PREVALENCE AND PATTERN OF ALBUMINURIA AND ITS
CLINICAL AND BIOCHEMICAL CORRELATES IN SICKLE
CELL ANAEMIA PATIENTS IN BENIN CITY

DISSERTATION SUBMITTED TO NATIONAL
POSTGRADUATE MEDICAL COLLEGE OF NIGERIA IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
FELLOWSHIP IN INTERNAL MEDICINE
(SUB-SPECIALTY: NEPHROLOGY)

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SUMMARY

Background: Sickle cell nephropathy is one the chronic complications of sickle cell disease and a major cause of mortality. The prevalence is likely to increase because sickle cell anaemia (SCA) patients now survive longer due to availability of better health care services which has led to improvement in their care. Albuminuria has been identified as an early marker of sickle cell nephropathy. The aim of the study was to determine the prevalence and pattern of albuminuria and its clinical and biochemical correlates in SCA patients in Benin city.

Methodology: This was a cross-sectional analytical study that involved consecutive 220 SCA patients attending sickle cell centre, Benin and 110 controls that fulfilled the inclusion criteria. The study was carried out over a 3 month period. Spot urine was assessed for albumin creatinine ratio in the study population and the prevalence and pattern of albuminuria were determined. Association was determined between albuminuria and age, body mass index, blood pressure, packed cell volume (PCV), serum creatinine, lactate dehydrogenase, aspartate transaminase, estimated glomerular filtration rate (GFR) and lipid profile in the SCA group. P –value of < 0.05 was taken as significant.

Results: The mean age of the SCA group was 23.08±8.68 years while that of the control was 24.31±4.95 years. The male:female ratio was 1:1.04 for both groups. The prevalence of normoalbuminuria, microalbuminuria and macroalbuminuria in the SCA vs control were 35.5% vs 90.1%, 50% vs 9.1% and 14.5% vs 0% respectively. There was significant association between albuminuria and dyslipidaemia, vaso-occlusive crises and blood transfusion in the SCA patients. There was significant positive correlation between albuminuria and serum creatinine, total cholesterol and low density lipoprotein cholesterol. There was significant negative correlation between albuminuria and high density lipoprotein cholesterol, PCV and estimated GFR. The significant predictors of albuminuria after multiple linear regression were PCV and serum creatinine.

Conclusion: There was significant association between albuminuria in SCA patients and age, serum creatinine, estimated GFR, PCV, severity of disease and dyslipidaemia in this study. Albuminuria may reflect an early stage of renal disease in SCA patients.
CHAPTER ONE

1.1 INTRODUCTION

Haemoglobinopathies are disorders affecting the structure, function or production of haemoglobin. These conditions are usually inherited and differ in severity. Different forms may present as haemolytic anaemia, cyanosis or vaso-occlusive crises (VOC). Haemoglobin is critical for normal oxygen delivery to tissues. It is also present in erythrocytes in such high concentrations that it can alter red cell shape and deformability.

Sickle cell disease (SCD) is the commonest structural haemoglobinopathy occurring in heterozygous form in 8% of American blacks. About 2% to 3% of American blacks carry haemoglobin C allele.\(^1\) Up to 200,000 cases of sickle cell anaemia (SCA) is recorded yearly in Africa.\(^2\) It is particularly common among people whose ancestors come from sub-Saharan Africa, India, Saudi Arabia and Mediterranean countries. Migration has raised the frequency of the gene in the American continent. The prevalence is 20 per 100 births in Nigeria and it constitutes greater than 9% of under-five mortality in West Africa.\(^2\) The median survival was estimated to be 42 years for men and 48 years for women in United States of America.\(^2\) This is slightly higher in Jamaica having 53 years for men and 58.5 years for women.\(^2\) There are no similar data on survival of patients with SCA in the African continent.

The management of SCA in most countries where it is prevalent is inadequate and systematic screening is not a common practice. Diagnosis is often made when patients present with severe complications.

Over the past 10 years, considerable progress has been made in several respects; such progress includes long term treatment with hydroxyurea and this has decreased instances of painful crisis
and improved quality of life.\textsuperscript{1} Others include bone marrow transplants for cure of SCA, better imaging studies, prompt management of life threatening complications like stroke and acute chest syndrome and regular blood transfusion programmes associated with iron chelation have been reported to prevent complications such as sickle cell nephropathy (SCN).\textsuperscript{2} However, these advances which are mainly applicable in high resource countries have unfortunately widened the gap in terms of quality of life between patients in developed and developing countries.\textsuperscript{2} These advances have also increased survival of SCA patients to adulthood with accompanying manifestation of end-organ damage such as renal failure.\textsuperscript{3} Thus, SCA patients now have better quality of life, increased survival due to better understanding of the pathogenesis and management of the disease and as a consequence of the above, the prevalence of chronic complications such as SCN has significantly increased.

The kidney is highly vascular receiving about 25\% of the total cardiac output. The low oxygen tension in the medullary interstitium makes it very vulnerable to vaso-occlusive events. Renal involvement in SCA patients that includes structural and functional abnormalities of the kidneys in the absence of any other aetiology is termed SCN.\textsuperscript{4} These abnormalities include glomerular disease presenting with albuminuria, tubular dysfunction presenting with renal tubular acidosis, increased sodium and phosphate reabsorption, medullary disease presenting with haematuria, renal papillary necrosis and loss of concentrating ability of the tubules and malignancy like renal medullary carcinoma. These abnormalities in renal function can progress and deteriorate to chronic kidney disease (CKD) and ultimately, end-stage renal disease (ESRD). Prevalence of renal failure in SCD ranges between 5\% and 18\% of the total population of SCA patients.\textsuperscript{5}
1.2 JUSTIFICATION FOR STUDY

Microalbuminuria (MA) has been identified as an early marker of glomerular injury in patients with SCA thus, an early marker of SCN.6 Factors such as age, gender, blood pressure (BP), anthropometry have been reported to be associated with renal function in SCA patients.7 Determining factors that are associated with albuminuria in SCA patients will serve as therapeutic targets in the management of albuminuria in these patients. This will be helpful in retarding the progression to end stage renal disease, reducing cardiovascular morbidity and overall mortality in these group of patients.

There are few available reports on the prevalence of MA amongst adult SCA patients in sub-Saharan Africa, particularly in Nigeria which has the highest prevalence and attendant burden of SCA. It is therefore, hoped that this study will contribute to filling part of the gap in the present knowledge, raise further in-depth research especially in Africa on SCN which will culminate in improved care.

1.3 AIMS AND OBJECTIVES

1.3.1 General Aim

The general aim of this study is to determine the level of albuminuria and its clinical and biochemical correlates in SCA patients in Benin City.

1.3.2 Specific Objectives

1.3.2.1. To determine the prevalence of normoalbuminuria, MA and macroalbuminuria among adult SCA patients.
1.3.2.2. To determine the relationship between albuminuria and age, gender, BP, chronic leg ulcer, haematocrit, serum creatinine, glomerular filtration rate (GFR), body mass index (BMI), waist; hip ratio and serum lipid profile in adult sickle cell anaemia patients.
CHAPTER TWO
LITERATURE REVIEW

Sickle cell nephropathy is one of the end-organ complications that may occur in patients with SCD. Platt et al reported that 18% of deaths could be ascribed to chronic end-organ involvement which predominantly is of renal cause. The prevalence of CKD among Nigerian SCA patients was reported to be 37.2% by Arogundade et al. The diagnosis of CKD in the SCA patients was determined in this study by the presence of proteinuria, haematuria or GFR of less than 60mls/min. This prevalence is likely to increase because SCA patients now survive longer due to availability of better health care services which has led to improvement in their care.

The hallmark of SCN is a combination of impaired renal concentrating capacity and normal diluting capacity. The relative hypoxia and hypertonicity in the renal medulla favour sickling of red cells in the vasa recta. This causes formation of intravascular microthrombi and obstruction of blood flow through the vasa recta, resulting in impairment of the counter current exchange mechanism.

There is also defective urinary acidification and potassium excretion in the distal tubules probably due to failure to maintain the electrochemical and hydrogen gradient along the collecting ducts due to impaired medullary blood flow and hypoxia. There is increase in renal blood flow, plasma flow and to a lesser extent, in GFR due to release of vasodilators such as nitric oxide and prostangladin. The hyperfiltration in the glomeruli leads to glomerulomegaly which causes glomerular injury when it is sustained, causing the characteristic classical focal segmental glomerulosclerosis.

Focal segmental glomerulosclerosis was reported as the commonest biopsy proven glomerular disease in SCA patients by Maigne et al, but Arogundade et al reported mesangioproliferative glomerulonephritis as the commonest glomerular disease in south-west Nigeria. Others are
thrombotic microangiopathy and less commonly minimal change disease. The classical mesangioproliferative is an immune complex disease that is associated with hypocomplementaemia, but this is different from the mesangioproliferative glomerulonephritis found in SCA patients where complement system does not play a role in the pathogenesis. Fragmented red cells lodge in the glomerular capillaries and they become phagocytosed by the mesangium which leads to mesangial expansion and duplication of the basement membrane. Chronic use of analgesics to relieve pain during VOC may also contribute to renal disease in SCA patients. They also receive frequent transfusions on account of severe anaemia during periods of crises and these expose them to hepatitis B and C infections which could also contribute to glomerular disease.

2.1 MICROALBUMINURIA/PROTEINURIA

Microalbuminuria has been identified as an early marker of glomerular injury in patients with SCA, thus an early marker of SCN. Microalbuminuria is albumin excretion in the urine above the normal range but below the clinically detectable level by standard dipstick screening test. It is defined as the presence of 20-200 microgram/minute or 30-300 milligram/day of albumin excretion in urine. It may also be defined as albumin:creatinine ratio (ACR) in a spot urine of equal or greater than 30-300 milligram/gram. There are different methods of quantifying urinary albumin such as immunoturbidimetry, immunonephelometry, radioimmunoassay or enzyme immunoassays. Though these methods vary in sensitivity, they are all considered equivalent when quality control is maintained. Potential confounders of MA are fever, severe hypertension, urinary tract infections, heart
failure, stress, menstruation, heavy exercise. All these factors may cause transient MA and must be taken into consideration when interpreting MA as a marker of kidney damage.

Albuminuria is an early marker of renal disease in patients with hypertension and diabetes mellitus (DM).\textsuperscript{17,18} Prevalence of MA varies in different groups of patients. It ranges between 10-42\% in diabetics, 11-40\% in hypertensives and 5-9\% in normal individuals.\textsuperscript{19} Microalbuminuria is the first sign of diabetic nephropathy in patients with DM and it has been reported that 20\% - 45\% of type I DM patients with MA will progress to overt nephropathy without intervention.\textsuperscript{10} Also, MA has been found to be an early marker of renal dysfunction in patients with essential hypertension. This may due to the effect of systemic hypertension in causing increase in intra-glomerular pressure, thereby causing endothelial injury and subsequent albuminuria. In a study done in the south-west of Nigeria, the prevalence rate of MA was reported to be 32.4\% in patients who were newly-diagnosed to have essential hypertension.\textsuperscript{19}

The Microalbuminuria: A Genoa Investigation on Complications (MAGIC) study also reported that the prevalence of MA in patients with essential hypertension who were not diabetic was 6.7\%. It showed that MA is associated with worse cardiovascular risk profile and is a concomitant indicator of early target organ damage including the kidneys and the heart.\textsuperscript{20}

Albuminuria has also been established as an early marker of SCN which occurs following hyperfiltration and early glomerular injury even when the serum creatinine is within normal limit.\textsuperscript{21}

Majority of the studies done in adult SCA patients have been on macroalbuminuria with only a few on MA. The prevalence of proteinuria in SCA has been reported to be 28\% in Kano, 50\% in Ile-Ife, 41\% in south province of Saudi Arabia while the prevalence of MA in adult SCA patients was found to be 26\% in Jamaica and 40\% in Brazil.\textsuperscript{22-26} A similar study done in
Abidjan, Ivory Coast on adult and paediatric SCA reported a prevalence rate of 17.4%.\textsuperscript{27} The varying prevalence rates from different studies and populations could possibly be explained on the basis of differences in patient’s characteristics, method of assessment of albuminuria and SCD behaviour in different parts of the world. The assessment of proteinuria in the study done in Kano was by the use of dipstick which is unable to detect MA unlike similar study in Brazil where quantitative assessment of albuminuria was used. Higher prevalence of albuminuria was reported in study done in Brazil compared to the study done in Ivory Coast where both paediatric and adult SCA were studied. The former study was done in adults which could account for the higher prevalence since albuminuria has been reported to increase with age.

It has also been observed that there are differences in severity and clinical presentation of SCA patients with similar origins studied at different centres.\textsuperscript{26} This may be explained by a complex interaction between environmental and genetic factors in patient’s population with SCD. The factors modifying differences in presentation include level of fetal haemoglobin, presence of alpha gene deletion, beta gene cluster haplotypes and other genetic and environmental factors that are yet to be identified.\textsuperscript{28-31} The co-inheritance of alpha- thalassemia has protective effect against albuminuria in SCA patients. It ameliorates the haematologic severity of SCA as indicated by higher haemoglobin levels and lower reticulocyte count. Improved rheology and decreased adhesiveness of the sickled erythrocyte related to the expression of alpha-globin genes have a less damaging effect on the renal endothelium and possibly ameliorates the degree of glomerular involvement.\textsuperscript{28-30} Sickle cell anaemia patients from bini tribe in south-south Nigeria were reported to have less severe disease compared to those from yoruba tribe in south-west Nigeria.\textsuperscript{31} This was explained by presence of significantly lower level of haemoglobin A2, higher level of fetal haemoglobin and lower incidence of alpha thalassemia in the bini SCA patients
compared to the yoruba SCA patients. The presence of certain haplotypes like Central Africa Republic beta-s- haplotype which is present in some African SCA increases the risk of developing irreversible complications such as SCN at an early age compared to those with Benin haplotype

The pathophysiology of MA is not completely understood, but it has been postulated that it may be the result of altered intra-renal haemodynamics with elaboration of widespread low level inflammatory processes in the body vasculature. In patients with hypertension with or without DM, increasing urine albumin excretion is associated with elevated level of inflammatory markers like C-reactive protein, endothelial dysfunction and platelet activation which are markers of increased cardiovascular risk.

The renin-angiotensin-aldosterone system (RAAS) is directly involved in the regulation of BP, fluid volume and vascular response to injury and inflammation. The inappropriate activation of this system causes fluid retention, hypertension, inflammatory, thrombotic and atherogenic effects that may contribute to end-organ damage. The increase in glomerular pressure and subsequent glomerular permeability may also be an explanation for MA. Microalbuminuria has been established as a marker of cardiovascular risk. Microalbuminuria causes endothelial injury leading to increased permeability to atherogenic lipids which initiate the atherosclerotic process. It also causes haemodynamic strain and instability which could contribute to atherosclerotic process and eventually lead to adverse cardiovascular events such as congestive cardiac failure, left ventricular hypertrophy, acute coronary syndrome and stroke. There have been well established evidences linking MA to increased cardiovascular risk irrespective of the mechanisms involved. Thus, preventing or treating MA will reduce cardiovascular risk in affected individuals.
2.2 CLINICAL CORRELATES OF MICROALBUMINURIA IN SICKLE CELL DISEASE

Microalbuminuria has been found to have certain clinical correlations in SCA patients, both in paediatric and adult populations. Studies have been able to establish a correlation between MA and age, gender, GFR, haematocrit, previous history of early blood transfusion, height, severity of disease and hydroxyurea use.\textsuperscript{38-42}

There is no clear association between gender and MA in most of the reports in literature, but a study done in Benin city showed that MA was commoner in females with SCA.\textsuperscript{38} They postulated that factors that confounded proteinuria (such as haematuria, urinary tract infection and leucocyturia) which are commoner in females including those with SCA may be responsible for a higher prevalence of MA in females. However, other factors might have been responsible for the significant gender difference in the prevalence of albuminuria in this study since these confounders were part of the exclusion criteria used in their study population. A similar trend was also reported by Dharnidharka et al who noted that males had a MA prevalence of 20.7% compared to 32.7% in females.\textsuperscript{39} Eke et al also reported a higher prevalence of MA in female SCA, though this was not statistically significant.\textsuperscript{40}

There are conflicting reports in literature about the correlation between age and MA in SCD patients. Abhulimen-Iyoh et al studying a paediatric population in Benin city, Nigeria reported a positive correlation between age and MA.\textsuperscript{38} Dharnidharka et al reported a MA prevalence rate of 26.5% and 40% in paediatric and adult SCA patients respectively.\textsuperscript{39} Similar correlations have also been reported in other studies\textsuperscript{7,22,41,42} However, a study done in Brazil did not find any correlation between MA and age of SCA patients.\textsuperscript{25} On the other hand, Aoki and Saad did not
find a correlation between MA and age in Brazilian SCA patients while Aleem et al\textsuperscript{24,26} however, found a borderline positive correlation between MA and age. Age-related hyperfiltration and increase in urinary albumin excretion may be responsible for the positive correlation between albuminuria and age in these SCA patients.

Anaemia has also been reported to be correlated with albuminuria in SCA patients in both paediatric and adult age groups. Low haematocrit has served as a protective mechanism in SCD by reducing blood viscosity and thus, reducing the frequency of vaso-occlusive crisis. Some reports have shown that haematocrit is inversely related to the degree of MA. Studies done in Kano, Ife, Jamaica and Benin have shown an inverse correlation between albuminuria and haematocrit.\textsuperscript{7,22,25,38} Conversely, other reports did not show any correlation between MA and haematocrit.\textsuperscript{26,43} The relationship between anaemia is not completely understood, but may relate to the effect of anaemia in causing vasodilatation which results in increased blood flow to the organs including the kidneys. The hyperfiltration that results then causes glomerular injury and this leads to albuminuria in these patients.

In early childhood, GFR has been found to be supra-normal in SCA patients as a result of increased renal plasma flow due to vasodilatation of renal vessels, but falls steeply as the disease progresses culminating in renal failure.\textsuperscript{25,43} Thus, similar to what is seen in early stages of diabetic nephropathy, hyperfiltration is associated with albuminuria in SCA patients as well.\textsuperscript{44-47}

In agreement with above report, the prevalence of hyperfiltration in an adult SCA population assessed in the modification of diet in renal disease (MDRD) study was found to be 51\% with 49\% having hyperfiltration alone, 36\% having hyperfiltration with MA and 15\% having hyperfiltration and microalbuminuria.\textsuperscript{47} Apart from hyperfiltration being associated with MA in SCD, a positive correlation between GFR and MA has been reported by Thompson et al.\textsuperscript{25}
Earliest clinical abnormality in SCN is albuminuria and immunoglobin G excretion. During this stage, the ultrafiltration coefficient is reduced even though GFR is preserved. \(^{20}\) Albuminuria establishes renal injury in SCA patients. \(^{47}\)

Blood pressure has been found to be lower in SCA patients when compared with normal individuals of similar age and gender. This has been corroborated by studies done in Nigeria. \(^{48-50}\)

The determinants of BP in SCA patients have been found to be haematocrit, body surface area, BMI and frequency of crises. \(^{48}\) However, Lamarre et al reported that there was no association between BP and painful VOC. \(^{51}\) They reported that the male gender, increased serum triglyceride level, BMI and blood viscosity are risk factors for developing hypertension in SCA. \(^{51}\) The chronic hypoxic state found in SCA leads to release of vasodilatory substances such as nitric oxide, endothelial relaxing factor which result in reduced peripheral resistance, thus contributing to lower BP in SCA patients. \(^{52}\) Salt and water wasting due to medullary defect and reduced vascular reactivity which occur in SCA patients also contributes to the relative resistance to development of hypertension in them.

In contrast to other glomerulopathies, development of systemic hypertension is uncommon with SCN. The onset of hypertension in SCA patients may signal the onset of severe renal impairment. Again, reports in literature about association between BP and MA in SCA patients are at variance. While some authors reported a positive correlation between BP and MA in SCA patients, others did not find association between BP and MA in SCA patients. \(^{25,28,38,43}\) The positive correlation between increase BP and albuminuria may be explained by the effect of increase BP on the glomeruli, causing glomerular injury with subsequent albuminuria.

There are few studies on correlation between MA and frequency of both VOC and aplastic crises in SCA. Majority of these studies were done in paediatric SCA patients. Ataga et al found a
positive association between frequency of priapism, which is a form of VOC and MA in SCA.\textsuperscript{41} Arogundade et al also established that SCA patients with increased frequency of crises and hospital admissions are more likely to develop renal complications.\textsuperscript{7} Eke et al also reported association between prevalence of MA and frequencies of hospital admission.\textsuperscript{40} There was no association between MA and frequency of crises from studies done by Dharnidharka et al and Mc Burney et al.\textsuperscript{39,53} A study of adult Jamaican SCD patients reported a higher prevalence of MA which was 40\% in those with previous history of chronic leg ulcers; defined as ulcer persisting for at least six months, compared to 20\% in those without chronic leg ulcers.\textsuperscript{54} Increase frequency of VOC crises is associated with medullary interstitial ischaemia which is key in the pathogenesis of SCN.

Only a few studies have been reported in literature regarding association of MA and anthropometric parameters in SCA patients. Although, there is a positive correlation between MA and BMI in group of patients who are diabetic and hypertensive, an inverse correlation was found in Jamaican sickle cell children.\textsuperscript{55} This is similar to report by Arogundade et al where he found a positive correlation between GFR and BMI and suggested that improving nutrition in SCA patients may serve of one of the strategies to improve renal function in them.\textsuperscript{7} Central obesity has been found to be an independent risk factor for MA in non-diabetic subjects.\textsuperscript{56} Visceral adipocytes produce leptin and other inflammatory cytokines that cause damage to both endothelial and mesangial cells in the kidneys which subsequently leads to MA.\textsuperscript{57,58} However, similar association have not been reported in SCA. A study done in Ivory Coast showed an inverse correlation between MA and height by both univariate and multivariate analysis, although there was no satisfactory explanation for this association.\textsuperscript{27}
Some reports have attempted to study the relationship between repeated early transfusion and albuminuria in SCA patients. While Alvarez et al reported that repeated early transfusion reduces albuminuria in SCA patients, Dharnidharha et al did not find any association.\textsuperscript{39,59} Repeated transfusion early in life is believed to be renoprotective by reducing the frequency of VOC and sickling in the vas recta and glomerular capillaries which are central in the pathogenesis of SCN. Hydroxyurea and angiotensin-converting enzyme inhibitor (ACEI) have been proven by some studies to reduce the prevalence of albuminuria in those who are regular on these medications, and they are therefore thought to be renoprotective. Mckie et al found that 44\% of SCA patients who were persistently on hydroxyurea and 56\% of those who were on ACEI who had MA returned to normoalbuminuria.\textsuperscript{60} Arogunadade et al reported a reduction in proteinuria and improvement in GFR in SCA patients after treatment with telmisartan which is an angiotensin receptor blocker (ARB) without compromising the BP.\textsuperscript{61} This may also be a pointer to the fact that glomerular hypertension may be one of the pathogenetic mechanism in the development of albuminuria and progressive renal damage in SCA patients. The renoprotective effect of hydroxyurea may result from its effect in increasing fetal haemoglobin level which results in less VOC with maintenance of the integrity of the medullary interstitium. Alvarez et al however, did not find hydroxyurea to be reno-protective in SCA patients.\textsuperscript{59}

2.3 BIOCHEMICAL CORRELATES OF MA IN SCA PATIENTS

The level of serum creatinine in SCA patients is lower than that of age and sex-matched normal individuals.\textsuperscript{52} This is due to reduced muscle mass found in SCA patients. There is also hyperfiltration and increased tubular secretion of creatinine, both leading to lower serum creatinine level compared to what is expected. Both MDRD and Cockcroft-Gault formulae have
not been validated in Nigerian SCA patients and they have been reported to significantly overestimate GFR compared to endogeneously derived creatinine clearance. Serum levels of creatinine greater than 1mg/dl in SCA patients represent significant renal insufficiency; thus, in early stages of SCN the serum creatinine may be normal.

Lactate dehydrogenase (LDH) is a marker of intravascular haemolysis and is associated with low level of haemoglobin, haptoglobin and high level of reticulocytes, bilirubin and aspartate transaminase. Levels of haemolysis vary amongst patients with SCA and there is existence of a phenotype of SCA patient with markedly elevated haemolysis. Lactate dehydrogenase enzyme is known to be a marker of severity in SCD. There are strong epidemiological associations between elevation of LDH and endothelial activation, pulmonary hypertension and end organ vasculopathy. Sickle cell anaemia patients with higher rate of haemolysis have increased mortality rate and risk of developing complications like chronic leg ulcers, priapism and pulmonary hypertension. Reports suggest that high LDH values may predict early mortality. Some studies have shown a strong positive correlation between MA and LDH in SCA patients. However, Asnani et al and King et al in separate studies done in Jamaica at different times did not show any association between MA and LDH. Day et al reported that there is a positive association between markers of haemolysis and albuminuria in haemoglobin SS patients but this does not occur in haemoglobin SC patients. This association may however be limited to those with phenotype that is characterized by marked haemolysis who are also more likely to have severe form of disease.

Studies on lipid profile in Nigerian SCA patients showed that they have low total cholesterol, high density lipoprotein cholesterol and low density cholesterol. A study done in Ilorin, Nigeria showed that hypertriglyceridaemia is a common finding in Nigerian SCA patients.
Nnodim et al however reported that there was no significant difference in mean values of triglyceride between SCA patients and non SCD controls. There is also a different pattern of lipid profile in SCA patients with or without macroalbuminuria. SCA patients with macroalbuminuria have significantly higher mean triglyceride, low density lipoprotein cholesterol (LDL-C), total cholesterol and lower high density lipoprotein cholesterol (HDL-C) than SCA patients with normoalbuminuria. The abnormal lipid profile reported in SCA patients which is characterized by elevated TG and reduced HDL-C is associated with increased atherogenic risk and this may significantly predispose them to cardiovascular disease.

Cystatin C, formerly known as postgamma-globulin, is a neuroendocrine basic polypeptide encoded by the CST3 gene with low molecular weight (13.3 kilodaltons), freely filtered by the kidneys and is a biomarker of kidney function. Its serum level increases as the GFR decreases and kidney function deteriorates, hence there is an inverse relationship between the GFR and serum level of cystatin C. It also plays a role in predicting new onset or deteriorating cardiovascular disease. Studies have shown that it is a more precise and accurate test in evaluating GFR and renal function. It is a predictor of early renal damage in patients with essential hypertension where it has a positive correlation with MA. Serum level of cystatin C has been shown to have positive correlation with MA in SCA patients and may be a reliable method of measure of renal function in them.
CHAPTER THREE
METHODOLOGY

3.1 Study Location

This study was carried out at the Sickle Cell Centre (SCC), Benin a state government health facility in Benin city, Edo state. The centre is in the south-south geo-political region of Nigeria and receives referral cases from Edo state and neighbouring states like Delta, Ondo, Kogi.

3.2 Study Design

This was a single centre, cross-sectional analytical study carried out over a three month period from April to June 2014. Consecutive SCA patients who were in steady state and met the inclusion criteria attending the SCC were recruited. For every two consecutive SCA patients, one control who was not a SCA, hypertensive, diabetic nor renal disease patient was recruited from the workers of the SCC and undergraduate students of University of Benin, Benin, Edo State.

3.3 Inclusion Criteria

The inclusion criteria for this study were:

- SCA patients who were 15 years and above
- SCA patients who were in steady state defined by absence of crises and blood transfusion in the last 3 months.
- SCA patients who consented to participate in the study

3.4 Exclusion Criteria

Persons with the presence of disease or factors known to cause albuminuria such as:

i) DM;

ii) Hypertension;
iii) Urinary tract infection;
iv) Haematuria
v) Recent vigorous exercise in the last 24 hrs
vi) Recent episode of febrile illness in the last 1 week
vii) SCA with history of CKD
viii) SCA patients not in steady state; and
ix) Non-consenting individuals

3.5 Sample Size

The sample size was determined using the Leslie and Kish formula as follows: 

\[ N = \frac{Z^2pq}{d^2} \]

Where

- \( N \) = Estimated sample size
- \( Z \) = Standard derivation usually set at 1.96, which corresponds to 95% confidence interval
- \( P \) = Prevalence of the disease under the study {which is 17.4% from previous study}\(^2\)
- \( q \) = 1 - \( p \)
- \( d \) = degree of accuracy required usually set at 0.05

Substituting the values:

\[ N = \left(\frac{1.96}{2}\right)^2 \times 0.174 \times 0.826 \]

\[ (0.05)^2 \]

\[ N = 217 \]

With an attrition rate of 10% =21.7

Minimum sample size =239

A total number of 240 subjects were recruited for the study, but only 220 had complete data at the end of the study.
3.6 Methods
All subjects were interviewed by me using a structured questionnaire (Appendix 1). Demographic information such as age, sex, occupation, religion and educational status were obtained. History of hypertension, DM and CKD was sought. All the subjects had fasting blood glucose or random blood glucose check to rule out DM. Anthropometric measurements such as weight was taken using a bathroom weighing scale made by U-MEC model 98114, height using a stadiometer, waist and hip circumference using a measuring tape.

BMI was calculated by this formula: \[ \frac{\text{Weight in Kg}}{\text{(height in cm)}^2} \]

Waist-hip ratio were calculated by this formula: \[ \frac{\text{waist circumference}}{\text{hip circumference}} \]

Blood pressure was measured in a sitting position after 5 minutes rest. BP was measured on the right arm with a mercury sphygmomanometer. The systolic and diastolic pressures were read to the nearest 2mmHg. Disappearance of the korotkoff’s sound (phase V) was used as the criterion for the diastolic blood pressure and the average of three consecutive BP readings were recorded.

10 mls of blood was collected from each study subject for lipid profile, PCV, creatinine estimation, serum LDH and AST assay.

Spot urine sample was tested for albuminuria. The principle of measurement of albuminuria was based on immuno-turbidimetry and that of creatinine on colorimetry. The values were used to determine the albumin: creatinine ratio (ACR). Participants who had leukocytes on using Combi-9 were assumed to have urinary tract infection and were excluded.
These investigations were carried out in the chemical pathology laboratory in UBTH, Benin.

The GFR was estimated using MDRD formula: $186 \times {\text{Serum (Cr)}}^{-1.154} \times {\text{(Age)}}^{-0.203} \times 0.742$ (if female) $\times 1.210$ (if black).\(^80\)

The packed cell volume (PCV) was determined by centrifugation method.\(^81\)

### 3.6.1 Definition of variables

Frequency of VOC in the last one year was used to define severity of disease. 1-2 VOC/year was mild, 3-5 was moderate while > 5 VOC per year was severe. Normoalbuminuria was defined as ACR of $< 30\, \text{mg/mmol}$, microalbuminuria as ACR of $30-300\, \text{mg/mmol}$ and macroalbuminuria as ACR of $> 300\, \text{mg/mmol}$\(^82\)

Dyslipidaemia was defined as any or combination of the following: Total cholesterol $> 5.17\, \text{mmol/l}$, HDL cholesterol $< 1.3\, \text{mmol/l}$ in females and $1.03\, \text{mmol/l}$ in males, LDL cholesterol $> 3.36\, \text{mmol/l}$ and TG $> 2.7\, \text{mmol/l}$\(^83\)

### 3.7 Data Analysis

Data generated from this study were entered and analysed using the statistical package for social sciences (SPSS) version 17.0. Results are presented in tabular and graphical forms. Univariate analysis was used in description of socio-demographic characteristics of the study population. Continuous variables are presented as mean and standard deviation for normally distributed data while median and interquartile range was used for skewed data. Discrete variables are presented as percentages. Chi-square test was used to determine the significance of the observed differences for categorical variables while chi-square with trend was used where the categorical variable was ordinal. Student t-test was used to compare mean values of the sub-groups for those with unskewed data while Mann Whitney U was used to compare skewed data. Pearson’s
correlation was used to determine association between continuous variables with unskewed data and Spearman’s correlation was used for skewed data P values < 0.05 was considered significant.
CHAPTER FOUR

RESULTS

A total of 220 SCA patients and 110 controls satisfied the inclusion criteria and completed the study over a 3 month period between April and June 2014.

4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION

Table 1 shows the socio-demographic characteristics of the study population. The SCA group were made up of 108 (49.1%) males and 112 (50.9%) females while the controls were made up of 54 (49.1%) males and 56 (50.9%) females. The male: female ratio was 1:1.04 for both groups. The age range of SCA group was 15-50 years with a mean age of 23.08±8.68 years while the age range of the control group was 17-50 years with a mean age of 24.31±4.95 years. Majority, 180 (81.8%) of the SCA patients were in their second and third decade of life. Two hundred (90.9%) of the SCA patients were single while the remaining twenty (9.1%) were married. Also, two hundred of the SCA patients were Christians while the remaining were Muslims. Eighty-two (37.3%) of the SCA had primary level of education, ninety-six (43.6%) had secondary level of education and the remaining 42(19.1%) had tertiary level of education. One hundred and thirty-two (60%) of the SCA patients were Bini, constituting the major ethnic group. A total of 163(74.1%) were unemployed while the remaining 57(25.9%) were employed.
Table 1: Socio-demographic characteristics of the study population

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>SCA group (n=220)</th>
<th>Control group (n=110)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age</td>
<td>23.08±8.68 years</td>
<td>24.31±4.95 years</td>
<td>0.169</td>
</tr>
<tr>
<td>15-20 years</td>
<td>94(42.7%)</td>
<td>47(42.7%)</td>
<td></td>
</tr>
<tr>
<td>21-30 years</td>
<td>86(39.1%)</td>
<td>43(39.1%)</td>
<td>0.945</td>
</tr>
<tr>
<td>31-40 years</td>
<td>26(11.8%)</td>
<td>13(11.8%)</td>
<td></td>
</tr>
<tr>
<td>40-50 years</td>
<td>12(6.4%)</td>
<td>6(6.4%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>108 (49.1%)</td>
<td>54 (49.1%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female</td>
<td>112 (50.9%)</td>
<td>56 (50.9%)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>200(90.9%)</td>
<td>80(72.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Married</td>
<td>20(9.1%)</td>
<td>30(27.3%)</td>
<td></td>
</tr>
<tr>
<td>Religion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christianity</td>
<td>200(90.9%)</td>
<td>110(100%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Islam</td>
<td>20(9.1%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>82 (37.3%)</td>
<td>24(21.8%)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>96 (43.6%)</td>
<td>76(69.1%)</td>
<td>0.435</td>
</tr>
<tr>
<td>Tertiary</td>
<td>42 (19.1%)</td>
<td>10(9.1%)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benin</td>
<td>132(60%)</td>
<td>75(68.2%)</td>
<td></td>
</tr>
<tr>
<td>Esan</td>
<td>46(20.9%)</td>
<td>20(18.2%)</td>
<td>0.838</td>
</tr>
<tr>
<td>Yoruba</td>
<td>12(5.5%)</td>
<td>5(4.5%)</td>
<td></td>
</tr>
<tr>
<td>Ibo</td>
<td>12(5.5%)</td>
<td>4(3.6%)</td>
<td></td>
</tr>
<tr>
<td>Etsako</td>
<td>18(8.1%)</td>
<td>6(5.5%)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>163 (74.1%)</td>
<td>90(81.8%)</td>
<td>0.130</td>
</tr>
<tr>
<td>Employed</td>
<td>57 (25.9%)</td>
<td>20(18.2%)</td>
<td></td>
</tr>
</tbody>
</table>
4.2 CLINICAL CHARACTERISTICS OF THE STUDY POPULATION

The mean body mass index, systolic blood pressure and diastolic blood pressure were significantly higher in the control group compared to the SCA patients. There was no significant difference in the mean waist-hip ratio between the two groups. (Table 2)

Table 2: Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SCA (n=220)</th>
<th>Control (n=110)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±Sd</td>
<td>Mean±Sd</td>
<td></td>
</tr>
<tr>
<td>Body mass index (Kg/m²)</td>
<td>17.31±3.57</td>
<td>23.96±4.37</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Waist-hip ratio (cm)</td>
<td>0.91±0.06</td>
<td>0.90±0.08</td>
<td>0.204</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>109.55±14.73</td>
<td>113.52±11.63</td>
<td>0.009*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>66.97±11.44</td>
<td>70.56±10.12</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

BP (blood pressure), GFR (glomerular filtration rate), WHR (waist hip ratio)

*(Significant p value of < 0.05)
4.3 THE BIOCHEMICAL AND HAEMATOLOGICAL CHARACTERISTICS OF STUDY POPULATION

The median value of serum HDL-C and mean value PCV were significantly lower in the SCA group while the median value of serum LDL-C, serum triglyceride and ACR were statistically higher in the SCA group compared to the control group. There was no significant difference in the median value of total cholesterol, mean values of estimated GFR and serum creatinine in the two groups. (Table 3)

Table 3: Biochemical and haematological characteristics of study population

<table>
<thead>
<tr>
<th>LIPID PARAMETER</th>
<th>SCA GROUP (n=220)</th>
<th>CONTROL GROUP (n=110)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±Sd/Median (IQR)</td>
<td>Mean±Sd/Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol† (mmol/l)</td>
<td>4.20(3.33-5.07)</td>
<td>4.36(3.66-4.36)</td>
<td>0.413</td>
</tr>
<tr>
<td>Triglyceride† (mmol/l)</td>
<td>1.02(0.90-1.11)</td>
<td>0.83(0.61-1.28)</td>
<td>0.025*</td>
</tr>
<tr>
<td>HDL-C† (mmol/l)</td>
<td>1.03(0.72-1.24)</td>
<td>1.79(1.47-2.01)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LDL-C† (mmol/l)</td>
<td>2.67(2.20-3.37)</td>
<td>1.88(0.97-2.61)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ACR † (mg/mmol)</td>
<td>1190(16-1935)</td>
<td>25(10-30)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>24.84±4.19</td>
<td>36.14±4.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine(mg/dl)</td>
<td>0.78±0.54</td>
<td>0.71±0.34</td>
<td>0.133</td>
</tr>
<tr>
<td>Estimated GFR(mls/min)</td>
<td>124.66±26.55</td>
<td>121.11±28.69</td>
<td>0.287</td>
</tr>
</tbody>
</table>

†Skewed data expressed in Median (IQR) and Man Whitney U test used.

Sd(Standard deviation) IQR (Interquartile range), HDL-C (high density lipoprotein cholesterol), LDL-C(low density lipoprotein cholesterol), ACR (albumin-creatinine ratio)

*(Significant p value of< 0.05)
4.4 PATTERN OF DYSLIPIDAEMIA OF THE STUDY POPULATION

The prevalence of low HDL-C was significantly higher in the SCA group compared to the control group with a value of <0.001 while the prevalence of high total cholesterol was significantly higher in the control group with a p value of 0.002. The prevalence of dyslipidaemia was significantly higher in the control group compared to the SCA group with a p value of <0.001. (Table 4)

Table 4: Pattern of dyslipidaemia of the study population

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n=110)</th>
<th>SCA (n=220)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High LDL-C (mg/dl)</td>
<td>18(16.4%)</td>
<td>44(20%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Low HDL-C (mg/dl)</td>
<td>14(12.7%)</td>
<td>124(56.4%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>High Triglyceride(mg/dL)</td>
<td>8 (7.2%)</td>
<td>8(3.6%)</td>
<td>0.18</td>
</tr>
<tr>
<td>High Total cholesterol(mg/dL)</td>
<td>24(21.8%)</td>
<td>20(9.1%)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>142(64.5%)</td>
<td>38(34.6%)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

HDL-C (high density lipoprotein cholesterol), LDL-C(low density lipoprotein cholesterol)

*(Significant p value of< 0.05)
4.5 PATTERN OF ALBUMINURIA IN STUDY POPULATION

The prevalence of normoalbuminuria, microalbuminuria and macroalbuminuria in the SCA patients were 35.5%, 50% and 14.5% respectively. The prevalence of normoalbuminuria and microalbuminuria in the control group were 90.9% and 9.1% respectively. None of the control subjects had macroalbuminuria. (Table 5)

Table 5: Pattern of albuminuria in study population

<table>
<thead>
<tr>
<th></th>
<th>SCA group</th>
<th>CONTROL group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoalbuminuria</td>
<td>78(35.5%)</td>
<td>100(90.9%)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>110(50%)</td>
<td>10(9.1%)</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>32(14.5%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>
4.6 PATTERN OF ALBUMINURIA IN SCA PATIENTS

The prevalence of normoalbuminuria, microalbuminuria and macroalbuminuria were 35.5%, 50% and 14.5% respectively in the SCA group. There was no significant difference in the prevalence rates between male and female SCA patients. (Table 6)

Table 6: Pattern of albuminuria in SCA patients

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoalbuminuria</td>
<td>78(35.5%)</td>
<td>38(48.7%)</td>
<td>40(51.3%)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>110(50%)</td>
<td>60(54.6%)</td>
<td>50(45.4%) 0.829</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>32(14.5%)</td>
<td>14(43.8%)</td>
<td>18(56.2%)</td>
</tr>
</tbody>
</table>
4.7 CLINICAL AND BIOCHEMICAL CHARACTERISTICS AMONG SCA PATIENTS ACCORDING TO THE LEVEL OF ALBUMINURIA

There was a significant difference in mean age of SCA patients in the normoalbuminuric, microalbuminuric and macroalbuminuric groups with mean age increasing with increasing albuminuria (p = 0.036). There was also significant difference in the mean PCV of SCA patients in the microalbuminuric and macroalbuminuric groups with a p value of 0.017. There was no significant difference in the blood pressure, serum creatinine and estimated GFR among the different groups of albuminuria. There was significant difference between mean value of TC, HDL-C and LDL-C across the different albuminuria group with p values of 0.016, <0.001 and 0.018 respectively (Table 7).

Table 7: Clinical and Biochemical characteristics of SCA patients according to the level of Albuminuria

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normoalbuminuria group (n=78)</th>
<th>Microalbuminuria group (n=110)</th>
<th>Macroalbuminuria group (n=32)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.11±7.27</td>
<td>23.57±9.40</td>
<td>25.53±8.76</td>
<td>0.036*</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.43±8.2</td>
<td>17.21±3.58</td>
<td>17.69±3.68</td>
<td>0.164</td>
</tr>
<tr>
<td>WHR (cm)</td>
<td>0.92±0.06</td>
<td>0.90±0.05</td>
<td>0.91±0.06</td>
<td>0.144</td>
</tr>
<tr>
<td>SBP(mmHg)</td>
<td>107.58±14.03</td>
<td>110.70±15.27</td>
<td>110.40±13.88</td>
<td>0.344</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>65.26±10.77</td>
<td>68.87±12.01</td>
<td>65.33±10.42</td>
<td>0.072</td>
</tr>
<tr>
<td>ACR† (mg/mmol)</td>
<td>17.8(15-25)</td>
<td>158(121-196)</td>
<td>459(354-913)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>25.00±4.15</td>
<td>25.03±4.05</td>
<td>22.87±4.31</td>
<td>0.017*</td>
</tr>
<tr>
<td>Serum creatinine eGFR(mls/min)</td>
<td>0.78±0.27</td>
<td>0.73±0.41</td>
<td>0.98±0.52</td>
<td>0.084</td>
</tr>
<tr>
<td>TC(mmol/l)</td>
<td>110.51±26.16</td>
<td>114.65±27.79</td>
<td>111.19±42.09</td>
<td>0.623</td>
</tr>
<tr>
<td>TG(mmol/l)</td>
<td>4.21±0.82</td>
<td>4.17±0.70</td>
<td>4.63±0.84</td>
<td>0.016*</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>0.99±0.15</td>
<td>0.95±0.21</td>
<td>1.04±0.16</td>
<td>0.053</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>1.20±0.41</td>
<td>0.94±0.31</td>
<td>0.96±0.47</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>2.63±0.86</td>
<td>2.86±0.68</td>
<td>3.11±1.04</td>
<td>0.018*</td>
</tr>
</tbody>
</table>

†Skewed data expressed in Median (IQR), Sd (Standard deviation), GFR (glomerular filtration rate), SBP(systolic blood pressure), DBP(diastolic blood pressure), PCV(packed cell volume), TC (total cholesterol), TG(triglyceride), HDL-C(high density lipoprotein-cholesterol), LDL-C(low density lipoprotein-cholesterol) IQR (Interquartile range) *(Significant p value of< 0.05)
4.8 MARKERS OF HAEMOLYSIS (SERUM LDH AND AST) AMONG THE SCA PATIENTS ACCORDING TO THE LEVEL OF ALBUMINURIA

There was no significant difference in the median value of serum LDH and AST across the different groups of SCA patients according to level of albuminuria. (Table 8)

Table 8: Markers of haemolysis (LDH, AST) in SCA patients according to level of Albuminuria

<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria Group(n=78)</th>
<th>Microalbuminuria Group(n=110)</th>
<th>Macroalbuminuria Group(n=32)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH† (IU/l)</td>
<td>78(42.5-108)</td>
<td>58(33-92.8)</td>
<td>76(41-107)</td>
<td>0.211</td>
</tr>
<tr>
<td>AST† (IU/l)</td>
<td>32(19.8-46.3)</td>
<td>29(17-42.5)</td>
<td>22(18-42)</td>
<td>0.488</td>
</tr>
</tbody>
</table>

†Skewed data expressed in Median (IQR).
Sd(Standard deviation), IQR (Interquartile range), AST(aspartate dehydrogenase), SCA (sickle cell anaemia)
4.9 PATTERN OF LIPID PROFILE IN SCA PATIENTS

The prevalence of low HDL-C increased significantly from normoalbuminuria to macroalbuminuria group with a p value of < 0.001. There was no significant difference in the prevalence of high LDL-C, total cholesterol and triglyceride across the groups. The prevalence of dyslipidaemia increased significantly from normoalbuminuria to macroalbuminuria group with a p value of 0.003. (Table 9)

Table 9: Pattern of dyslipidaemia in SCA patients

<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria (n=78)</th>
<th>Microalbuminuria (n=110)</th>
<th>Macroalbuminuria (n=32)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High LDL-C (mg/dL)</td>
<td>14(17.9%)</td>
<td>20(18.2%)</td>
<td>10(31.2%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Low HDL-C (mg/dL)</td>
<td>30(38.5%)</td>
<td>74(67.3%)</td>
<td>20(62.5%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>High Triglyceride</td>
<td>2(2.6%)</td>
<td>2(1.8%)</td>
<td>4(12.5%)</td>
<td>0.91</td>
</tr>
<tr>
<td>High Total cholesterol</td>
<td>10(12.8%)</td>
<td>4(3.6%)</td>
<td>6(18.8%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>38(48.7%)</td>
<td>82(74.5%)</td>
<td>22(68.8%)</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

HDL-C (high density lipoprotein cholesterol), LDL-C (low density lipoprotein cholesterol)
4.10 ASSOCIATION BETWEEN LEG ULCER, TRANSFUSION, VOC AND ALBUMINURIA IN SCA PATIENTS

There was no significant association between leg ulcer and degree of albuminuria. There was significant association between frequency of VOC and history of transfusion in the last one year with degree of albuminuria with p-values of 0.021 and 0.007 respectively. (Table 10)

Table 10: Prevalence of leg ulcer, transfusion and frequency of VOC according to level of albuminuria

<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria Group (n=78)</th>
<th>Microalbuminuria Group (n=112)</th>
<th>Macroalbuminuria Group (n=32)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg ulcer</td>
<td>12(15.4%)</td>
<td>16(14.5%)</td>
<td>6(18.8%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Transfusion</td>
<td>48(61.5%)</td>
<td>92(83.6%)</td>
<td>24(75%)</td>
<td>0.007</td>
</tr>
<tr>
<td>VOC frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2/ year</td>
<td>44(56.4%)</td>
<td>68(61.8%)</td>
<td>18(56.3%)</td>
<td></td>
</tr>
<tr>
<td>3-5/ year</td>
<td>24(30.8%)</td>
<td>40(36.4%)</td>
<td>10(31.2%)</td>
<td>0.021</td>
</tr>
<tr>
<td>≥6/ year</td>
<td>10(12.8%)</td>
<td>2(1.8%)</td>
<td>4(12.5%)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant p value of < 0.05
4.11 CORRELATION BETWEEN ALBUMINURIA AND CLINICAL AND BIOCHEMICAL PARAMETERS IN SICKLE CELL ANAEMIA PATIENTS

There was significant positive correlation between albuminuria and serum creatinine (p= <0.001, r= 0.349), LDL-C (p= <0.001, r= 0.361) and total cholesterol (p= <0.001, r =0.335). There was also significant negative correlation between albuminuria and PCV (p= <0.001, r = -0.251), estimated GFR (p= 0.003, r = -0.207), and HDL-C (p= 0.016, r = -0.170). These are shown in table 11.

Table 11: Correlation between albuminuria, clinical and biochemical characteristics in SCA patients

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>P VALUE</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.106</td>
<td>0.113</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>0.372</td>
<td>-0.063</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.075</td>
<td>0.124</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.881</td>
<td>-0.010</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>&lt;0.001*</td>
<td>-0.251</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>&lt;0.001*</td>
<td>0.349</td>
</tr>
<tr>
<td>Estimated GFR (mls/min/1.73m$^2$)</td>
<td>0.003*</td>
<td>-0.207</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>&lt;0.001*</td>
<td>0.335</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>0.073</td>
<td>0.125</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>0.016*</td>
<td>-0.170</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>&lt;0.001*</td>
<td>0.361</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>0.781</td>
<td>-0.020</td>
</tr>
<tr>
<td>Aspartate transaminase(U/L)</td>
<td>0.137</td>
<td>0.111</td>
</tr>
</tbody>
</table>

HDL-C (high density lipoprotein cholesterol), LDL-C(low density lipoprotein cholesterol)
*(Significant p value of< 0.05)
p = 0.003, r = -0.207

Fig 1: Correlation between estimated GFR and albuminuria

p = <0.001, r = 0.349

Fig 2: Correlation between serum creatinine and albuminuria
p = <0.001, r = -0.251

Fig 3: Correlation between PCV and albuminuria
4.12 MULTIPLE LINEAR REGRESSION TO DETERMINE PREDICTORS OF ALBUMINURIA

The significant predictors of albuminuria in the SCA patients were packed cell volume and serum creatinine with p value of 0.009 and 0.004 respectively following a multiple regression analysis. (Table 12)

Table 12: Multiple linear regression of predictors of albuminuria in SCA patients

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>(Constant</td>
<td>55.854</td>
<td>279.089</td>
<td>.200</td>
<td>0.842</td>
</tr>
<tr>
<td>PCV</td>
<td>-14.218</td>
<td>5.420</td>
<td>-0.181</td>
<td>-2.623</td>
</tr>
<tr>
<td>SBP</td>
<td>-0.334</td>
<td>1.507</td>
<td>-0.016</td>
<td>-0.222</td>
</tr>
<tr>
<td>SCR</td>
<td>165.462</td>
<td>57.337</td>
<td>0.305</td>
<td>2.886</td>
</tr>
<tr>
<td>TC</td>
<td>2.028</td>
<td>1.947</td>
<td>0.197</td>
<td>1.041</td>
</tr>
<tr>
<td>HDL</td>
<td>-2.853</td>
<td>2.489</td>
<td>-0.139</td>
<td>-1.146</td>
</tr>
<tr>
<td>LDL</td>
<td>0.629</td>
<td>2.017</td>
<td>0.064</td>
<td>0.312</td>
</tr>
<tr>
<td>EGFR</td>
<td>1.060</td>
<td>1.010</td>
<td>0.102</td>
<td>1.050</td>
</tr>
</tbody>
</table>

*(Significant p value of < 0.05)
CHAPTER FIVE
DISCUSSION

Using ACR as a measure of proteinuria, the prevalence, pattern and correlates of albuminuria were studied in SCA patients. Albuminuria was more prevalent in SCA patients than controls. Majority (81.8%) of the patients were in their second and third decade of life. This may be a reflection of the reduced life expectancy in SCA patients compared to the general population. This reduction is worse in African SCA patients compared to such patients in developed countries mainly due to poor environmental sanitation, unaffordability and inaccessibility to good quality health services in the developing countries, although these factors were not evaluated in this present study.

Majority of the SCA patients were Christians and of Bini origin; and this could be explained by the fact the Christianity and Bini are the predominant religion and tribe respectively in Edo State where this study was carried out. Majority of the study population were single and unemployed students and this is also a reflection of the age distribution of the study population.

The prevalence rate of normoalbulminuria, MA and macroalbuminuria in SCA patients were 35.5%, 50% and 14.5% while that of the control group were 90.9%, 9.1% and 0% respectively. The prevalence of albuminuria in the SCA patients was significantly higher compared to the control group. The prevalence of MA in SCA patients in this study is higher than 17.4% and 26% reported in Ivory Coast and Jamaica respectively but is comparable to a prevalence of 40% reported in Brazil.25,26,29 The prevalence of macroalbuminuria was similar to 16.8% reported in Ile–Ife, but lower than 26% reported previously in Kano.23,22 The varied prevalence from reports
across different parts of the continents could possibly be explained by the differences in patient’s characteristics and SCD behaviour in different parts of the world. This is evidenced by the observation that variable associations have been found in patients with similar origins studied at different centres.\textsuperscript{26} The factors modifying differences in presentation are level of fetal haemoglobin, presence of alpha gene deletion, beta gene cluster haplotypes and other genetic and environmental factors that are yet to be identified.\textsuperscript{28-31}

There was significant difference in the mean age of the different groups of SCA patients based on the level of albuminuria. The mean age of the SCA patients with macroalbuminuria was higher than those with normoalbuminuria and MA. This trend agrees with some previous studies that reported association between albuminuria and age.\textsuperscript{38,39} This study showed age related increase in albuminuria in SCA patients which is similar to what is observed in diabetics. This may be explained by age related hyperfiltration and subsequent glomerulosclerosis which leads to albuminuria. It has been thought from available literature that sickle cell glomerulopathy could evolve in five clinical stages: stage 1:normoalbuminuric stage, stage 2: microalbuminuric stage, stage 3: macroalbuminuric stage with preserved GFR, stage 4: macroalbuminuric stage with progressive renal insufficiency and stage 5: ESRD.\textsuperscript{84} However, this classification still remains a hypothesis since evidence of progression through these 5 stages is still not sufficient.

There was no statistically significant difference in the prevalence of albuminuria between both gender in this study. This finding is similar to the report by Eke et al but different from reports of Abhulimen-Iyoha and Dharnidharka et al who found significantly higher prevalence of albuminuria in females.\textsuperscript{38-40} Confounders of proteinuria such as haematuria and urinary tract infections which are known to be commoner in females were part of the exclusion criteria in this study. This may be responsible for absence of significant gender difference in the prevalence of
albuminuria, although these confounders were also said to have been excluded in the previous studies that reported gender difference in the prevalence of albuminuria.

This study showed that there was significant negative correlation between PCV and the degree of albuminuria and that SCA patients with higher degree of albuminuria also tend to have lower PCV. Similar findings have been reported in both paediatric and adult SCA populations. ²²,²³,²⁵,³⁸ Conversely, other reports did not show any correlation between MA and haematocrit. ²⁶,⁴³ This study showed that PCV was a predictor of albuminuria in SCA patients. The higher the severity of the anaemia, the more pronounced the effect of dilatation of the blood vessels from effect of chronic hypoxia which will lead to increase blood flow to the kidneys, hyperfiltration, glomerular injury and subsequently leads to albuminuria. Also, one of the mechanism of anaemia in SCA patients is repeated VOC which is also central in the pathogenesis of glomerular injury and albuminuria in SCA. Severe anaemia may be also be a feature of severe form of SCA which is more commonly associated with end organ damage such as SCN. These reasons may therefore explain the association between albuminuria and haematocrit in this study.

There was a positive correlation between albuminuria and systolic BP, though not statistically significant. This finding is similar to report by Abhulimen-Iyoha but is at variance with reports of Thompson and Guash et al who found a positive correlation between albuminuria and BP. ²⁵,²⁷,³⁸ The mean values of BP parameters in the SCA patient were significantly lower than the control group and this agreed with previous reports. ⁴⁸-⁵⁰ Sickle cell anaemia is characterized by chronic hypoxic state which leads to vasodilatation from the effects of nitric oxide and this contributes significantly to relative resistance to development of hypertension. ⁵² The prevalence of dyslipidaemia was 34.6% in the SCA patients which was significantly lower than 64.5% in the control group. The commonest type of dyslipidaemia in the SCA group was
low HDL-C which was present in 56.4% of the SCA patients and this is similar to previous reports.\textsuperscript{68-71} The median value of serum triglyceride was significantly higher in the SCA patients compared to the control group. This is similar to report of Erastus et al, however Nnodim et al did not find significant difference in TG levels between SCA and control group.\textsuperscript{68,71} The median value of serum LDL-C was significantly higher in SCA group compared the controls. This study showed significant association between dyslipidaemia and albuminuria. There was significant positive correlation between total serum cholesterol, LDL-C and albuminuria. There was also a significant negative correlation between albuminuria and serum HDL-C. This study also showed significantly worsening lipid profile in the different groups of SCA patient based on the level of albuminuria which is similar to report of Emokpae et al.\textsuperscript{72} Most of previous studies on albuminuria in SCA patients did not study association between albuminuria and lipid parameters. Dyslipidaemia is associated with glomerulosclerosis which may lead to albuminuria and progressive renal damage. HDL-C has both anti-inflammatory and anti-oxidant functions.\textsuperscript{85} In the presence of low HDL-C, there is LDL-C oxidation which causes inflammation of the glomerular endothelium. This causes glomerular injury which consequently leads to albuminuria. Low HDL-C has also been associated with severe anaemia, higher leukocyte and platelet count in SCA compared with those with normal HDL-C.\textsuperscript{86} All these haematological parameters may also contribute to vasodilatation, VOC in the medullary vasculature and glomerular capillaries. Treatment of dyslipidaemia as seen in other causes of CKD may help reduce albuminuria and retard progression to ESRD in patients with SCA.

The serum creatinine and GFR were higher in the SCA patients compared to the controls, though was not statistically significant. The mean value of serum creatinine was higher in the macroalbuminuria group compared to the other groups, though this difference was not
significant. There was a positive correlation between albuminuria and serum creatinine on one hand and a negative correlation between albuminuria and GFR on the other. Serum creatinine was also found to be a predictor of albuminuria in the SCA patients in this study. Thus, worsening albuminuria in this study was a pointer to worsening renal function and hence increasing albuminuria in SCA patients can predict progressive renal damage. Microalbuminuria may be used as an early marker of SCN just like in diabetics. Institution of treatment of MA with ACEI or ARB in SCA patients may help to reverse or slow progression to ESRD.

There was a positive association between frequency of VOC and albuminuria in this study. This finding agrees with reports from Arogundade et al who found that increased frequency of VOC and hospital admissions in SCA were associated with renal complications. Ataga et al also reported association between priapism, a form of VOC and albuminuria in SCA patients. Repeated VOC in the glomeruli have been associated with glomerular disease which may present with albuminuria. Fragmented red cells during episodes of VOC lodge in the glomerular capillaries and they become phagocytosed by the mesangial cells leading to mesangial expansion and duplication of the basement membrane. This leads to membranoproliferative glomerulonephritis with normal level of complements. There was also positive association between transfusion and severity of albuminuria in this study. The need for recurrent transfusion and increased frequency of VOC in SCA may indirectly reflect the severity of the disease. Repeated early blood transfusion in SCA patients early in life has been found to be protective against development of SCN by reducing the numbers of sickled red cells and VOC which are central to the pathogenesis of SCN. Darbari et al reported that severe painful VOC is a marker of SCA severity and premature mortality. Hence SCA patients with recurrent VOC have severe
disease which is associated with end organ damage that includes SCN. Hence, it may be inferred from this present study that albuminuria is associated with severity of SCA.

There was no association between LDH and AST as markers of haemolysis and albuminuria in this study. Elevation of LDH has been closely associated with endothelial activation and pulmonary hypertension. The association between these markers of haemolysis and albuminuria is still debatable. The finding in this study is similar to some previous reports. The association between these markers and albuminuria may not be prominent in the SCA patients who participated in this study because they were in steady state. This finding may also be due to the fact that albuminuria and pulmonary hypertension do not share the same pathogenesis in SCA patients. However, some authors have reported association between LDH and albuminuria in some SCA patients.
CONCLUSION

There were significant association between albuminuria in SCA patients and age, serum creatinine, estimated GFR, PCV and dyslipidaemia in this study. Albuminuria may reflect early stages of renal disease in SCA patients. Instituting measures to manage dyslipidaemia and prevent severe anaemia early in SCA patients may help to reduce albuminuria and subsequent progression to ESRD in addition to the use of established therapeutic agents such as ACEI, ARB and hydroxyurea.

LIMITATIONS OF THE STUDY

The following limitations were encountered in the course of this study.

1. Albuminuria was assessed once for each subject in this study. This made it impossible to confirm persistent albuminuria, which ought to be re-confirmed three months after initial assessment.

2. The cross-sectional design of this study did not allow for determination of full significance of albuminuria in the development of CKD.

3. Due to financial constraints, cystatin C could not be assessed in this study. Lactate dehydrogenase and AST as markers of haemolysis were only assessed in the SCA patients.

RECOMMENDATIONS
1. Albuminuria should be regularly assessed in SCA patients especially as they get to second and third decade of life so as to detect early kidney damage and effective therapeutic measures should be instituted as appropriate.

2. Lipid profile should be included as the part of routine investigations in SCA patients with aggressive treatment using dietary and therapeutic modalities so as retard worsening albuminuria and subsequent progression to overt renal failure.

3. Measure should be put in place to prevent or treat severe anaemia early in SCA patients.
APPENDIX I

Questionnaire on prevalence and pattern of albuminuria and its clinical and biochemical correlates in Sickle cell anaemia Patients in Benin City.

A. Demographic Information

Name (Initials) 
Serial No.:  
Hospital No. 
Contact Phone No.

Tribe 

1. Sex: M [ ] F [ ]
2. Age: ________________ years
3. Marital status: Single [ ], Married [ ], Divorced [ ], Widowed [ ]
4. Religion: Christian [ ], Muslim [ ], Others ______________
5. Occupation: _______________________
6. Educational status: Primary [ ], Secondary [ ], Tertiary [ ], None [ ]

B. History:

Symptoms:
Facial swelling Yes / No
Leg swelling Yes/ No
Nocturia Yes / No
Frothiness of urine Yes / No
Reduction in urine output Yes / No
Dysuria Yes / No
Loin Pain Yes / No
Urinary frequency Yes / No

Medical History
Diabetes mellitus Yes / No
Hypertension Yes / No
Previous blood transfusion Yes / No
If yes, how many times? __________________
Chronic leg ulcer Yes / No
Drug History
Hydroxyurea use: Yes / No
If yes:
  Are you regular? Yes / No
  For how long have you been on hydroxyurea?
Lipid lowering drug: Yes / No
If yes:
  What type? ....................................
  Are you regular? Yes / No
  For how long have you been on it?
ACEI or ARB: Yes / No
If yes:
  What type?
  Are you regular?
  For how long have you been on it?

Physical Examination:

1. Anthropometry
Weight: ______kg
Height: ______m
BMI: ________kg/m²
Waist Circumference: ______cm
Hip Circumference: ______cm
Waist-Hip Ratio

2. Physical Examination
Pallor: Yes / No
Dehydration: Yes / No
Facial swelling: Yes / No
Leg swelling: Yes / No
Blood Pressure:
  Systolic [ ] mmHg
  Diastolic [ ] mmHg

D. Laboratory Evaluation

1. Urinalysis
Albuminuria: Leucocyturia
Negative [ ] Negative [ ]
Trace [ ] Trace [ ]
1+ [ ] 1+ [ ]
2. Albumin Creatinine Ratio: ........................mg/mmol
3. Serum Electrolyte, Urea and Creatinine
   a. Urea: ___________ mg/dl
   b. Creatinine: ______mg/dl
   c. Sodium: ________ mmol/L
   d. Potassium: ______mmol/L
   e. Chloride: _______mmol/L
   f. Bicarbonate: _____mmol/L
   g. eGFR: ________ml/min

4. Random Blood Glucose [ ] mg/dl

5. Serum Lipid Profile
   a. Total cholesterol _________mmol/l
   b. HDL-Cholesterol__________mmol/l
   c. LDL-Cholesterol___________mmol/l
   d. Triglycerides______________mmol/l

6. Packed cell volume
   PCV___________________%
APPENDIX 2

The urine albumin was determined as follows;

1. A working standard of 200 microgram/ml was prepared from the stock (1mg/ml)
2. A reagent mixture was made by adding 1.0ml of 10% CuSO4 to 1.0ml of 2% sodium potassium tartrate and 100mls of a solution containing 20g of anhydrous sodium bicarbonate and 4g of sodium hydroxide per litre.
3. 5mls of reagent was added to all test tubes (standard, blank, control and unknown) with incubation at room temperature for 10 minutes.
4. 0.5mls of Folin-Ciocalteau’s phenol reagent was added to the test tubes, mixed immediately and incubated at room temperature for 30 minutes.
5. Absorbance was read at 750nm in a spectrophotometer using a reagent blank.
6. A calibration curve of absorbance at 750nm against albumin concentration (microgram/ml) was plotted. The calibration curve was used to determine the urine albumin.

Total serum cholesterol and triglycerides were determined by enzymatic estimation, while HDL was determined by precipitation. The cholesterol esters of the sample were hydrolyzed with cholesterol esterase. The free cholesterol released by cholesterol oxidase then forms 4-cholesten-3-one and hydrogen peroxide ($H_2O_2$). Hydrogen peroxide in the presence of phenol and 4-amino-antipyrine was treated with peroxidase. This resulted in the formation of a red quinonimine derivative. The absorbance of this derivative was measured with the spectrophotometer to determine the cholesterol level in serum using the following equation:
Cholesterol level (mg/dl) = \frac{\text{ABSORBANCE OF SAMPLE}}{\text{ABSORBANCE OF STANDARD}} \times 300

An enzyme solution was prepared by dissolving enzyme reagent in 100 ml of the cholesterol buffer supplied in a purchased cholesterol kit. The standard, sample and blank were prepared by adding 0.02 ml of each to 3 mls of enzyme solution. The sample and blank solutions were incubated in a 37°C water bath for five minutes. The absorption spectra of the standard and sample were determined using a spectrophotometer. The cholesterol level was calculated. LDL was determined from the values of the aforementioned using the Friedewald formula.\(^7^9\)

The HDL-C was determined in the laboratory by:

1. Labelling of test tubes: control, blank, standard and unknown.
2. Mixing equal volumes of serum and HDL cholesterol precipitating reagent in the glass tube vigorously i.e. 0.2mls serum + 0.2mls HDL precipitant reagent.
3. Centrifuging for 10 minutes at 1500-2000g.
4. Separating supernatant from precipitate. The supernatant fraction contained HDL.

The triglycerides (TG) was determined in the laboratory by:

1. Reconstituting the TG reagent according to the vial label instructions.
2. Labelling of test tubes: blank, standard, control, and unknown.
3. Pipetting 1ml of reagent into all test tubes.
4. Placing all test tubes in a 37°C heating block for at least 4 minutes.
5. Add 0.01ml of sample to respective tubes and mixing.
6. Incubating all the test tubes for 5minutes at 37°C.
7. The spectrophotometer was zero at 520nm with reagent blank.
8. Absorbance (s) of all test tubes were read and recorded.
TG of unknown = Absorbance of unknown X concentration of standard

Absorbance of standard

Serum creatinine was measured using Jaffe’s alkaline picrate method. The principle of picrate method involved reaction between creatinine and sodium picric acid in alkaline condition to form a colour complex which absorbs at 510nm. The rate of formation of colour was proportional to the creatinine concentration in the sample. The procedure involved:

1. Combining and mixing equal volumes of creatinine picric acid reagent and creatinine buffer reagent.
2. Labelling test vial, reagent blank, standard, control and unknown test tubes.
3. Pipetting 3.0mls of working reagent into test tubes.
4. Transferring 0.1ml of sample to its respective tube, distilled water to reagent blank and mix.
5. Placing all test tubes in 37°C heating bath for 15 minutes.
6. Setting wavelength of the spectrophotometer at 510nm and zero the instrument with the reagent blank. Absorbance of all the tubes were read and recorded.
7. Calculating the creatinine value of the unknown by comparing its absorbance change with that of the known standard.
CONSENT FORM

Dear Participant,

I invite you to be a part of this study titled; PREVALENCE AND PATTERN OF ALBUMINURIA AND ITS CORRELATES IN SICKLE CELL PATIENTS IN BENIN, being conducted by me, Dr Adejumo Oluseyi Ademola, a resident in the Nephrology unit, Department of Medicine, University of Benin Teaching Hospital.

This study will have no financial implication on you as the study will be totally funded by me. The purpose of this study is to determine the prevalence and the correlates of microalbuminuria among sickle cell anaemia patients. This will help to detect early stages of sickle cell nephropathy and ensure early intervention is instituted, hence prevention progression to sickle cell nephropathy.

The information you provided will be treated with strict confidentiality. Participation is voluntary and you are under no obligation to partake and can withdraw at any point with no consequences.

The study will require that you give me some information about yourself and the illnesses either by filling a form me or I filling the form myself on your behalf, collecting blood samples which will be analyzed.

Your participation will be highly appreciated, however there will be compensation for participation, but I will remain grateful to you. Nonparticipation nor withdrawal from this study will not in any way affect your treatment.

My contact details are Department of Internal Medicine, UBTH. Contact phone number is 08034225294 and contact email address is ceeward2010@yahoo.com.
Please if you are willing to participate, kindly sign this form and you will be given a questionnaire to fill.

............................

Sign

Participant
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