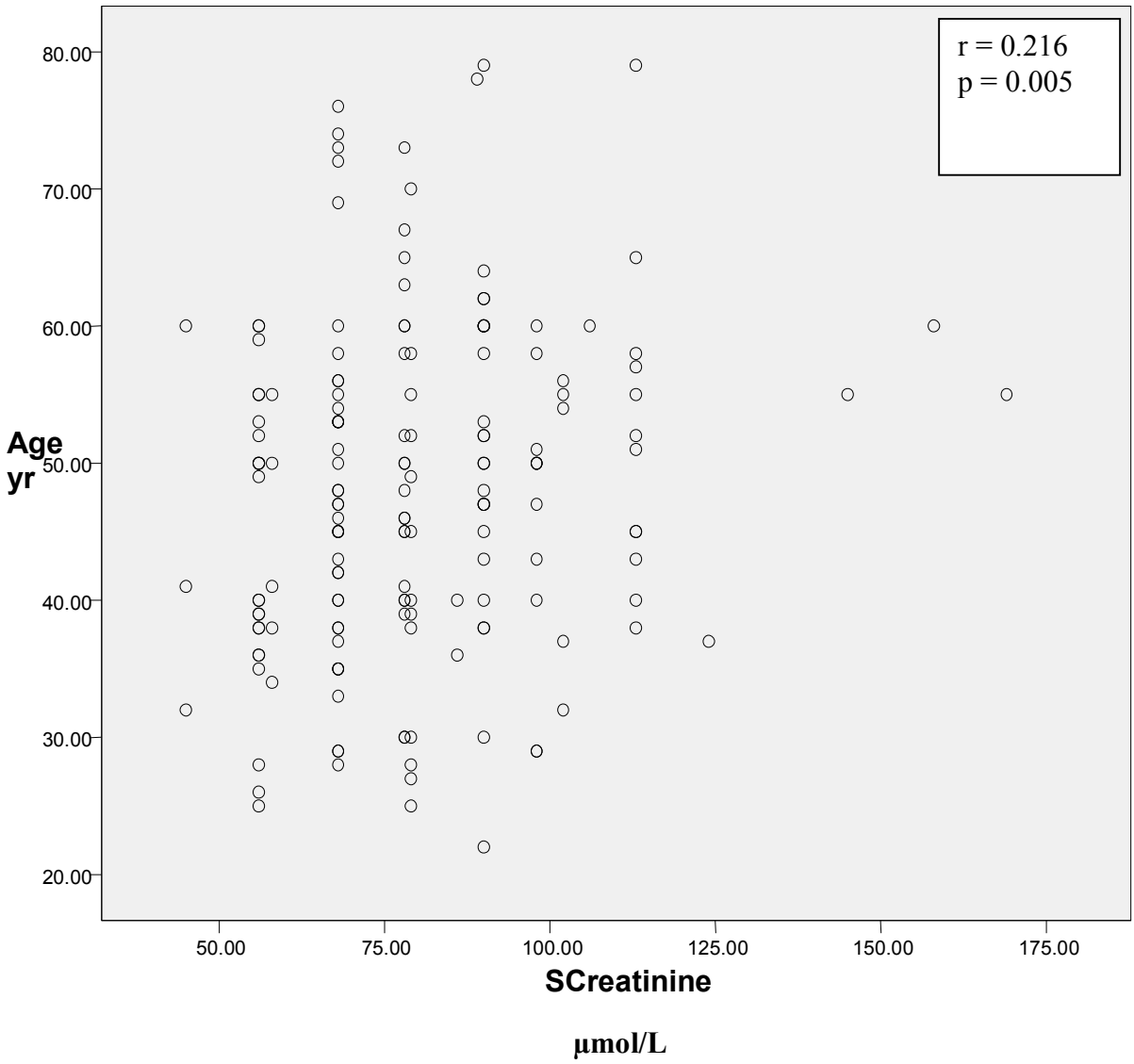


Fig 4.2, Relationship between Age and serum creatinine in patients group

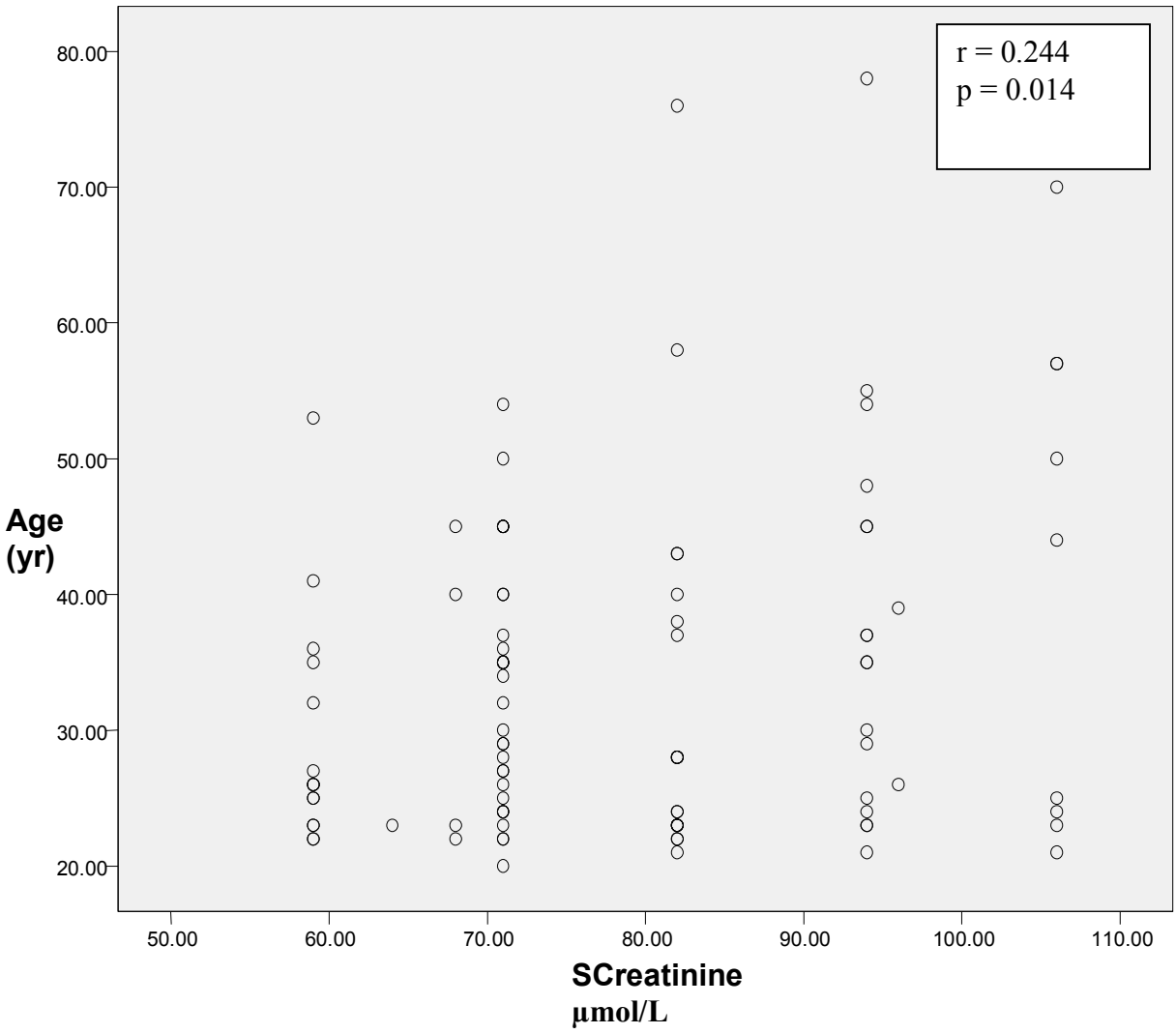


Fig. 4.3 Relationship between Age and Serum Creatinine in Controls

control (r=0.233, p=0.020) as shown in figure 4.4. There was a negative and statistically insignificant correlation between age and albumin-creatinine ratio in patients (r= -0.045, p=0.556) and controls (r=0.058, p=0.564).

4.10 RELATIONSHIP BETWEEN DURATION AND BIOCHEMICAL ANALYTES IN DIABETIC PATIENTS

There was no statistically significant correlation between duration and fasting blood glucose (r=0.027, p=0.728). A non statistically significant correlation was found between duration and urinary creatinine (r=0.034, p=0.658). A positive and statistically significant correlation was observed between duration and serum creatinine (r=0.152, p=0.047) as in fig. 4.4. A negative and non statistically significant relation was observed between duration and urinary albumin in patients (r= -0.380, p=0.623). There was no statistically significant relation between duration and *glycated* haemoglobin in patients (r=0.077, p=0.321). A negative but statistically insignificant correlation was observed between duration and albumin-creatinine ratio (r= -0.015, p=0.847).

4.11 RELATIONSHIP BETWEEN BMI AND BIOCHEMICAL ANALYTES IN BOTH PATIENTS AND CONTROLS

There was no statistically significant relation between BMI and FBG in patients and controls, r=0.082, p=0.290 and r=0.196, p=0.290 respectively. A positive and statistically significant relation were observed between BMI and SBP (r = 0.273, p = 0.001) and DBP (r = 0.183, p = 0.017). A statistically insignificant correlation was observed between BMI and serum creatinine in both patients (0.040, p=0.603) and controls (r= - 0.020, p=0.844). There was no statistical significant relation between BMI and urinary albumin in patients and control, (r=0.069, p=0.375 and r=0.080, p=0.426) respectively. A negative but

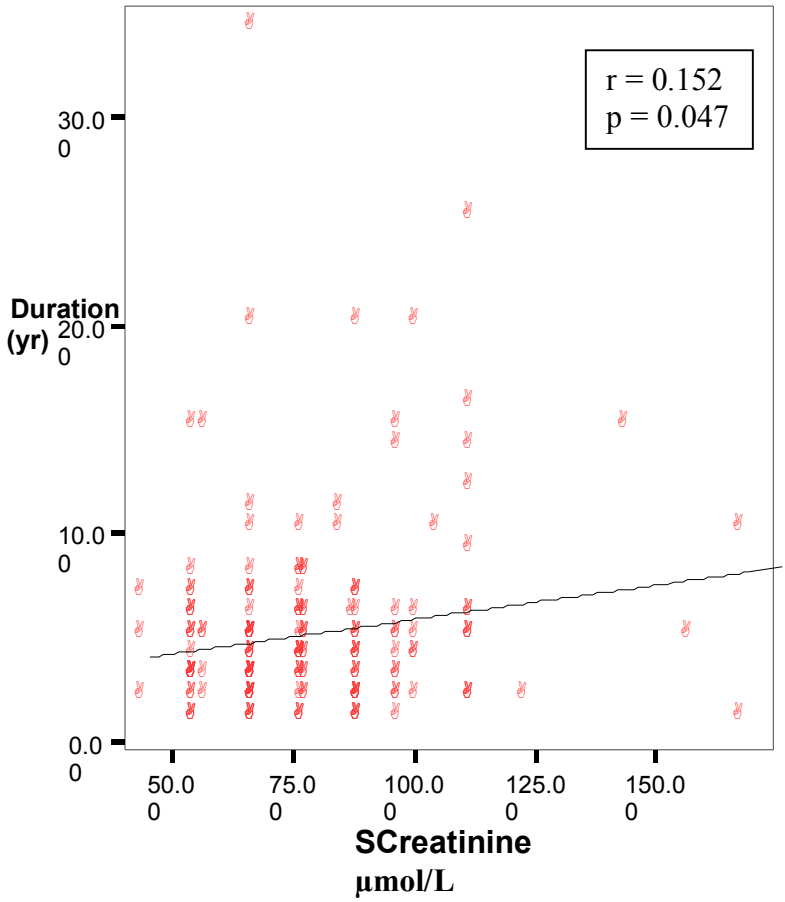


Fig. 4.4 Relationship between Duration and serum Creatinine in Patients

statistically insignificant relation was observed between BMI and *glycated* haemoglobin in both patients (r= - 0.067, p=0.385) and controls (r= - 0.054, p=0.591). A non-statistically significant difference was observed between BMI and ACR in patients (r=0.021, p=0.782) but statistically significant in controls (r=0.216, p=0.031).

4.12 RELATIONSHIP BETWEEN BMI AND BIOCHEMICAL ANALYTES AMONG NORMO- AND MICROALBUMINURIC PATIENTS AND CONTROLS

There was no statistical relation observed between BMI and FBG in normo-albuminuric patients (r=0.092, p=0.289) and controls (r=0.965, p=0.05). Similarly, no significant relation was observed with UCr, SCr, UAlb, GHbA1c and ACR (p= >0.05).

In the albuminuric class of the diabetic patients, similar non-statistical significant correlations were observed between BMI and the biochemical analytes ($p \geq 0.05$).

4.13 RELATIONSHIP BETWEEN AGE AND BIOCHEMICAL ANALYTES AMONG NORMOALBUMINURIC AND MICROALBUMINURIC DIABETIC PATIENTS AND CONTROLS

Among the normoalbuminuric class there was a non-statistically significant relation between age and FBG in both patients (r=0.053, p=0.542) and controls (r=0.004, p=0.965). A negative statistical significant relation was observed between age and urinary creatinine in patients (r= - 0.182, p=0.034) and controls (r= - 0.254, p=0.011). Another significant relation was seen in age and SCr in patients (r=0.215, p=0.012). A non-statistically significant correlation was observed in both patients and controls (r=0.037, p=0.667 and r=0.061, p=0.544 respectively) between age and ACR. A statistically significant relation was observed between age and UAlb in patients (r=0.181, p=0.035) and negative but non-statistically significant in controls (r= - 0.064, p=0.526).

In relating age with GHbA1c, a non-statistically significant relation was observed in patients (r=0.098, p=0.225) but a significant relationship was observed in controls (r=0.217, p=0.03) as in fig. 4.5.

The correlation between age and biochemical analytes among microalbuminuric patients, no positive or negative relation was obtained between age and all the biochemical analytes ($p > 0.05$).

4.14 RELATIONSHIP BETWEEN *GLYCATED* HAEMOGLOBIN AND OTHER BIOCHEMICAL ANALYTES

A negative and statistically significant relation was observed with UCr (r= -0.157, p=0.040). Positive and statistically significant relation was observed with FBG (r=0.189, p=0.013) and negative statistically insignificant correlation with SCr (r=0.124, p=0.107). A positive and statistically significant correlation was also observed with ACR (r=0.375, p=0.001) as shown in fig. 4.6.

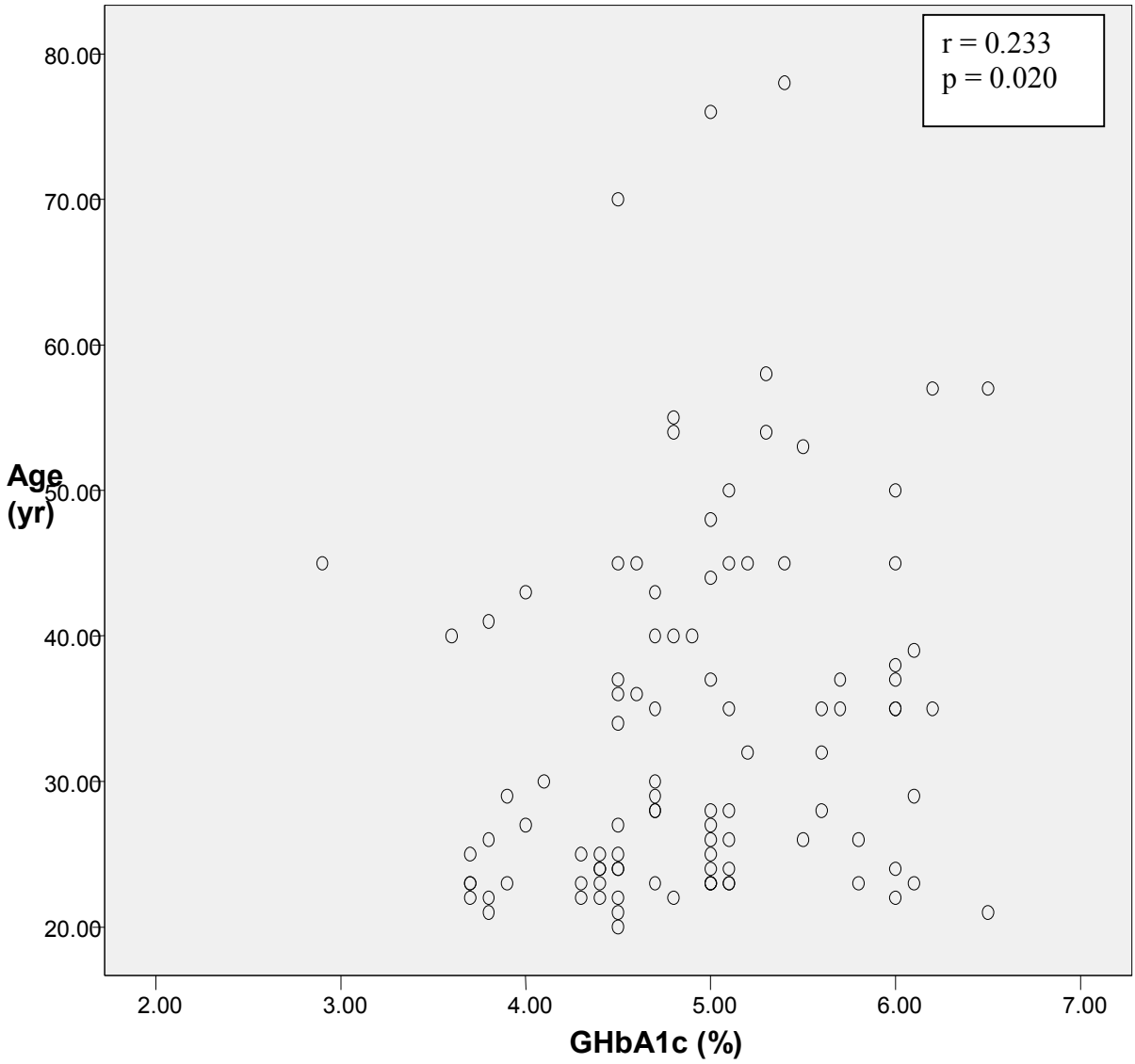


Fig.4.5 Relationship between Age and Glycated haemoglobin in Normo-Albuminuric controls

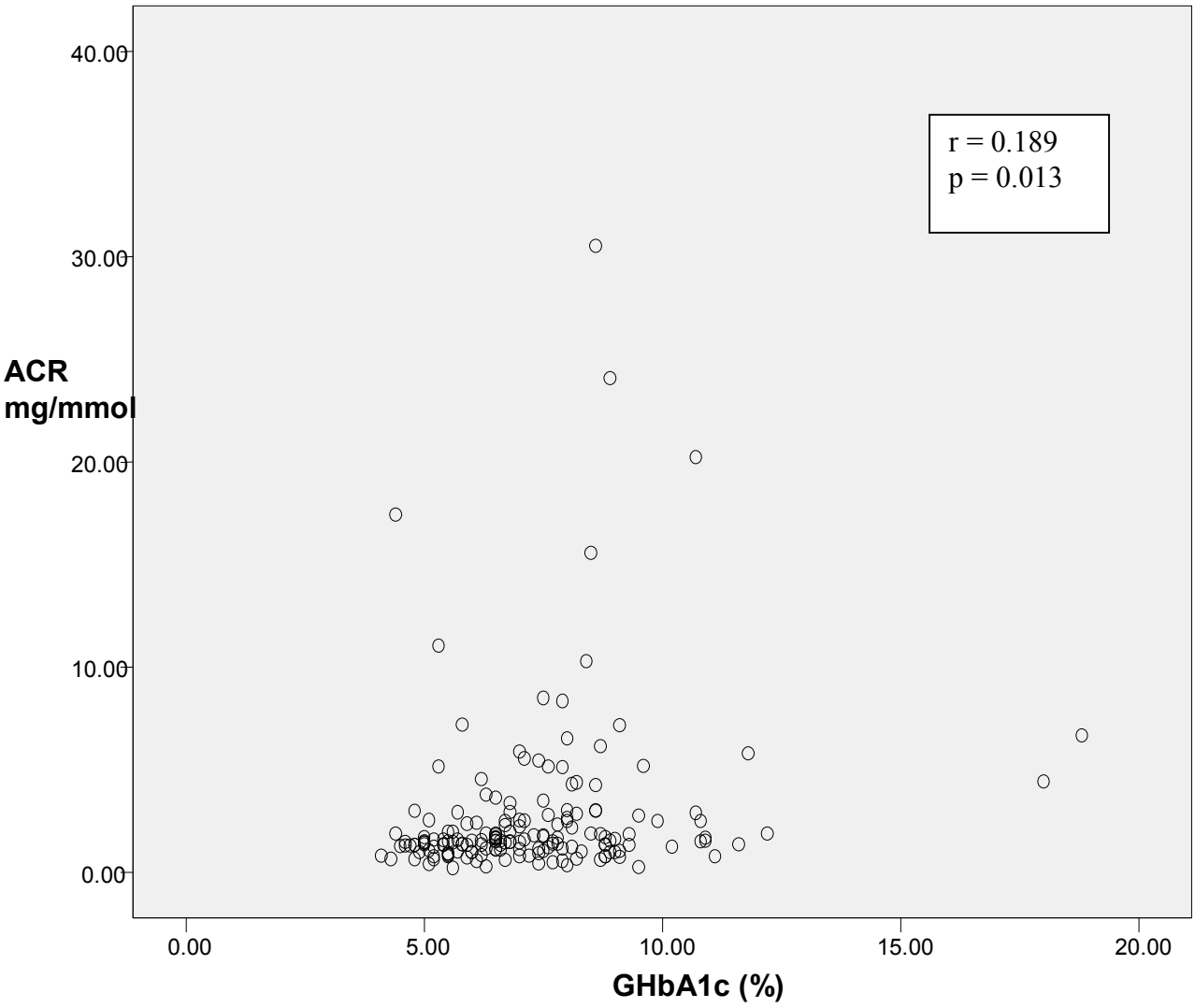


Fig 4.6 Relationship between ACR and Glycated haemoglobin in patients

CHAPTER FIVE

5.0 DISCUSSION

Diabetes Mellitus is a common condition worldwide. One of the complications is diabetic nephropathy which is detected in its early stage by the presence of microalbuminuria. Microalbuminuria, a sign of endothelial dysfunction may herald the impending and serious renal impairment. In the present study, microalbuminuria occurred in 23% of diabetic patients comparable to a findings among the Caucasian type II diabetic patients in whom a prevalence of 21% was reported by Khikhava *et al* (1989), 20% by Torffvitt *et a,* (1991) and 22% as reported by Parving *et al* (1988). This is lower than the prevalence of 38% reported by Orluwene *et a,* (2008) in Port Harcourt, 37.6% in Lagos by Iwalokun *et al* (2006), 50% reported in Benin by Unuigbe *et al* (2001) and 52% by Erasmus *et al* (1992) in a study conducted in Ilorin. Most of the Nigerian studies quoted above used "MICRAL" a strip for urinary albumin assessment. The MICRAL test strip was less specific than the immunoturbidimetric method employed in the present study as found by Lutale *et al* (2007). Differences in design, setting, methods for collection of urine and assessment of microalbuminuria, and patient populations may contribute to the differences found between these studies.

Poor glycaemic control is a well defined contributor to the development and progression of microalbuminuria among Type 2 patients as well as Type 1 patients (DCCTs 1993, UKPDS 1998, Rakesh *et al,* 2009, ADA 2009). Of the total 170 patients assessed, 131 (77%) were normoalbuminuric and 39 (23%) had microalbuminuria. Of these, 35.9% were in good control having GHbA1c < 6.39%, 24.7% at a borderline with GHbA1c between 6.39-7.13% and 39.4% had poor glycaemic control. Among these categories,

microalbuminuria was found to be 11.5%, 14.3% and 28.4% respectively. Thus, there is a simple linear relationship between GHbA1c and microalbuminuria. The mean value of GHbA1c was significantly higher in patients (7.2±0.16) than controls (4.9±0.07) ($p < 0.001$). This signified the clinical usefulness of GHbA1c assay in evaluating glycaemic control among diabetic patients in this environment. Comparing the mean values of GHbA1c between normo-albuminuric and microalbuminuric diabetic patients, a higher and statistically significant difference was observed in microalbuminuric than normo-albuminuric patients ($p<0.05$). This suggests that control of GHbA1c is a strong risk factor associated with progression of microalbuminuria and that controlling hyperglycaemia will lead to halting or slowing the progression of diabetic nephropathy to ESRF. In contrast, most of the available studies from sub-Saharan African (Lutale *et al,* 2007) fail to demonstrate any significant relationship between the level of glycaemic control and microalbuminuria.

Duration of diabetic diagnosis has been found to be a risk factor in the development of microalbuminuria in diabetic nephropathy. Out of the total patients in the present study, 118 (69.4%) fell within 0-5years of diagnosis and 25(21%) of them had abnormal ACR. Thirty seven (21.8%) had a DOD of >5 to ≤11 years and 10(27%) of them had abnormal ACR while 15(8.8%) of those with equals to or greater than 11years of diagnosis had 6(40%) of ACR abnormality. This agrees with the relative finding of Rakesh *et al* (2008) in UK and Iwalokun *et al* (2006) at Lagos where they observed a linear relation between duration of diabetes and the incidence of microalbuminuria.

Hypertension could occur as a coexisting condition or develop as a complication of diabetes mellitus. Elevated blood pressure is documented as the most significant

contributing factor in the pathogenesis and the progression of abnormal ACR and eventually development of diabetic nephropathy in both Type 1 and Type 2 diabetic patients (Timothy *et al,* 2000 and Mogensen, 2003). In the present study, the mean SBP and DBP were lower among control subjects than diabetic patients group. When SBP and DBP of patients with normal ACR were compared with those with abnormal ACR, a higher BP was noticed in those with abnormal ACR but there was no significant difference. This agrees with the report of Lutale *et al* (2007).

Obesity is probably the most important modifiable acquired risk factor in the aetiology of type II diabetes found in many cross sectional studies. The resultant changes lead to mal development in the endocrine pancreas and the liver, leading to reduced capacity for insulin secretion and also insulin resistance. This leads to an increased susceptibility to type II diabetes in adult life. Assessment involves the estimation of body mass index (BMI) as a ratio of weight and height. A significantly higher BMI was observed in patients with diabetes mellitus than in controls ($p<0.001$). This is supported by the fact that obesity leads to an increase susceptibility to type II diabetes mellitus, which if not properly controlled may lead to diabetic nephropathy. Comparing BMI between normo- and microalbuminuric patients revealed higher among microalbuminuric but no significant difference. Comparison made between male and female patients revealed no statistically significant differences among all the clinical and biochemical analytes except BMI ($p=0.001$). This agreed with previous finding in this environment by Bakari *et al,* 2006. This could be explained partly, apart from the genetic factor, due to the cultural preference to remain in-doors with less physically active engagement.

Major clinical complications were encountered in a study of 747 diabetic patients in Zaria, among which nephropathy accounted for 3.9% (Bakari *et al,* 2006) as second to hypertension with 47% as a cause of chronic renal failure. These results excluded microalbuminuria as a risk factor of renal failure in diabetes mellitus.

Therefore, from the present study, it has been shown that detection of microalbuminuria as early as possible in the course of the disease is very important in the prevention of diabetic nephropathy. Measurement of microalbuminuria is therefore an adjunct in this direction.

CONCLUSION

The conclusion that could be drawn from the study includes;

1. Reference values of 0.14-1.78 mg/mmol and clinical decision limit of 2.19 mg/mmol for microalbuminuria was established.
2. The observed prevalence of microalbuminuria in the present study is 23%.
3. Reference values of 3.43 – 6.39 % and a clinical decision limit of 7.13 % for glycated haemoglobin was established.
4. Chronic poor glycaemic control was the only modifiable factor associated with the presence of microalbuminuria.
5. In diabetic patients the duration in diabetes, age, GHbA1c and serum creatinine relate positively in the progression of microalbuminuria in diabetic patients.

RECOMMENDATIONS

1. Measurement of micro-albuminuria and glycated haemoglobin should be included in the routine investigations for the management of diabetic patients. This will help to reduce the incidence of diabetic nephropathy and the rate of mortality and morbidity among these patients.
2. Since microalbuminuria was found to be influenced by hypertension, its assessment may assist in detecting those at risk of developing kidney problem at an early stage for prevention and or treatment.
3. Diabetic patients at risk of developing micro-cardiovascular disease and retinopathy should have their microalbuminuria assessed early for good management and control.

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APPENDICES

CONSENT FORM

Serial No……..

Hospital No……..

Age……..

Consent Form to participate in a study

The research is designed to determine the PREVALENCE OF MICROALBUMINURIA AMONG DIABETIC PATIENTS ATTENDING ABUTH, SHIKA-ZARIA. Blood and urine will be required for the study.

The results obtained will be treated with confidentiality.

You are free to participate or decline in this study without any consequence.

This study will hopefully improve your present health status and that of others in the future.

If you desired to participate in the study please kindly sign the attached form below.

Thank You.

Appendix (i)

INFORMED WRTTEN CONSENT

I…….....of……...

agreed to participate in this study of Prevalence of Microalbuminuria among Diabetic Patients Attending ABUTH, Shika - Zaria. The full procedure and the probable benefits of the test were fully explained to me.

I was informed that a sample of my blood and urine will be collected for the test. If I so wish, the test results will be communicated to me in confidence.

I made this consent willingly without being subjected to any pressure.

Participant Name……..... Signature……

Witness Name…… Signature……...

Researchers Name……. Signature……

Appendix (ii)

PROFORMA Date……....

S/No…… Diabetes type……..

Hospital No……. Ethnicity……...

Age……. Sex…….

Weight…… BMI……

Height……. Blood Pressure……

Sex…….

Education: Primary Secondary Tertiary

Occupation……

MedicalCondition……

……

Drugs on Use……..

RESULTS:

Albuminuria..... …….....(ug)

Serum Urea…….(mmol/l)

Serum Creatinine…….(umol/l)

Urine Creatinine……..(umol/l)

HbA1c……(%)

Fasting Blood Glucose……..(mmol/l)

URINALYSIS.....

.....

.....

Appendix (iii)

Random Blood Glucose…….(mmol/l)

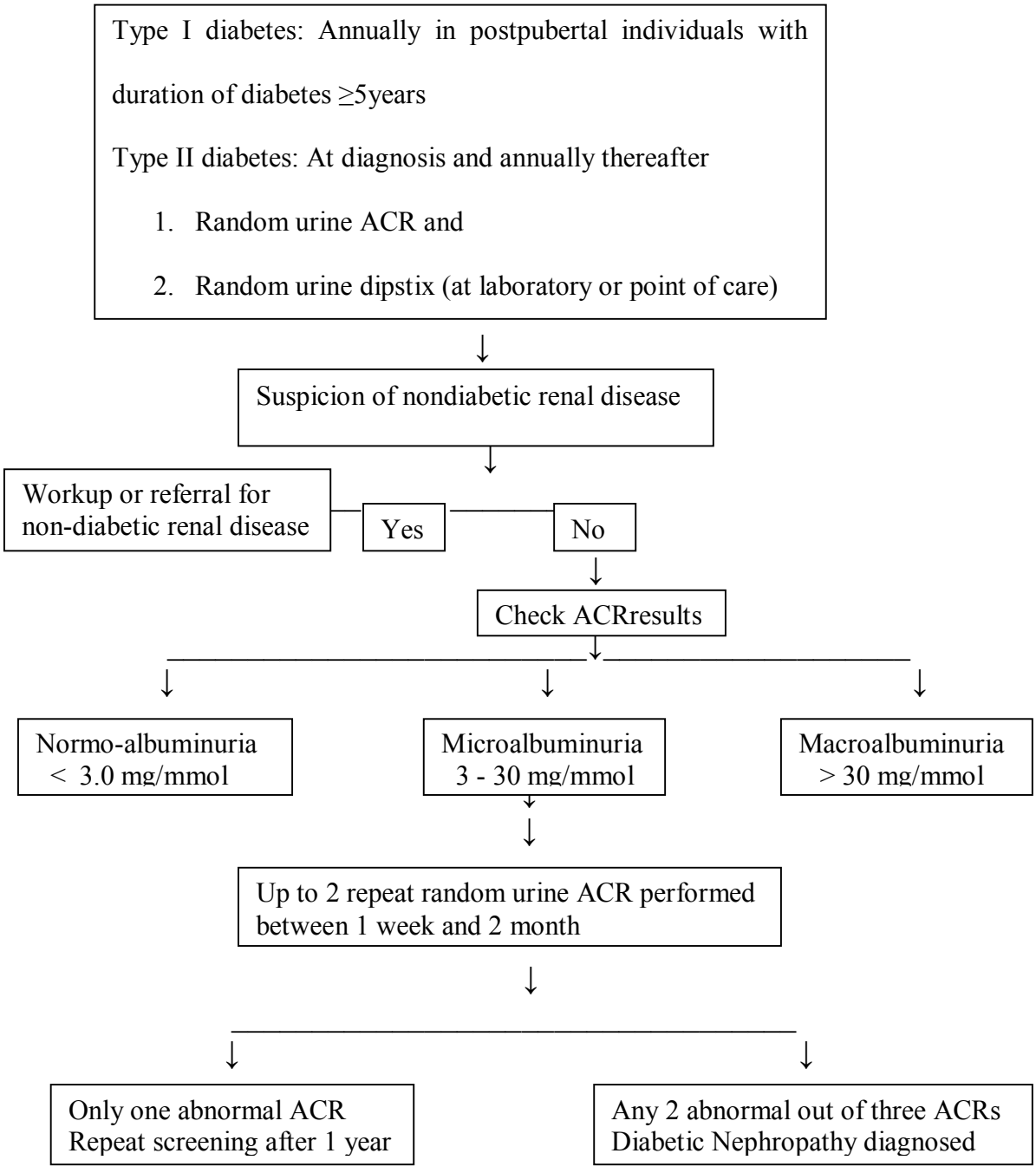
Sodium……..(mmol/l)

Potassium……..(mmol/l)

Chloride…….(mmol/l)

Bicarbonate……(mmol/l)

SCREENING FOR DIABETIC NEPHROPATHY AND NONDIABETIC RENAL DISEASE



Appendix (iv)