CORRELATION BETWEEN HIGH DENSITY LIPOPROTEIN CHOLESTEROL AND KIDNEY FUNCTION IN TYPE 1 DIABETES MELLITUS AS A PREDICTOR OF DIABETIC NEPHROPATHY IN HUMAN SUBJECTS

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Medical Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University

APRIL, 2016
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

To my lovely wife Anne Alindah and son Jansen Peters. My parents and family for their encouragement and moral support.
ACKNOWLEDGEMENT

It is with pleasure and deep sense of indebtedness that I acknowledge the invaluable help of these extra ordinary individuals who, all in small or large part put their shoulders to the wheel and indeed their noses to the “grindstone” by contributing immensely to the fulfillment of the mandate of this study. It scarcely needs mention that although a single name appears as the author, the enormous task of technical work, analysis, guidance, counsel and indeed typing was made possible by dedication from these team hence no words can adequately express the depth of my gratitude and the significance to me of their graciously offered support and humility.

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ABREVIATIONS AND ACRONYMS

AACE
American Association of Clinical Endocrinologists

ACAT
Acyl-CoA cholesterol acyltransferase

ACE
Angiotensin-converting enzyme

ADA
American Diabetes Association

ARB
Angiotensin receptor blockers

BMI
Body mass index

CAD
Coronary artery disease

CETP
Cholesterol ester transfer protein

CKD
Chronic kidney disease

CRF
Chronic renal failure

CVD
Cardiovascular disease

DN
Diabetic nephropathy

eGFR
Estimated glomerular filtration rate

ESRD
End stage renal disease

GFR
Glomerular filtration rate

GLMP
Guidelines laboratory medicine practice

HBA1C
Glycated hemoglobin

HDL-C
High density lipoprotein cholesterol

KNH
Kenyatta National Hospital

LCAT
Lecithin cholesterol acyltransferase

LDL-C
Low density lipoprotein cholesterol

MDRD
Modification of diet in renal diseases

NCEP
National cholesterol education program

NKDEP
National kidney disease education program

NKF
National kidney foundation
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>RAS</td>
<td>Rennin angiotensin system</td>
</tr>
<tr>
<td>UACR</td>
<td>Urine albumin-creatinine ratio</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>Very low density lipoprotein cholesterol</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

Diabetic nephropathy (DN) is a common microvascular complication occurring five years from onset of type 1 diabetes mellitus, leading to renal failure and death. It is characterized by albuminuria and a reduced glomerular filtration rate. Although much emphasis has been placed on screening for albuminuria and estimated glomerular filtration rate (eGFR) to predict or diagnose DN, less attention has been focused on the role of high density lipoprotein cholesterol (HDL-C) in risk assessment of DN onset. The aim of the present study was to evaluate the association between HDL-C levels and the markers of kidney functions, urinary albumin creatinine ratio (UACR) and eGFR, in patients with type 1 diabetes mellitus. In total, 89 type 1 diabetic mellitus patients attending Kenyatta National Hospital were recruited. A questionnaire was administered to ascertain age, gender, marital status, education level, family history of kidney disease and the diabetes duration. The following parameters were measured: blood pressure, body mass index, UACR, eGFR, total and HDL cholesterol. Data analysis was done using SPSS version 20.0. Chi squared test was used to analyse categorical variables of states of UACR, HDL-C and eGFR against the demographic and clinical risk factors. While for analysis of between group continuous variables, t test, one way ANOVA and Pearson correlation statistics were applied. The values of UACR, HDL-C and eGFR levels in the study population ranged from 3–300 mg/g, 0.45–3.45 mmol/l and 29.1–240.5 ml/min/1.73m², respectively. The number of participants with abnormal levels of UACR, HDL-C and eGFR were 45%, 14%, and 22%, respectively. The UACR and HDL-C values were significantly associated with the risk factors of: duration of diabetes, systolic and diastolic blood pressure (p < 0.05). The confounding factor of marital status was only significantly associated with UACR levels. On the other hand eGFR was not associated with any of the patients tested characteristics (p > 0.05). The HDL-C values were significantly lower in the subjects with albuminuria compared to normoalbuminuric group (p = 0.001). There was no significant association or correlation between HDL-C and eGFR values (r = 0.029; p > 0.05). Therefore, eGFR does not add predictive value of diabetic nephropathy among patients with type 1 diabetes mellitus. However, there was a significant inverse correlation between HDL-C and UACR level (r = -0.394; p < 0.05). Therefore, HDL-C has the potential to alternately predict the development of nephropathy levels among patients with a long standing case of type 1 diabetes mellitus.
CHAPTER ONE: INTRODUCTION

1.1 Background information

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Symptoms of diabetes include polyuria, polydipsia, weight loss, sometimes with polyphagia and blurred vision (ADA, 2010). The disease is broadly classified into four types: diabetes mellitus type 1, which is caused by lack of production of insulin by the pancreas resulting from a cellular autoimmune destruction of the beta cells of the pancreas. This disease usually presents acutely in younger people. Type 2 diabetes mellitus, caused by poor or lack of response to insulin hormone, it is mostly evident from age 40 years. Type 3 diabetes mellitus also known as gestational diabetes, which involves a combination of relatively inadequate insulin secretion and responsiveness and it usually improves or disappears after delivery (Gardener and Dolores, 2011). The fourth type is the secondary diabetes, arising from other diseases such as chronic pancreatitis, Cushing’s syndrome, acromegaly and also occurs following pancreatic surgery (Marshall, 1988).

Diabetes mellitus is a major cause of morbidity and mortality with much of the economic and social costs attributed to long term microvascular and macrovascular complications and diminished quality of life. The injurious effects of microvascular complications include diabetic nephropathy (DN) leading to renal failure, retinopathy with potential loss of vision, peripheral neuropathy with risks of foot ulcers, amputations and charcoat
joints. Macrovascular complications include coronary artery disease, peripheral arterial disease, stroke and sexual dysfunction (Fowler, 2008).

Recent estimates indicate, that there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 (Fowler, 2008; ADA, 2010). In Africa, about 9.4 million people are affected by diabetes mellitus and it is estimated to increase to 12.7 million, an increase of about 35% by 2015 (Naicker, 2013). The National costs of diabetes in the USA for 2002 were estimated at $ US 132 billion and these are expected to rise to $ 192 billion in 2020 (ADA, 2010).

Diagnosis of diabetes mellitus depends upon the demonstration of hyperglycemia in patients with classical symptoms and signs, or asymptomatic. A fasting plasma glucose concentration of ≥ 7.0 mmol/l (126 mg/dl), or a random plasma concentration value of ≥ 11.1 mmol/l (200 mg/dl) is diagnostic (WHO, 2006). Diabetes has been associated with high risk for development of progressive renal disease. After 5 to 10 years of type 1 diabetes mellitus, 40% of individuals begin to suffer from microalbuminuria, a condition characterized by excretion of small amounts of albumin in urine (Bishop et al., 2005; Oerfelt and Regitz, 2012). Kidney disease in diabetic patients is clinically characterized by increasing rates of urinary albumin excretion starting from normoalbuminuria, which progresses to microalbuminuria, macroalbuminuria and eventually to end stage renal disease (ESRD) (Venugopal and Iyer, 2010).
Uncontrolled hyperglycemia is the most important contributing risk factor to the development of diabetic nephropathy among the type 1 diabetic patients. However, glycemic control cannot be the only determinant of who develops diabetic nephropathy as only about one third of individuals with type 1 diabetes develop nephropathy regardless of glycemic control (Molitch et al., 2006). Afkhami-Ardekani et al. (2008) observed that arterial hypertension and abnormalities of blood lipid concentration and structures are also important antecedents of renal complications in diabetes mellitus. Several studies have confirmed reduced high density lipoprotein cholesterol (HDL-C) level as an important cardiovascular risk factor. Similarly, the cardioprotective attributes of HDL-C have also been elucidated by many studies. However, it is still not clear whether normal HDL-C levels have independent protective effects on kidney function (NCEP, 2001).

1.2 Problem statement and justification

Diabetic nephropathy is a common complication of diabetes and a leading cause of end-stage renal disease. End stage renal disease is the stage of kidney impairment which is irreversible, cannot be controlled by conservative management alone, and requires dialysis or kidney transplantation to maintain life. The diseases accounts for a significant number of morbidity and mortalities among the diabetics. It also exerts a huge social and economic burden on the overall cost of the healthcare system and the community through large numbers of hospital admissions and specialized medical care including dialysis and kidney transplant. It is, therefore, important to generate more information on the risk factors that compound this disorder to encourage preventive care.
Microalbuminuria measurement is currently the gold standard for detection or prediction of DN. However, its predictive powers have limitations as it has been reported that a decline in the renal function of patients with diabetes is not always accompanied by increased urine albumin creatinine ratio (UACR) and in other cases, one may have microalbuminuria that is not related to the decline of renal function. Therefore, markers that offer greater predictability and sensitivity to development of DN need to be identified to overcome the problems inherent in measurement of UACR. This study, therefore, aimed to test whether measurement of HDL-C levels can add more predictive value and be a more beneficial alternative marker to UACR in predicting development of DN among type 1 diabetic patients. It is envisaged that information generated by this study will further improve our knowledge on the predictors of diabetic nephropathy and also the risk factors that confound the type 1 diabetes mellitus patients to development of diabetic nephropathy.

1.3 Null hypothesis
There is no correlation between HDL-C levels and markers of kidney functions in the prediction of development of diabetic nephropathy amongst type 1 diabetic patients.

1.4 Objectives
1.4.1 Main objective
To determine the correlation between HDL-Cholesterol levels and kidney function in predicting diabetic nephropathy among type 1 diabetic mellitus patients.
1.4.2 Specific objectives

i) To determine the concentrations of HDL-C, UACR and eGFR in patients with type 1 diabetes mellitus and establish their association with the risk factors of age, gender, marital status, literacy, family history of kidney disease, diabetes duration, body mass index and blood pressure.

ii) To determine the association between the levels of HDL-C and UACR in predicting diabetic nephropathy among type 1 diabetes mellitus patients.

iii) To determine the association between the levels of HDL-C and eGFR in predicting diabetic nephropathy in patients with type 1 diabetes mellitus.

1.5 Significance of the study

It is hoped that the information generated by this study will further advance our knowledge on the understanding of the best approaches for early detection and greater predictability of DN. This will help inform the operations in our health care system with regard to prevention, early detection or delaying the onset of renal disease in type 1 diabetes and also improve the monitoring and management strategies for those already with diabetic nephropathy.
CHAPTER TWO: LITERATURE REVIEW

2.1 Definition and history of diabetic nephropathy

Diabetic nephropathy has classically been defined as increased protein excretion in urine characterized by a set of structural and functional kidney abnormalities in patients with diabetes. Structural abnormalities include hypertrophy of the kidney, increase in glomerular basement membrane thickness, nodular and diffuse glomerulosclerosis, tubular atrophy and interstitial fibrosis, while the functional alteration includes an early increase in glomerular filtration rate with intraglomerular hypertension and eventual loss of renal function (Ayodele et al., 2004). Diabetes nephropathy is also known as Kimmelstiel-Wilson syndrome or nodular diabetic glomerulosclerosis or intercapillary glomerulonephritis. The syndrome was first described by a British physician Clifford Wilson (1906 – 1997) and American physician Paul Kimmelstiel (1900 – 1970) in 1936 (Vujicic et al., 2012).

The natural history of diabetic nephropathy in individuals with type 1 diabetes is clinically characterized initially with a small increase in urinary albumin excretion referred to as microalbuminuria also called incipient nephropathy; and subsequently a more advanced disease defined by the presence of macroalbuminuria or proteinuria referred to as overt nephropathy; with a progressive decline in glomerular filtration rate leading to end stage renal disease (Zelmanovitz et al., 2009; Ahmedani et al., 2012). Ahmedani et al. (2012) stated that with microalbuminuria, the decline in renal functions
varies but average reduction in glomerular filtration is around 10-12 ml/min/year. Approximately, 40% of all type 1 diabetic patients ultimately develop the clinical syndrome of diabetic nephropathy associated with a progressive increase in urinary albumin excretion, accompanied with a rise in blood pressure and a relentless decline in glomerular filtration culminating eventually into end stage renal failure (Gupta et al., 2013).

2.2 Prevalence and risk factors of diabetic nephropathy

Diabetic nephropathy is one of the dreaded complications of diabetes mellitus affecting approximately 40% of type 1 and 2 diabetic patients globally (Gross et al., 2005). The European Diabetes (EURODIAB) prospective complications study group and the 18 – years Danish study showed that the overall occurrence of microalbuminuria in patients with type 1 and 2 diabetes mellitus is (after 7.3 years) 12.6% and 33%, respectively (Vujicic et al., 2012). Evans and Capell (2000) stated that approximately 40% of patients with type 1 diabetes and 5 – 15% of patients with type 2 diabetes eventually develop ESRD, although the incidence is substantially higher in certain ethnic groups. This makes patients with type 1 diabetes to be at the highest individual risk of nephropathy, though those with type 2 diabetes are also at significant risk. In Sub-Saharan Africa, the prevalence of diabetic nephropathy ranges from 6% to 16% (Naicker, 2013). Lutale et al. (2007), in their study on microalbuminuria among type 1 and type 2 diabetic patients of African origin in Dar es Salaam, observed an overall prevalence of microalbuminuria of 11% and macroalbuminuria 5% whereby in type 1 patients, microalbuminuria was
present in 12% and macroalbuminuria in 1%, while among the type 2 patients, 10% and 7% had microalbuminuria and macroalbuminuria, respectively. Afkhami-Ardekani et al. (2008) observed that after 5 – 10 years of type 1 diabetes mellitus, 40% of individuals become microalbuminuric, and when macroalbuminuria sets in, 50% of the cases reach end stage renal disease in 7 – 10 years.

The two main risk factors associated with development of diabetic nephropathy are hyperglycemia and arterial hypertension. Several studies have shown significant correlation between increased arterial pressure and glycated hemoglobin which is an indicator for poorly controlled sugar with the development of diabetic nephropathy (Celepkolu et al., 2014; vujicic et al., 2012). But also of great importance is dyslipidemia which is characterized by increased serum triglyceride, LDL cholesterol and decreased HDL cholesterol (Bamashmoos and Ganem, 2013; Celepkolu et al., 2014). Genetic predisposition has also been said to be a substantial determinant of the occurrence and severity of DN. Whereby it is more likely in siblings and children of parents with proteinuria independently of the type of diabetes, with 14% probability of a child from parents without proteinuria, 23% probability incase one of the parents has proteinuria and 46% probability incase both parents have proteinuria, although the exact genetic model underlying DN susceptibility is uncertain (Zelmanovitz et al., 2009; Vujicic et al., 2012). Other risk factors are smoking, glomerular hyperfiltration, dietary factors, age, duration of diabetes, gender and overweight (Viswanathan et al., 2010; Agarwal et al., 2011).
Important clinical concomitants of diabetic nephropathy are retinopathy and cardiovascular disease. Nearly all patients with diabetic nephropathy will also have developed retinopathy. This has important implications for screening and attention to preventive measures in the care of patients with diabetes. Furthermore, diabetic renal disease is closely associated with coronary artery disease as the presence of microalbuminuria is a powerful predictor of death from cardiovascular disease. Thus, the presence of diabetes, especially if accompanied by microalbuminuria, is a signal for very aggressive attention to all cardiovascular disease risk factors and for improvement to the fullest extent possible by lifestyle modification and pharmacotherapy when appropriate (Evans and Capell, 2000).

2.3 Pathogenesis of diabetic nephropathy

Diabetic nephropathy is a progressive kidney disease characterized by microangiopathic damage of glomerular vessels, which typically manifests with proteinuria and diffuse glomerular sclerosis (Oerfelt and Regitz, 2012). Diabetic nephropathy in individuals with type 1 diabetes is initially characterized by thickening of the glomerular and tubular membrane, with progressive mesangial expansion leading to the progressive reduction of glomerular filtration surface. Concurrent interstitial morphological alterations also occur as well as hyalinization of the afferent and efferent glomerular arterioles (Zelmanovitz et al., 2009).
Afkhami-Ardekani et al. (2008) observed that hyperglycemia; arterial hypertension and dyslipidemia cause disorders of the albumin excretion rate by damaging the podocyte and slit diaphragm protein scaffold with overproduction of and extracellular release of oxygen radical species at the glomerular level. In a study conducted in Pima Indians, an ethnic group highly susceptible to development of diabetic nephropathy, a smaller number of podocytes per glomerulus was the greatest predictor of increased urinary albumin excretion and progression to clinical nephropathy, whereby when this finding was present in normoalbuminuric individuals, they were at a higher risk of progressing to renal disease than those who did not have a podocyte lesion (Zelmanovitz et al., 2009).

Boyle (2007) elucidated the generalized narrowing of arterial walls throughout the body of the diabetic patient following oxidation, which accumulate in the endothelial walls of arteries. Monocytes then infiltrate the arterial wall and differentiate into macrophages which accumulate oxidized lipids to form foam cells. Once formed, foam cells stimulate macrophage proliferation and attraction of T-lymphocytes. The T-lymphocytes in turn induce smooth muscle proliferation in the arterial walls and collagen accumulation resulting in formation of a lipid rich atherosclerotic lesion. The pathological changes to the kidney include increased glomerular basement, membrane thickness, microneurysm formation and mesangial nodule formation called kimmelsteil-wilson bodies (Fowler, 2008). The development of diabetic nephropathy (DN) is characterized by a progressive increase in excretion of protein, particularly albumin, an early and continuing rise in systemic blood pressure and late decline in glomerular filtration rate (GFR) leading to
eventual end stage renal failure (Hamed et al., 2002). Albuminuria also activates a series of inflammatory pathways through tubular cells and feeds this process. In addition, the mechanical stress resulting from renal hyperperfusion induces the release of cytokines (TNF-α), growth factors (VEGF, TGF-β), cholesterol and local triglycerides that induce the accumulation of proteins from extracellular matrix leading to mesangial expansion and glomerulosclerosis (Sharma et al., 1999). Vujicic et al. (2012) and Mongesen et al. (1983) outlined five stages of development of diabetic nephropathy:

i) **Early hypertrophy stage:** This stage lasts approximately five years from the onset of the disease. It is characterized by increase in the size of the kidneys by approximately 20% and an increased renal plasma flow of 10%-15%, while albuminuria and blood pressure remains within the normal range. Glomerular filtration rate (GFR) may be normal or increased.

ii) **Silent stage:** This stage starts approximately two years after the onset of the disease and is characterized by subtle morphological changes of the kidney including thickening of the glomerular basement membrane, glomerular hypertrophy, mesangial and tubulointerstitial expansion. There are still no clinical signs of the disease at this stage, though GFR is increased. A number of patients remain in this stage until the end of their life.

iii) **Incipient diabetic nephropathy:** This stage is characterized by microalbuminuria (UACR 30 – 299 mg/g) and it is the first clinically detectable sign of glomerular damage. Blood pressure may be increased or normal. It usually occurs five to ten
years after the onset of the disease. Approximately 40% of patients reach this stage.

iv) **Overt diabetic nephropathy**: It is characterized by macroalbuminuria (UACR ≥ 300 mg/g), the urine dipstick tests positive for proteinuria while GFR decreases below 60 mL/min/1.73 m², and blood pressure increases above normal values.

v) **End stage renal disease**: It is characterized with uremia, otherwise referred to as terminal kidney failure (TKF). The GFR decreases below 15 mL/min/1.73 m² and the patients may require kidney replacement therapy (peritoneal dialysis, hemodialysis, kidney transplantation).

### 2.4 Screening and diagnosis of diabetic nephropathy

Diabetic nephropathy is generally defined by a rise in urinary albumin excretion and reduced renal function as reflected by rise in plasma creatinine concentration, reduced calculated creatinine clearance or decreased glomerular filtration rate. Therefore, measurement of urinary albumin excretion, glomerular filtration rate (GFR) including determination of various risk factors is essential in predicting and diagnosing diabetic nephropathy (Fineberg et al., 2013).

Currently, microalbuminuria is used as the gold standard marker for diabetic nephropathy however, it is far from ideal as a risk marker or predictor of nephropathy as it often regresses to normoalbuminuria as noted by Tabaei et al. (2001). Gross et al. (2005) also observed that some patients with either type 1 or type 2 diabetes have decreased
glomerular filtration rate (GFR) in the presence of normoalbuminuria, traditionally, GFR is expected to decrease when proteinuria is established, but not before. Bamashmoos and Ganem (2013) also observed that about 10% type 2 diabetic subjects could have diabetic nephropathy, low GFR without increased urinary albumin excretion which is also true among the type 1 diabetics. Klassen (2003) and Miller et al. (2009) highlighted a number of variables affecting measurement of urinary albumin excretion accuracy, and as such, GFR should routinely be estimated and urinary albumin excretion routinely measured for a proper screening of diabetic nephropathy. ADA (2010) and NKF (2007) recommended annual screening for kidney damage starting at the diagnosis of patients with type 2 diabetes and five years after diagnosis of type 1 diabetes to allow early detection of development of diabetic nephropathy, and the following parameters are recommended.

2.4.1 Urinary albumin excretion

One of the central functions of the kidneys is the excretion of low molecular weight, water soluble, plasma waste products into urine, whereas macromolecules the size of albumin and larger are retained. Glomerular damage usually results in glomerular proteinuria due to both increased permeability to plasma proteins that are normally not freely filtered through glomerulus such as albumin and transferin and increased excretion of extracellular matrix proteins (Afkhami-Ardekani et al., 2008).

After 5-10 years of type 1 diabetes mellitus, 40% of individuals begin to excrete small amounts of albumin in the urine (microalbuminuria). Microalbuminuria is the first
clinically detectable sign of renal involvement and once present, it progresses over 5 – 10 years to macroalbuminuria (Afkhami-Ardekani et al., 2008; Ahmedani et al., 2012). The term microalbuminuria has been used to describe an amount of albumin in the urine which is less than can be detected by ordinary clinical dipstick tests such as albustik (Hamed et al., 2002). According to ADA (2010), microalbuminuria is defined as an albumin excretion rate of 30–299mg/24hours (incipient nephropathy), while albumin excretion rate of ≥ 300 mg/24 hours is macroalbuminuria (overt nephropathy). Albumin is normally present in urine at a concentration of less than 30 mg/g of creatinine (Table 2.1). Presence of macroalbuminuria heralds the onset of proteinuria which is a sign of established kidney damage and plays a direct pathogenic role in the progression of renal and cardiovascular disease (Ruggeneti and Remuzzi, 2006). Microalbuminuria or elevated urine albumin creatinine ratio (UACR) is currently the gold standard marker of glomerular damage as well as an established early marker and an independent risk factor for development of both renal disease and cardiovascular disease (CVD) among the diabetic and the general population (Matheson et al., 2010).

Different types of urine specimens including spot collection, 24 hour collection and timed collection may be used for determination of urinary albumin excretion. However, engaging in exercise within the last 24 hours prior to testing, infection, fever, congestive heart failure, marked hyperglycaemia, marked hypertension, urinary tract infection, haematuria and menstruation can all increase urinary albumin over baseline level and confound the diagnosis of diabetic nephropathy. Owing to this variability in urinary
albumin excretion, measurement of albumin to creatinine ratio on random spot urine sample is preferred because it helps to compensate for patients with such underlying conditions (ADA, 2004; Fineberg et al., 2013).

Although microalbuminuria is currently the gold standard for detection or prediction of diabetic nephropathy, its predictive powers have limitations. In an extensive review of the issues associated with measurement of UACR, Miller et al. (2009) listed a number of variables affecting urinary albumin excretion. This included; gender, age, ethnicity, muscle mass, diet, time of collection and inter-method variations. Klassen (2003) also observed that microalbuminuria is not always a predictor of progressive DN as it may be seen transiently during pregnancy, after exercise and with protein loading including day to day as well as diurnal variation.

It is therefore, imperative to come up with alternative markers that offer greater predictability and sensitivity to the development of nephropathy to overcome the problems inherent in UACR measurement. In order to address these inadequacies, this study seeks to evaluate whether measurement of HDL-C levels can be used as an alternative marker that will offer greater sensitivity and predictability of DN development in type 1 diabetes patients.
Table 2.1: Definitions of abnormalities in albumin excretion.

<table>
<thead>
<tr>
<th>Category</th>
<th>Spot collection (mg/g creatinine)</th>
<th>24 hr collection (mg/24h)</th>
<th>Timed collection (µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 30</td>
<td>&lt; 30</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30 – 299</td>
<td>30 -299</td>
<td>20 – 199</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>≥ 300</td>
<td>≥ 300</td>
<td>≤ 200</td>
</tr>
</tbody>
</table>

Source: ADA (2010).

2.4.2 Glomerular filtration rate

Glomerular filtration rate is accepted as the best measure of overall kidney function in health and disease. In principle, glomerular filtration rate (GFR) is the product of the number of nephrons. Chronic kidney disease progressively destroys the nephrons hence reducing the GFR of the subject (Afkhami-Ardekani et al., 2008). An accurate measure of GFR can be undertaken using low molecular weight markers of kidney function such as inulin, iohexol or technetium (labelled DTPA), however, these methods are time consuming, expensive and generally not available. The regular measurement of serum creatinine levels is simple to perform and is currently the most common method. However, because creatinine is invariably reabsorbed by the renal tubules, serum creatinine and creatinine clearance measurements tend to underestimate the GFR in the context of hyperfiltration and over estimate the GFR in the context of hypofiltration and is subject to errors in urine collection unless great care is taken (Chadban et al., 2010).
For optimal approximation of GFR from serum creatinine measurements, allowances need to be made for age, gender, race, height and weight of the individual as in the Modification of Diet in Renal Diseases (MDRD) equation which is a satisfactory index of GFR (Levey et al., 1999).

In microalbuminuric patients, GFR may remain stable, but a subset of patients has shown a rapid decline (Gross et al., 2005). In approximately half of the patients with type 1 diabetes lasting up to five years, GFR value is approximately 25 – 50% above normal range and these patients have a higher risk of developing diabetic nephropathy (Vujicic et al., 2012). Type 1 diabetics often have an initial elevation in their GFR, which may exceed 150 ml/min, in stage one of glomerular hypertrophy and hyperfiltration and it is significantly related to the increase in the kidneys size. However, with the increase in protein excretion, there is a tendency of GFR to fall to lower levels during the second and the third stages of diabetic nephropathy. Persistent proteinuria results in progressive fall of GFR culminating into ESRD in months to a year if not managed (Hamed et al., 2002).

The most convenient method for appraising the kidney function is the estimated glomerular filtration rate (eGFR) which is used to stage the level of chronic kidney disease. The eGFR is usually based on serum creatinine levels, age, sex and race of an individual. The recommended equation by the National Kidney Foundation is that of the MDRD which is the most widely used for computing eGFR for people above 18 years old (Gross et al., 2005). This calculator is available on National Kidney Disease Education Program website at http://www.nkdep.nih.gov/professionals/gfr-
While, for determination of eGFR for the 17 years old and below, Counahan-Barratt method is used and it is available as the Steven Fadem calculator at [www.nephron.com](http://www.nephron.com) (Counahan et al., 1976; Levey et al., 1999; Bakris et al., 2011). The reference range of GFR values in young individuals is from 90 to 130 ml/min/1.73m², declining at approximately 10 ml/min/decade after 50 years of age (Gross et al., 2005; NKF, 2007). The GFR levels depicting renal changes in patients are presented in Table 2.2. Although the measurement of albuminuria is essential to diagnose DN, there are some patients who present decreased GFR when urinary albumin excretion values are normal. Based on this, the classification of National Kidney Foundation can also be used to stage chronic kidney disease in such patients (Zelmanovitz et al., 2009).

**Table 2.2: Stages of glomerular filtration rate levels in the development of renal changes**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
<th>GFR (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or raised GFR</td>
<td>≥90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild decrease in GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>3</td>
<td>Moderate decrease in GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>4</td>
<td>Severe decrease in GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney Failure</td>
<td>&lt;15</td>
</tr>
</tbody>
</table>

*Source: NKF, K/DOQI clinical practice guidelines (2002)*
2.4.3 High density lipoprotein cholesterol

High density lipoprotein (HDL) in particular HDL-C, is synthesized primarily in the liver and to a lesser extent in small intestinal cells as a precursor comprising phospholipid, cholesterol, apo-E and apo-A (Marshall, 1988). Higher density lipoprotein is classified into two density classes, HDL$_2$ and HDL$_3$. The former is the larger of the two particles and has an estimated molecular weight of 360,000 kd compared with 175,000 kd for HDL$_3$. The HDL$_2$ is also associated with a larger lipid mass of 60% compared with 45% in HDL$_3$ (Burtis and Ashwood, 1994).

During the synthetic process, phospholipid and free cholesterol undergo extensive compositional and structural modifications after secretion. Most important of these is the esterification of free cholesterol to form cholesteryl ester by an enzymatic reaction catalysed by Lecithin cholesterol acyltransferase (LCAT) which is activated by its cofactor apo-A-1. Persons with LCAT deficiency have an accumulation of cholesteryl ester deficient particles in plasma (Kaplan et al., 2003). This is followed by increased density of HDL particles which are thus converted from HDL$_3$ to HDL$_2$. While some of the cholesteryl esters remain in HDL, much is transferred to other lipid particles such as chylomicrons, very low-density lipoproteins (VLDL-C) and low density lipoprotein (LDL-C) which reaches the liver indirectly. Thus HDL-C has an important function in removing cholesterol from the circulation and in facilitating its excretion (Marshall, 1988; Kaplan et al., 2003).
2.4.3.1 Homeostatic role of HDL-cholesterol in the body

The efflux capacity of HDL-C prevents pathogenesis of atherosclerosis responsible for coronary artery diseases (CAD), by reverse cholesterol transport which removes excess cholesterol, such as the atherogenic low density lipoprotein-cholesterol (LDL-C) particle, from peripheral cells and transports it in plasma for hepatic delivery, excretion in bile and intestinal loss (Khera et al., 2011). HDL-C is now emerging as a key entity in both determining risk and providing protection against renal damage (Blaton, 2009; Trevisan et al., 2006). Muntner et al. (2000) observed that people with low HDL-C and hypertriglyceridemia at baseline have a higher risk for loss of renal function. Similarly, Wang et al. (2013) observed that low HDL-C levels are independently associated with arterial stiffness which is a major cause of reduced kidney function due to renal artery stenosis. Yamamoto et al. (2012) also observed that HDL-C has pleiotropic effects such as antioxidative, antinflammatory and anti-apoptotic effects, possibly protecting the kidney function.

Finally, Blaton (2009) proposed three mechanisms through which dyslipidemia accelerate progression of renal disease; reabsorption of fatty acids and phospholipid by tubular epithelial cells forming foam cells, accumulation of lipoproteins in glomerular mesangium and acquired lecithin cholesterol acyltransferase deficiency due to chronic insufficiency and nephritic syndrome. In an extensive study by Morton et al. (2012), he observed that low HDL-C was a significant independent prognostic factor for the development of renal events, in particular new-onset albuminuria in type 2 diabetes.
2.4.3.2 Desired Lipid goals for management of dyslipidemia

Dyslipidemia is a comorbidity commonly associated with type 1 diabetes. Diabetic dyslipidemia is characterized by elevated triglyceride, low density lipoprotein cholesterol and low high density lipoprotein cholesterol levels (Zelmanovitz et al., 2009). Guidelines issued by American Association of Clinical Endocrinologists (AACE) outline the desired lipid goals for management of dyslipidemia as; LDL-C < 100 mg/dl (< 2.6 mmol/l), triglyceride < 150 mg/dl (< 1.7 mmol/l) and HDL-C > 150 mg/dl (1.7 mmol/l) (AACE, 2012).

2.5 Prevention and treatment strategies for diabetic nephropathy

According to recommendations by American Diabetes Association, patients with type 1 diabetes mellitus of more than 5 years after diagnosis should undergo annual screening for development of microalbuminuria, for early detection or prediction of nephropathy to enable timely medical interventions (ADA, 2010). The prevention and treatment strategy should focus on lifestyle modification, control of hypertension and dyslipidemia. Lifestyle modification is a first step in diabetes management, irrespective of presence of chronic kidney disease (CKD) and should comprise of measures to encourage smoking cessation, weight loss, and increased physical activity as well as dietary changes.
Strict glycemic control is one of the logical measures that help to prevent development of DN according to various clinical trials (Zelmanovitz et al., 2009). The ADA (2010) and NKF (2007) recommend achieving HBA$_1$C of 7.0% in patients with diabetes irrespective of presence of CKD. Guidelines from AACE vary slightly, endorsing a more stringent HBA$_1$C goal of 6.5%. Dietary approach and therapeutic intervention involving insulin use is utilized to achieve this goal.

Intensive blood pressure control is another strategy used to prevent development of nephropathy. Blood pressure goals currently recommended are 130/80 mm Hg for patients with both type 1 and type 2 diabetes irrespective of CKD (NKF, 2007; ADA, 2010). Use of anti hypertensive drugs such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) which are renin-angiotensin system blockers confers an additional benefit on renal function as several studies have demonstrated that, treatment of hypertension produced a beneficial effect on albuminuria (Gross et al., 2005; Fowler, 2008).

Management of the diabetic patients with respect to dyslipidemia should be focused on raising the ‘good cholesterol’ HDL-C and lowering the ‘bad cholesterol’ LDL-C through dietary modifications which includes reduction in saturated fats, trans fat and cholesterol intake together with increases in omega 3 fatty acids, viscous fiber, plant stanoll/ sterols, weight loss and increased physical activity (Bakris, 2011). If this fails, a combination
therapy involving statin and other drugs, such as fibrates or niacin may be necessary to achieve ideal lipid control, but patients should be monitored closely for possible adverse reactions of therapy (Fowler, 2008; Zelmanovitz et al., 2009).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design

This was a descriptive cross-sectional study of type 1 diabetic patients attending the diabetic clinic at Kenyatta National Hospital (KNH), Kenya, performed from September 2014 to May 2015. The clinic received, on average, a daily attendance of about 40 cases, both new and old diabetic patients. The specimens collected from the participants were analysed for various biochemical profiles at the Clinical Chemistry laboratory of the Department of Human Pathology, University of Nairobi.

3.2 Study population

Subjects were recruited from a population of about 600 type 1 diabetes mellitus patients on consecutive sampling basis, by use of patients’ files to establish diagnosis and duration of diabetes. Patients’ records were accessed after obtaining permission from the Hospital’s Research and Programs Department. The subjects were of both genders, aged between 13-48 years.

3.3 Inclusion criteria

The inclusion criterion for the subjects was set as follows;

1. Patients who had been diagnosed as type 1 diabetic for the last 5 or more years.
2. Patients aged from 13 years and above.
3. Patients who were not on lipid lowering agents.
4. Patients attending KNH diabetic clinic regularly for the last two months.

5. Patients who were not pregnant.

6. In non-fasting state for the purpose of standardization of lipid results according to Sindhu et al. (2012).

7. Signed informed consent to participate in study.

3.4 Exclusion criteria

The exclusion criterion for the subjects was set as follows;

1. Patients diagnosed as type 1 diabetes mellitus with less than 5 years into diagnosis.

2. Patients on lipid lowering agents.

3. Patients who were pregnant.

4. Patients on dialysis or confirmed to have chronic kidney disease

5. Patients demonstrating urinary tract infections.

3.5 Sample size determination

Calculation of sample proportion was based on formulae by Fisher et al. (1991) and Israel. (1992).

\[ n = \frac{z^2pq}{e^2} \]

n = desired sample size

p = prevalence of DN in type 1 diabetic patients (6.1%) (Naicker, 2013)

q = 1 - p which is (1 – 0.061)

e = desired level of precision at 5% (0.05)

z = area under normal curve, 1.96
\[ n = (1.96^2) \times 0.061 \times (1 - 0.061)/0.05^2 \]

\[ n = 89 \]

Therefore 89 participants were recruited in the study.

### 3.6 Ethical consideration of the study

Approval to carry out the study was sought and granted by the Kenyatta National Hospital/University of Nairobi Research and Ethics Committee (Appendix I). Every prospective patient was given a complete explanation directly or through their guardians about the nature and purpose of the study by the study nurse. They were then allowed to ask questions before signing the consent form. Confidentiality of participating subjects was maintained, as patients laboratory evaluation findings were communicated to the attending clinicians for continued timely management.

### 3.7 Recruitment and consenting of the subjects

Recruitment of study participants was done by consecutive sampling basis. Patients meeting the requirements of screening were taken through the consenting process by the study nurse or investigator as per the Appendix II.

### 3.8 Data collection

#### 3.8.1 Questionnaire for demographic details of subjects

Demographic details of age, gender, smoking status, family history of kidney disease, duration of diabetes and medication use of the participants was obtained using a
standardized self-report questionnaire (Appendix III), which was administered on face to face counseling method by the study nurse or the investigator.

3.8.2 Physical examination of the subjects

The physical examination of the participants was done by the study nurse, and it included anthropometric and hemodynamic measurements. The height and weight of the patients were measured using balance beam scale with height road (Seca 700 medical scale, United Kingdom). Body mass index was calculated as the weight in kilograms divided by height of the patient in meters squared (kg/m²), BMI less than 25 kg/m² was considered to be normal weight while levels more than 25.1 were considered as overweight (Rosenbaum et al., 1997). The blood pressure measurements for the participants were taken using a sphygmomanometer (Omron healthcare Europe B.V, global) in supine position. The patients were rested for five minutes to allow blood pressure to stabilize before the measurements were taken to minimize biases occasioned by physical activity or anxiety among the patients. Two readings of blood pressure were made from each participant in space of two minutes to minimize bias due to single reading. The mean value of blood pressure was used for the statistical analysis. Patients were categorized as hypertensive if the systolic blood pressure was above 130 mmHg or if diastolic blood pressure was above 85 mmHg (Afkhami-Ardekani et al., 2008). A study flow-chart showing how the patients were processed is presented in Table 3.1.
Table 3.1: Study flow-chart

KNH diabetic clinic: Patient & patient file; screening by study nurse

Type 1 diabetes → Patient screening and classification → Type 2 diabetes

Type 1 diabetes with ≥ 5 years into diagnosis

Consenting: by nurse/investigator

Consented → Type 1 diabetes with < 5 years into diagnosis

Not consented

Type 2 diabetes

Questionnaire: by study nurse/investigator

Specimen collection: study nurse/investigator

Laboratory: Specimen analysis

Patient notified of availability of results by

Clinic registry: Results received from lab

Copy to the patient file

Patient

Result

Doctor
3.9 Specimen collection

3.9.1 Blood sample collection
A 3 ml venous blood sample was collected from non-fasting patients in a sterile plain vacutainer tube by venipuncture and delivered to the Clinical Chemistry Laboratory, University of Nairobi, for subsequent processing and analysis.

3.9.2 Urine sample collection
Spot urine samples were collected from the subjects in universal urine collection bottles at random during medical visit, and then examined physically for evidence of urinary tract infection and hematuria. The samples were thereafter taken to Clinical Chemistry Laboratory for the measurements of urine albumin to creatinine excretion ratio (UACR).

3.9.3 Handling of collected samples
Samples were delivered to the laboratory and analysed on the same day of collection. In cases of delay in the analysis, serum was kept frozen at -30 °C while urine was stored at 2-8 °C.

3.10 Laboratory analysis of samples
Determination of the concentrations of total cholesterol, HDL-C and creatinine on the subjects’ blood specimen was done using Human diagnostic reagent kits (Human Gesellschaft fur Biochemica und Diagnostica MBH, German) and the results evaluated by an automated Selectra pro S series clinical chemistry analyser (Elitech group clinical systems, Netherlands). The sera creatinine results were used in the computation of eGFR.
results of the subjects. Dyslipidemia was defined as an elevation of serum total cholesterol of $\geq 6.1$ mmol/l and decreased HDL-C of $< 1.04$ mmol/l, while eGFR value of $\leq 90$ ml/min/1.73m$^2$ indicated reduced renal function (NCEP, 2001; Renal association, 2013). On the other hand, UACR levels were determined on subjects’ urine samples using Clinitek 50® system (Bayer healthcare LLC, USA). Diabetic nephropathy was indicated based on albuminuria of $\geq 30$ mg albumin/g creatinine (ADA, 2012).

3.10.1 Total cholesterol determination

3.10.1.1 Principle of assay

Total cholesterol was assayed using Chod-pap® method, which is an enzymatic colorimetric test with lipid clearing factor. Cholesterol is determined after enzymatic hydrolysis and oxidation, the indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The absorbance of this complex of quinoneimine is proportional to the cholesterol concentration in the sample (Human Gesellshaft fur Biochemica und Diagnostica MBH, German).

3.10.1.2 Total cholesterol assay

An aliquot of 5 µl of standard and sample was mixed in different cuvettes with 500 µl of working reagent of cholesterol and incubated for 10 minutes at 20-25°C. The concentration of the complex was then measured against the reagent blank at the wavelength of 546 nm on Selectra clinical chemistry analyser (Elitech group clinical
systems, Netherlands). The details of the chemical reactions were as per the test protocol (Appendix IV).

3.10.2 HDL cholesterol determination

3.10.2.1 Principle of assay

The HDL-C levels were assayed by precipitant and standard enzyme calorimetric method whereby chylomicrons, VLDL and LDL are precipitated by addition of phosphotungstic acid and magnesium chloride. After centrifugation the supernatant fluid contains the HDL fraction, which is assayed for HDL cholesterol with the cholesterol liquicolor test kit (Human Gesellschaft fur Biochemica und Diagnostica MBH, German).

3.10.2.2 HDL cholesterol assay

An aliquot of 200 µl of patient’s serum was mixed with 500 µl of the precipitant and incubated for 10 minutes at room temperature, followed by centrifugation for 2 minutes at 10,000 g. The clear supernatant was separated from the precipitate within 1 hour. A 50 µl volume of distilled water (blank), standard, and HDL supernatant was pipetted into different cuvettes and mixed with 500 µl of the cholesterol working reagent then incubated at 20-25 °C for 10 minutes. After incubation, the concentrations of the standard and the sample were measured against reagent blank at a wavelength of 546 nm on the Selectra clinical chemistry analyzer (Elitech group clinical systems, Netherlands). The details of the chemical reactions were as per the test protocol (Appendix V).
3.10.3 Serum creatinine determination

3.10.3.1 Principle of assay

Serum creatinine concentrations were determined by Jaffe-reaction photometric colorimetric test for kinetic measurements using human creatinine liquicolor reagent. Creatinine forms an orange-red colored complex with picric acid in alkaline solution. The absorbance of this complex is proportional to the creatinine concentration in the sample (Human Gesellschaft fur Biochemica und Diagnostica MBH, German).

3.10.3.2 Serum creatinine assay

A 500 µl volume of creatinine working reagent was incubated at 37°C for about two minutes, and then mixed with 50 µl of patient’s serum or standard as during calibration. The reactants were incubated within the programmed lag time of 30 seconds, and a read time of 120 seconds after which the clinical chemistry analyzer displayed the concentration of creatinine at a wavelength of 510 nm. The details of the chemical reactions were as per the test protocol (Appendix VI).

3.10.4 Computation of estimated glomerular filtration rate

Estimated glomerular filtration rate for participants aged 18 years and above, was computed using Modification of Diet in Renal Diseases (MDRD) formula available on National Kidney Disease Education Program website at http://www.nkdep.nih.gov/professionals/gfr-calculator/org-con.htm:
eGFR (ml/min/1.73m²) = 175 x (serum creatinine level)^{1.154} x (age^{-0.203} x (1.742 if female) x (1.212 if African American) (Levey et al., 1999).

While for the participants under the age of 18 years, Counahan-Barratt method (Counahan et al., 1976) was applied using the Steven Fadem calculator available online at www.nephron.com. eGFR (ml/min/1.73m²) = (0.43 x length) / serum creatinine level.

A normal eGFR was taken to be above 90 mls/min/1.73m² according to Levey et al (1999) and Renal Association (2013).

3.10.5 Analysis of urine albumin to creatinine excretion rate

The measurement of urine albumin and creatinine excretion rate was done by Clinitek 50® system (Bayer healthcare LLC, USA). It is a semi automated bench top instrument designed to read microalbumin 2 reagent strips calorimetrically to give results of urinary albumin and creatinine excretion rate.

3.10.4.1 Principle of assay

Albumin determination is based on albumin binding a high affinity sulfonephthalein dye at a constant pH to produce a blue color while creatinine reacts with disopropylbenzene dihydroperoxide and 3,3’,5,5’- tetramethylbenzidine using a catalyst copper creatinine complex to produce colored complex ranging from orange through green to blue. The intensity of these colors is determined calorimetrically to give the actual concentration of albumin and creatinine (Bayer healthcare LLC, USA).
3.10.4.2 Assay of urine albumin-creatinine excretion rate

The strip was dipped in the patient’s urine wetting the test pads then removed immediately dragging the edge of the strip against the rim of the urine container to remove excess urine. The start button on the Clinitek 50® system was switched on while blotting the reagent strip on a paper towel after which it was placed on the instrument’s test/feed table with the reagent pads facing up. The table was then automatically moved into the instrument’s reader position where the strip was identified and read, displaying or printing the results as soon as they were available. The details of the chemical reactions were as per the test protocol (Appendix VII).

A diagnosis of microalbuminuria was made when the ratio of urinary albumin to creatinine (UACR) was 30-299, whereas macroalbuminuria was indicated when the UACR was above 300 mg/g. Normoalbuminuria was said to exist if UACR was less than 30 mg/g (ADA, 2012).

3.11 Quality control and interpretation of patients’ results

Commercial control materials were used whereby, for each analyte, the control was treated the same way as patient’s sample. High and low range commercial controls were run before sample analysis, the control values, the lot number and the date of the run was recorded in the control log sheet. The patients’ samples were only run and reported following acceptable quality control performance and interpreted based on the reference ranges as provided by National Cholesterol Education Program (NCEP, 2001) for lipids, ADA (2010) for UACR and NKF (2008) for eGFR.
3.12 Statistical analysis

Continuous variables were expressed as means and standard deviation while categorical variables were expressed as percentages. The UACR values were used to categorize patients into various nephropathy states. Subjects with UACR ≤ 29 mg albumin/g creatinine were classified as normoalbuminuria (no nephropathy) while those with UACR ≥ 30 mg albumin/g creatinine were categorized as albuminuria (nephropathy). Chi square tests (Pearson $X^2$, likelihood ratio and Fishers Exact Test) were used to analyse categorical variables of states of UACR, HDL-C, and eGFR levels of the subjects against the demographic, clinical and biochemical risk factors. On the other hand, between group comparison for continuous variables, unpaired t test and one way ANOVA were applied.

To evaluate correlation between HDL-C with UACR and eGFR, Pearson’s correlation analysis for continuous variables was used, while the statistical comparison of the HDL-C group means in UACR and eGFR states was undertaken using the unpaired t test. $P < 0.05$ was defined as the limit of statistical significance. All data entry and management activities were undertaken and analysed on spreadsheet for IBM Statistical Package for Social Scientists (SPSS) version 20.0. The descriptive summary statistics were presented in forms of tables and figures.
CHAPTER FOUR: RESULTS

4.1 UACR levels in diabetic patients

A descriptive summary of demographic, clinical and biochemical characteristics of type 1 diabetic patients in different states of UACR levels categorized as normoalbuminuria (UACR ≤29 mg/g) or albuminuria (UACR ≥30 mg/g) is presented in Table 4.1. Patients’ individual data is presented in Appendix VIII. Out of the 89 subjects, 49 (55%) had normoalbuminuria, while 40 (45%) had increased UACR levels designated as albuminuria which comprised 35 microalbuminuric and 5 macroalbuminuric subjects.

Amongst the 49 normoalbuminuric subjects, 43% were male while 57% were female. Out of the 40 albuminuric subjects, 40% and 60% were male and female, respectively. No statistically significant association was found between the incidence of albuminuria and the gender of the patients. Analysis of the subjects’ marital status showed that among the normoalbuminuric group, 47% were married while 53% were single. For the albuminuric group, 25% were married while 75% were single. Being single was therefore a significant confounding factor for nephropathy in the subjects (p = 0.033).

Analysis of the subjects’ on the basis of literacy standards as measured by the education level attained showed that, of the 49 normoalbuminuric subjects, 28% had attained primary level of education whereas, 45% and 27% had attained secondary and tertiary
levels of education, respectively. For the albuminuric participants 25% had attained primary level of education while 52% and 23% had attained secondary and tertiary levels of education, respectively. Statistical analysis showed no significant association between development of albuminuria and the level of education attained ($p = 0.426$).

Four of the subjects with normoalbuminuria had history of kidney disease in their families as opposed to only two in the albuminuria state. No statistical significant association was found between incidence of albuminuria and the family history of kidney disease ($p = 0.687$). The mean age for the subjects was $25 \pm 8.9$ years; the two groups, normoalbuminuria ($25 \pm 8.2$ years) and albuminuria ($26 \pm 9.7$ years), were virtually identical with respect to age hence the difference was not statistically significant ($p > 0.05$). On the other hand, the mean duration of diabetes for the albuminuric group was significantly higher than the normoalbuminuric group ($p < 0.05$).

The average BMI for all the subjects was $22.5 \pm 3.6$ kg/m$^2$ with no significant statistical difference between the subjects with normoalbuminuria and albuminuria ($p > 0.05$). Participants’ blood pressure averaged 120.7 mmHg and 76.2 mmHg for systolic and diastolic, respectively. Blood pressure (sBP and dBP) for the subjects with albuminuria was significantly higher than for those with normoalbuminuria ($p < 0.05$).
The participants’ had mean HDL-C value of $1.33 \pm 0.38$ mmol/l. The subjects with albuminuria had mean HDL-C level of $1.19 \pm 0.27$ mmol/l, which was significantly lower than the normoalbuminuric group at $1.47 \pm 0.41$ mmol/l ($p < 0.05$). On the other hand, total cholesterol levels were not statistically different between the normoalbuminuria and the albuminuria group ($p = 0.155$). Mean eGFR values for the participants was $115.3 \pm 38.6$ ml/min/1.73m$^2$; and the difference between the UACR states was not significant ($p > 0.05$).

It was noted that subjects with albuminuria were generally older, had longer duration of diabetes with higher systolic and diastolic blood pressure but reduced levels of HDL-C and eGFR.
Table 4.1: Characterization of patients’ data within levels of UACR states

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Urine albumin creatinine ratio states</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normoalbuminuria</td>
<td>Albuminur</td>
</tr>
<tr>
<td></td>
<td>89</td>
<td>49 (55)</td>
<td>40 (45)</td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
<td>37 (42)</td>
<td>21 (43)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>37 (42)</td>
<td>21 (43)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>52 (58)</td>
<td>28 (57)</td>
</tr>
<tr>
<td>Marital status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td></td>
<td>56 (63)</td>
<td>26 (53)</td>
</tr>
<tr>
<td>Married</td>
<td></td>
<td>33 (37)</td>
<td>23 (47)</td>
</tr>
<tr>
<td>Education level (%)</td>
<td></td>
<td>25 (28)</td>
<td>15 (31)</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td>25 (28)</td>
<td>15 (31)</td>
</tr>
<tr>
<td>Secondary</td>
<td></td>
<td>40 (45)</td>
<td>19 (38)</td>
</tr>
<tr>
<td>Tertiary</td>
<td></td>
<td>24 (27)</td>
<td>15 (31)</td>
</tr>
<tr>
<td>Family history of kidney disease %</td>
<td></td>
<td>6 (7)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>With</td>
<td></td>
<td>6 (7)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Without</td>
<td>83 (93)</td>
<td>45 (92)</td>
<td>38 (95)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>25 ± 8.9</td>
<td>25 ± 8.2</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>25 ± 8.9</td>
<td>25 ± 8.2</td>
<td>26 ± 9.7</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td></td>
<td>8.6 ± 4.7</td>
<td>6.8 ± 2.3</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td></td>
<td>22.5 ± 3.6</td>
<td>22.7 ± 4.1</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td>22.3 ± 2.8</td>
<td>22.3 ± 2.8</td>
</tr>
<tr>
<td>Systolic</td>
<td>120.7 ± 19.9</td>
<td>113.9 ± 16.0</td>
<td>128.7 ± 21.3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76.2 ± 11</td>
<td>72.5 ± 11.8</td>
<td>80.1 ± 9.4</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.33 ± 0.38</td>
<td>1.47 ± 0.41</td>
<td>1.19 ± 0.27</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.40 ± 1.11</td>
<td>4.23 ± 0.98</td>
<td>4.58 ± 1.24</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>115.3 ± 38.6</td>
<td>121.9 ± 34.9</td>
<td>105.7 ± 41.9</td>
</tr>
</tbody>
</table>

Data are percentage, mean ± SD; normoalbuminuria (UACR, ≤ 29 mg/g), albuminuria includes patients with microalbuminuria (UACR, 30-299 mg/g) and macroalbuminuria (UACR, ≥300 mg/g), p values are based on chi-squared test for proportions and t test for means. Statistical significance was denoted at p < 0.05 [*]. Values in parenthesis are percentages.
4.2 HDL-C levels in diabetic patients

The participants’ demographic, clinical and biochemical data in three different levels of HDL-C states which were classified as normal (≥ 1.55 mmol/l), borderline low (1.05 - 1.54 mmol/l) and low (≤ 1.04 mmol/l) are presented in Table 4.2. Of the 89 patients, 17% had normal levels of HDL-C, 67% had borderline low and 16% had low levels.

Of the 15 subjects with normal levels of HDL-C, 33% were males while 67% were females. Married subjects in this group were 40% while 60% were single. Categorization based on the highest level of education attained by the subjects showed that 20%, 47% and 33% had attained the primary, secondary and tertiary levels of education, respectively. Further analysis of this group revealed that only one subject had a family history of kidney disease while the rest did not.

The borderline low HDL-C group had 60 subjects, 48% of whom were males and 52% were females. Only 40% of the subjects in this group were married. Categorization of the subjects based on education level showed that 32%, 38% and 30% had attained primary, secondary and tertiary levels of education, respectively. Of the 60 subjects, only 8% had history of kidney disease in their families.
Out of the 14 subjects in the group with low levels of HDL-C, 21% were males while 79% were females. Only 21% of the subjects in this group were married. Characterization based on highest level of education attained by the subjects showed that 22% had primary education level, while 71% and 7% had secondary and tertiary levels of education, respectively. None of the subjects in this group had a history of kidney disease in their families. All the subjects in the three states of HDL-C levels did not significantly differ with respect to gender, marital status, education level and the history of kidney disease in their families ($p > 0.05$).

The means of age for the subjects in the three levels of HDL-C states ranged from $26 \pm 6.6$ years for the normal HDL-C state to $25 \pm 9.1$ years for borderline low and $28 \pm 10.4$ years for the group with low HDL-C levels. No significant difference was found between the three groups with respect to age ($p = 0.517$). On the other hand, HDL-C levels of the subjects reduced significantly with the increase in the duration of diabetes ($p < 0.05$).

Analysis of BMI for the subjects in the three states of HDL-C levels showed that the subjects were virtually identical with respect to BMI (normal, $22.9 \pm 3.3$; borderline low, $22.4 \pm 3.9$; low, $22.3 \pm 2.8$ kg/m$^2$; $p = 0.903$). While evaluation of blood pressure (sBP and dBP) for the three states of HDL-C showed a statistically significant increase of both systolic and diastolic blood pressure, with the highest mean of blood pressure recorded in the lower state of HDL-C and the lowest mean in the normal HDL-C state ($p < 0.05$).
On analysis of the subjects’ biochemical parameters between the three states of HDL-C levels, a statistically significant difference between the means of UACR levels was found in the three states of HDL-C. There was a negative relationship between UACR and HDL-C whereby, as the HDL-C levels reduced, UACR levels increased ($p < 0.05$). On the other hand, the means for the patients total cholesterol levels were virtually identical with respect to the three states of HDL-C levels ($p > 0.05$). Similarly, no statistical difference was found between the three states of HDL-C levels with respect to the subjects’ eGFR values; normal HDL-C state had average eGFR of 109.6 ± 30.8; borderline low state had 120.2 ± 41.1 and low HDL-C state had 95.9 ± 31.9 ml/min/1.73m$^2$ ($p > 0.05$).

Generally, subjects with low HDL-C levels were likely to be older, had longer duration of diabetes, and had higher systolic and diastolic pressure with lower UACR and eGFR levels.
### Table 4.2: Characterization of patients’ data within levels of HDL-C states

<table>
<thead>
<tr>
<th></th>
<th>High density lipoprotein cholesterol states</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Borderline low</td>
</tr>
<tr>
<td>n=89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (17)</td>
<td>60 (67)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (67)</td>
<td>31 (52)</td>
</tr>
<tr>
<td>Marital status (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>9 (60)</td>
<td>36 (60)</td>
</tr>
<tr>
<td>Married</td>
<td>6 (40)</td>
<td>24 (40)</td>
</tr>
<tr>
<td>Education level (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>3 (20)</td>
<td>19 (32)</td>
</tr>
<tr>
<td>Secondary</td>
<td>7 (47)</td>
<td>23 (38)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>5 (33)</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Family history of kidney disease (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>1(7)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Without</td>
<td>14 (93)</td>
<td>55 (92)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26 ± 6.6</td>
<td>25 ± 9.1</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>7 ± 2.1</td>
<td>8 ± 4.1</td>
</tr>
<tr>
<td>Body mass index (Kg/m²)</td>
<td>22.9 ± 3.3</td>
<td>22.4 ± 3.9</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>113.9 ± 22.3</td>
<td>119.4 ± 15.7</td>
</tr>
<tr>
<td>Diastolic</td>
<td>69 ± 15.7</td>
<td>77.12 ± 10.3</td>
</tr>
<tr>
<td>UACR (mg/g)</td>
<td>14.3 ± 6.5</td>
<td>44.9 ± 52.1</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.38 ± 0.84</td>
<td>4.47 ± 1.23</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>109.6 ± 30.8</td>
<td>120.2 ± 41.1</td>
</tr>
</tbody>
</table>

Data are percentage, mean ± SD, normal HDL-C (≥ 1.55 mmol/l), borderline low HDL-C, (1.05 –1.54 mmol/l) and low HDL-C, (≤ 1.04 mmol/l), p values were based on chi-squared test for proportions and one way Anova test for means. Statistical significance was denoted at p < 0.05[*]. Values in parenthesis are percentages.

### 4.3 eGFR levels in diabetic patients

The values of the estimated glomerular filtration rate are presented in Table 4.3. Patients with values greater than 90.1 ml/min/1.73 m² were classified as normal while those ≤ 90 ml/min/1.73 m² were in the abnormal region. Of the 89 patients, 69 (78%) had normal levels and 20 (22%) had abnormally reduced levels of eGFR.
In the normal eGFR group, which constituted 78% of the patients, the distribution was as follows; males 31 (45%) and females 38 (55%), married 26 (38%) and single 43 (62%). Classification of the subjects based on the highest level of education attained by individual participants showed that, at primary level there were 27.5%, secondary level at 45% and tertiary level at 27.5%. Subjects with family history of kidney disease were only 6%.

The abnormal eGFR group had 20 participants and their demographic distribution was as follows; males 30% and females 70%; married participants were 35% while 65% were single. Categorizations based on the level of education attained by the subjects were 30%, 45% and 25% for primary, secondary and tertiary levels of education, respectively.

Statistically, there was no association between the prevalence of abnormally reduced eGFR levels in the subjects with respect to their gender, marital status, education level attained and the history of kidney disease in their families ($p > 0.05$).

The mean age for the subjects in normal and abnormal eGFR groups was $25 \pm 8.2$ and $26 \pm 11.6$ years, respectively, while the mean diabetes duration of the patients in normal eGFR was $8 \pm 3.9$ years and abnormal eGFR was $10.6 \pm 7.2$ years. Although both the means of age and diabetes duration for the abnormal eGFR group were slightly higher
than for the normal eGFR group, the differences were not statistically significant ($p > 0.05$).

The two states of eGFR levels, were virtually identical with respect to BMI, (normal eGFR state, 22.4 ± 3.6 vs. abnormal eGFR state, 22.6 ± 3.8 kg/m$^2$; $p = 0.867$). On the other hand, although the systolic and diastolic blood pressure for the normal eGFR group was higher compared to that of the abnormal eGFR group, the differences were statistically insignificant ($p > 0.05$).

In both normal and abnormal states of eGFR, subjects did not significantly differ with respect to their biochemical variables, UACR, HDL-C and T. Cholesterol (UACR, $p = 0.089$; HDL-C, $p = 0.631$; Total cholesterol $p = 0.332$). It was noted that subjects with lower eGFR were generally older, had longer duration of diabetes, higher systolic blood pressure, total cholesterol, UACR and lower HDL-C compared to those with normal eGFR, but the differences were not statistically significant ($P > 0.05$).
### Table 4.3: Characterization of patients’ data within states of eGFR levels

<table>
<thead>
<tr>
<th></th>
<th>States of estimated glomerular filtration rate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal (low)</td>
<td></td>
</tr>
<tr>
<td>n = 89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (45)</td>
<td>6 (30)</td>
<td>0.432</td>
</tr>
<tr>
<td>Female</td>
<td>38 (55)</td>
<td>14 (70)</td>
<td></td>
</tr>
<tr>
<td>Marital status (%)</td>
<td></td>
<td></td>
<td>0.791</td>
</tr>
<tr>
<td>Single</td>
<td>43 (62)</td>
<td>13 (65)</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>26 (38)</td>
<td>7 (35)</td>
<td></td>
</tr>
<tr>
<td>Education level (%)</td>
<td></td>
<td></td>
<td>0.888</td>
</tr>
<tr>
<td>Primary</td>
<td>19 (27.5)</td>
<td>6 (30)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>31 (45)</td>
<td>9 (45)</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>19 (27.5)</td>
<td>5 (25)</td>
<td></td>
</tr>
<tr>
<td>Family history of kidney disease (%)</td>
<td></td>
<td></td>
<td>0.599</td>
</tr>
<tr>
<td>With</td>
<td>4 (6)</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>65 (94)</td>
<td>18 (90)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>25 ± 8.2</td>
<td>26 ± 11.6</td>
<td>0.581</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>8 ± 3.9</td>
<td>10.5 ± 7.2</td>
<td>0.053</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>22.4 ± 3.6</td>
<td>22.6 ± 3.8</td>
<td>0.867</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>119.9 ± 16.8</td>
<td>122.3 ± 30.2</td>
<td>0.661</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76.4 ± 10.5</td>
<td>72.4 ± 13.6</td>
<td>0.254</td>
</tr>
<tr>
<td>UACR (mg/g)</td>
<td>46.0 ± 59.6</td>
<td>76.8 ± 13.6</td>
<td>0.089</td>
</tr>
<tr>
<td>HDLC (mmol/l)</td>
<td>1.35 ± 0.35</td>
<td>1.31 ± 0.38</td>
<td>0.631</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.32 ± 1.13</td>
<td>4.62 ± 1.09</td>
<td>0.332</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>States of estimated glomerular filtration rate</th>
<th></th>
</tr>
</thead>
</table>
| Data are percentage, mean ± SD; normal eGFR (≥ 90.1 ml/min/1.73m²) and abnormal eGFR (≤ 90 ml/min/1.73m²); p values were based on chi-squared  test for proportions and t test for means. Statistical significance was denoted at p < 0.05[*]. Values in parenthesis are percentages.

### 4.4 Association of HDL-C and UACR levels

The distribution of the subjects in various states of urinary albumin excretion based on their HDL-C values is presented in Figure 4.1. Of the 49 participants with normoalbuminuria, 14 (29%) had normal HDL-C values, 33 (67%) had borderline low and 2 (4%) had low HDL-C values. The albuminuric group which comprised of 40 subjects’ had only one (2%) patient with normal HDL-C value, 27 (68%) had borderline...
low and 12 (30\%) had low HDL-C values. The mean HDL-C value for the subjects with normoalbuminuria was significantly higher than for the subjects with albuminuria (1.47 mmol/l vs. 1.19 mmol/l, \( p < 0.05 \)).

Continuous line ______ mean level of HDL-C for all the subjects in UACR states

Broken line …………… categorizations of the subjects based on their levels of HDL-C states

Figure 4.1 Distribution of HDL-C measurements in states of UACR levels
4.4.1 Correlation between HDL-C and UACR values

A bivariate Pearson correlation analysis between HDL-C and UACR values for all the participants is presented in Figure 4.2. The lowest value of HDL-C for the subjects was 0.45 mmol/l with a corresponding UACR level of 300mg/g. On the other hand, the highest value of HDL-C was 3.45 mmol/l with a corresponding UACR value of 10 mg/g. Therefore, there was a negative relationship between the HDL-C levels of the participants and their UACR values whereby, when the levels of the former declined, there was a complimentary increase in the levels of the latter. Statistically, there was a significant inverse correlation between HDL-C and UACR values of the participants ($r = -0.394$, $p < 0.05$).

![Figure 4.2: Correlation between HDL-C and UACR levels](image)
4.5 Association of HDL-C and eGFR levels

The distribution of the subjects in various states of eGFR based on their HDL-C values is presented in Figure 4.3. It is shown that of the 69 subjects with normal eGFR, 11 (16%) had normal HDL-C, while 49 (71%) and 9 (13%) had borderline low and low HDL-C values, respectively. On the other hand, of the 20 patients with abnormal eGFR, four (20%) had normal values of HDL-C, 11 (55%) were in the borderline low and 5 (25%) had low HDL-C values. Although the mean of HDL-C values for the subjects with normal eGFR was higher than those in abnormal eGFR state, 1.35 mmol/l vs 1.30 mmol/l, the difference was not statistically significant ($p > 0.05$).

Figure 4.3: Distribution of HDL-C measurements in eGFR states
4.5.1 Correlation between HDL-C and eGFR values

A bivariate Pearson correlation analysis between HDL-C and eGFR levels for all the participants is shown in Figure 4.4. The subject with the lowest value of HDL-C had 0.45 mmol/l with a corresponding eGFR value of 56.80 ml/min/1.73m$^2$, while one with the highest value of HDL-C had 3.45 mmol/l and a corresponding eGFR value of 99.0 ml/min/1.73m$^2$. A poor positive correlation between HDL-C and eGFR values of the subjects was found, whereby, when HDL-C levels decreased, there was a complimentary decrease of the eGFR levels. However, the correlation was not statistically significant ($r = 0.029, p > 0.05$).

![Figure 4.4 Correlation between HDL-C and eGFR levels](image-url)
CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATION

5.1 Discussion

This study sought to evaluate HDL-C as an alternative early predictor of diabetic nephropathy among the type 1 diabetes mellitus patients, by correlating with the analytes for kidney function used as predictors or markers of DN. This included UACR and eGFR levels. Also studied were the associations with the selected demographic confounders and risk factors for the development of DN.

Evaluation of the participants’ demographic and clinical characteristics with respect to UACR showed that subjects with normoalbuminuria and albuminuria did not significantly differ with respect to the education level attained by the subjects which implies that it is a weak confounder for DN. Schillinger et al. (2006) observed that literacy could mediate the effect of education on glyceamic control, since successful diabetes care involves interactive communication, participatory decision making and activation of patients with regard to goal setting. The present findings showed that the subjects had the requisite literacy standards to enable them cope with the demands of strict diabetes management plan. On the other hand, marital status of the subject emerged as an important confounding variable for DN, since a significant large number of the single subjects presented with albuminuria compared to the married ($P < 0.05$). This finding is in agreement with August et al. (2010), who recommended that marital status and gender differences should be considered in the delivery of healthcare services and
design of programs to help diabetic patients. The reduced incidences of albuminuria amongst the married subjects could be attributed to the social support received from their spouse with regard to management especially dietary and treatment adherence.

Evaluation of the subjects in normoalbuminuria and albuminuric states showed that there was no significant difference with respect to their family history of kidney disease, gender, age, BMI, total cholesterol and eGFR levels which suggests that they are low risk factors unlikely to be associated with diabetic nephropathy. These findings are consistent with those reported in past studies. Tabaei et al. (2001) and Afkhami-Ardekani et al. (2008) found no significant difference between normoalbuminuric and microalbuminuria subjects with respect to age, gender, BMI and family history of kidney disease. Also, Molitch et al. (2006), El-wakf et al. (2011) and Celepkolu et al. (2014) found no significant differences between the normoalbuminuria and albuminuria subjects on analysis of age, BMI, sex, total cholesterol and eGFR levels. But they contrast with those of Agarwal et al. (2011) and Gross et al. (2005), who observed that being male, having family history of kidney disease, aging and high BMI increases the risk of developing nephropathy. Variations between these studies could be attributed to differences of study populations with respect to age distribution, nutritional status, and proportions of subjects with history of kidney disease in their families, also at play could be differences in the methods used for the measurement of albuminuria and the type of urine specimen used.
Significant differences were found on analysis of duration of diabetes, systolic and diastolic blood pressure and HDL-C levels of the subjects between the two groups. This implied that they are high risk factors that may be associated with the development of diabetic nephropathy. The finding on duration of diabetes was in agreement with the observations of Varghese *et al.* (2001), Gupta *et al.* (2013) and Bamashmoos *et al.* (2013), who reported an increased frequency of microalbuminuria with the increase in the duration of diabetes. These observations lend credence to the fact that type 1 diabetic patients develops microalbuminuria five years from onset of diabetes and in ten years, progress to macroalbuminuria and eventually to ESRD (Ahmedani *et al.*, 2012).

There were significantly higher levels of both systolic and diastolic blood pressure in albuminuric subjects compared to the normoalbuminuric group, which was in accord with findings of Ahmedani *et al.* (2012). Agarwal *et al.* (2011) also reported increased incidence of DN in subject with high systemic blood pressure compared to the subjects with low systemic blood pressure. This observation supports the concept that glomerular hypertension is an important antecedent towards the initiation and progression of diabetic nephropathy and that raised albumin excretion and blood pressure are concomitant phenomena in type 1 diabetic patients progressing to albuminuria (Afkhami-Ardekani *et al.*, 2008).

The association of levels of UACR states and HDL-C showed that albuminuric subjects had significantly lower HDL-C levels than those with normoalbuminuria, a finding consistent with the reporting of Molitch *et al.* (2006) who showed that patients with
increased HDL-C levels were associated with lower likelihood of having albuminuria. This finding agrees with the fact that low HDL-C level represents an established risk factor for atherosclerosis which is a major cause of reduced kidney function due to renal artery stenosis (Wang et al., 2013). This finding therefore, enables us to achieve a more definitive association between the low levels of HDL-C in type 1 diabetic subjects with development of albuminuria, and as such, it can be a useful predictor for the likelihood of diabetic nephropathy onset in such patients.

Analysis of the participants’ characteristics at the three states of HDL-C levels showed that subjects did not differ with respect to gender, education level, family history of kidney disease, age, BMI, total cholesterol and eGFR level. However, significant differences were found with duration of diabetes, systolic and diastolic blood pressure and UACR levels \( (p < 0.05) \). Findings on gender are consistent with findings of Molitch et al. (2006) and Afkhami-Ardekani et al. (2008) who found no significant difference in distribution of gender with regard to various nephropathy states. However, Morton et al. (2012) reported that men were at higher risk of reduced HDL-C levels. Inter-study variations in findings could be due to differences of gender proportions among studies, the methods and the type of specimen used for determining HDL-C levels.

In the present study, no significant correlation was found between the marital status of the subjects and the reduced HDL-C levels \( (p = 0.393) \). August et al. (2010) observed
that within the social support network, the spouse is the most important source of support, especially on health related issues. However, lack of significant difference between categories of marital status with regard to levels of HDL-C in this study could be because the subjects with single marital status found social support from within the social network other than spouse.

Participants with family history of kidney had insignificant association with the prevalence of low HDL-C levels, which is in agreement with the findings reported by Ahmedani et al. (2005). However, Gross et al. (2005), in his review on diabetic nephropathy observed that many previous epidemiologic studies have shown that familial predisposition to kidney disease increases the risk of developing nephropathy. The explanation of the current findings can possibly be due to the small proportion of the subjects who had history of kidney disease in their families.

The subjects’ age within the three states of HDL-C showed no significant statistical difference. This is in agreement with the finding of Jia et al. (2014), although Morton et al. (2012) reported a significant association between reduced HDL-C levels and the age of the patients, where the elderly patients were more likely to have reduced levels of HDL-C. The inter-study variations are probably due to differences in age distribution among the study populations.
Subjects in the low HDL-C state had significantly higher duration of diabetes (12 ± 7.3 years) compared to the borderline low (8 ± 4.1) and normal HDL-C states (7 ± 2.1 years; p < 0.05) an observation that is in concordance with that of Morton et al. (2012). This finding is also in accordance with the previous evidence that diabetic dyslipidemia is associated with the time course of the diabetes among patients with type 1 diabetes. On the other hand, BMI of the subjects showed no significant difference with respect to the three HDL-C states under analysis, which is in agreement with the earlier finding by Ahmedani et al. (2005). In contrast, Morton et al. (2012) found a significant correlation between low HDL-C and high BMI. The inter-study variations may be due to differences in study design and population distribution.

It was observed that subjects with high blood pressure (sBP and dBP) were more likely to have low HDL-C values than normotensive. Morton et al. (2012) found a significant association between high systolic blood pressure and low HDL-C levels. Similarly, Agarwal et al. (2011) reported increased incidence of nephropathy with increase in blood pressure. This finding supports the concept that dyslipidemia accelerates progression of renal disease and contributes to atherogenic diathesis and the high risk of cardiovascular disease in diabetic patients.

A significant association was found between UACR and HDL-C levels, where the low state of HDL-C had higher values of UCAR compared to borderline and normal states (p < 0.05). This observation is in concordance with findings of Gupta et al. (2013) and
Molitch et al. (2014). However, no significant association was found between total cholesterol and eGFR with respect to the three states of HDL-C. The findings from the current analysis further lend credence to the fact that diabetic dyslipidemia is a predictor of rapid progression of microalbuminuria.

There was no significant association between gender and the prevalence of reduced eGFR ($p = 0.432$) which is in keeping with findings of Kong et al. (2006) and Wang et al. (2013). This result implies that individuals with type 1 diabetes may not be predisposed to decline of eGFR levels on the basis of their gender. There was also no significant association between family history of kidney diseases and eGFR levels which was in accord with the observation of Shen et al. (2009). However, Mattix et al. (2002) observed a significant difference between non-Hispanic whites and non-Hispanic blacks with respect to the creatinine levels. Furthermore, Gross et al. (2005) also noted that many previous epidemiologic studies have shown that familial predisposition to kidney disease increases the risk of developing nephropathy. Lack of difference between subjects in the normal and abnormal eGFR states with respect to family history of kidney disease was probably due to the small proportion of subjects who had history of kidney disease in their families.

The subjects’ age was also found not to be a risk factor for the decline of eGFR levels which is discordant with the findings reported by Wang et al. (2013) and Agarwal et al.
(2011) who found that diabetic patients in low eGFR quartiles had significantly higher age than those in normal quartile \( (p < 0.05) \). The variation between the current study and the others could probably be due to the differences in the distribution of age of subjects’ in respective studies.

In the current analysis, although the subjects with low eGFR had longer duration of diabetes than the normal eGFR group, the difference was not statistically significant an observation in concordance with findings by Boer et al. (2014). However, Kong et al. (2006) reported a significant difference in duration of diabetes with respect to eGFR levels, whereby subjects’ with longer duration of diabetes had reduced levels of eGFR. The inter study variations are probably due to difference in the distribution of duration of diabetes of the study population but more importantly.

The BMI of the subjects with normal eGFR \( (22.4 \pm 3.6 \text{ kg/m}^2) \) was identical to the subjects with abnormal low eGFR \( (22.6 \pm 3.8 \text{ kg/m}^2) \) which is in keeping with findings of past studies (Jia et al., 2009; Wang et al., 2013; Boer et al., 2014). However, Kong et al. (2006) reported association between reduced eGFR with increased BMI. Variations in findings between studies may be attributed to the nutritional status of the subjects. Poor nutrition leads to weight loss exposing such patients with low BMI to the risk of progression to low eGFR and nephropathy.
This study found no significant association between blood pressure and eGFR levels \((p > 0.05)\), which contrasted findings from past studies. Wang et al. (2006) and Kong et al. (2006) found that only systolic but not diastolic was significantly associated with reduced eGFR. However, Boer et al. (2014) found that both systolic and diastolic blood pressure were significantly associated with reduced eGFR levels. These contrasting results could in part be due to the differences in study design, methods of blood pressure determination and interpretation and other potential biases.

The findings of the differences between eGFR states with respect to the levels of UACR, HDL-C, and total cholesterol were not statistically significant, \((p > 0.05)\), which is concordant with findings by Jia et al. (2009) who reported no significant association between HDL-C \((P = 0.180)\) and total cholesterol \((p = 0.113)\) with reduced eGFR. However, many studies have made discordant observations with Wang et al. (2013) and Kong et al. (2006) reporting significant association between UACR, HDL-C and total cholesterol with reduced eGFR levels. The findings in the present study indicate that UACR, HDL-C and total cholesterol levels are not independently associated with eGFR. Furthermore, the inter study variations in the association between the biochemical parameters and the states of eGFR can be attributed to several factors such as differences in populations, types of specimens and collection method used, methods of measurement used to determine levels of UACR, HDL-C and total cholesterol.
A bivariate analysis between UACR and HDL-C values for all the participants yielded a significant inverse correlation \((r = -0.394; p < 0.05)\) which is in concordance with the findings of past studies (Aziz et al., 2011; Gupta et al., 2013; Zhang-suo and Dong-wei, 2013). The inverse relationship between UACR and HDL-C can be attributed to a wide range of glomerular lesions which develops among patients with longstanding type 1 diabetes mellitus leading to persistent microalbuminuria even if treatment is administered. At the same time, the glomerular lesions reduce the kidney function thereby affecting the regulation of HDL-C metabolism leading to reduced levels of HDL-C. Thus in such patients, microalbuminuria may be a marker rather than a predictor of renal structural changes. Measurement of HDL-C levels is not affected by lesions in the glomerular and as such, the significance of testing HDL-C levels in predicting diabetic nephropathy cannot be gainsaid. The findings of the present study are pertinent as it enhances our knowledge and understanding of HDL-C as potential predictor and biomarker for the development of diabetic nephropathy in patients with long standing type 1 diabetes. To affirm this view, Morton et al. (2012), in his large study, perhaps provided the strongest evidence that HDL-C level is an independent risk factor for the development of diabetic nephropathy in diabetic patients.

The correlation between HDL-C and eGFR values was not statistically significant \((r = 0.029; p > 0.05)\). Wang et al. (2013) reported that HDL-C and eGFR levels were not independently associated, but a significant correlation between these variables could only be achieved on the influence of confounders such as age, blood pressure and lipid
parameters. In general, for a population with roughly normal kidney function, the cause of correlation between HDL-C and eGFR may be the reduced renal protective effects of lower HDL-C levels as opposed to reduced kidney function. However, this kind of correlation differs from that between eGFR and HDL-C in patients with moderate to severe kidney dysfunction (Wang et al., 2013). The present findings may be explained in part due to the fact that for microalbuminuric patients, glomerular filtration rate (GFR) may remain stable due to the hepatoprotective effects of HDL-C. According to Levey et al. (1999), only a subset of microalbuminuric patients has shown a rapid decline in GFR whether measured or estimated. Since majority of the study population were either normoalbuminuric or had microalbuminuria, they were therefore largely in the region of stable eGFR.

5.2 Conclusions

The hypothesis evaluated by this study, that there is no correlation between HDL-C levels and markers of kidney functions in predicting development of diabetic nephropathy amongst type 1 diabetic patients was rejected. Subsequently, from this study, it can be concluded that;

i. The values of UACR, HDL-C and eGFR levels in the study population ranged from 3–300 mg albumin/g creatinine, 0.45–3.45 mmol/l and 29.1–240.5 ml/min/1.73m^2, respectively. The number of participants with abnormal levels of UACR, HDL-C and eGFR were 45%, 14%, and 22%, respectively. The UACR and HDL-C values were significantly associated with the risk factors of: duration
of diabetes, systolic and diastolic blood pressure. Besides, single marital status was also found to be a significant confounder of nephropathy. While eGFR levels associated with neither of the patients’ variables studied.

ii. The HDL-C values were significantly lower in the subjects with albuminuria compared to normoalbuminuric group \((p = 0.001)\). Similarly, there was a significant inverse correlation between HDL-C and UACR level. Therefore, HDL-C has the potential to alternately predict the development of nephropathy among type 1 diabetes mellitus patients; hence it may be used to compensate for the specificity issues inherent in UACR measurements.

iii. There was no significant association or correlation between HDL-C and eGFR values. Therefore, eGFR is a poor predictor of diabetic nephropathy in type 1 diabetes mellitus patients.

### 5.3 Recommendation

Determination of HDL-C levels accords significant predictive value to the onset of diabetic nephropathy among type 1 diabetic mellitus patients of four years into diagnosis hence it can be used in place of UACR levels for the purpose of predicting DN. However, for optimal management of type 1 diabetes mellitus with regard to DN onset, both HDL-C & UACR levels should be determined concurrently, to improve on predictive precision and efficiency.
5.3.1 Future research

1. This study does not show the renal protective effects of the desirable levels of HDL-C, hence the need for a deliberate study to evaluate the mechanisms through which HDL-C impacts on the glomerulus.

2. To study the causal relationships between the associations of HDL-C and the various risk factors and confounding variables of diabetic nephropathy.

3. Need for large long-term observational studies which will account for all the risk factors in helping to derive the predictive index for diabetic nephropathy among the diabetics.
REFERENCES


Renal association, 2013. [www.renal.org/information/the.uk.eckd-guide/normal-gfr](http://www.renal.org/information/the.uk.eckd-guide/normal-gfr)


Appendix I: Ethical approval letter

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19574 Code 00202
Telegrams: varsity
(254-020) 2753600 Ext 44555

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

Ref: KNH-ERC/A/304
Link: www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 725308-9
Fax: 725727
Telegrams: MADSUP, Nairobi

Wanyama Francis Mugeni
156/CTY/PT/23313/2012
School of Pure and Applied Sciences
Kenya University

10th September 2014

Dear Francis

RESEARCH PROPOSAL: EVALUATION OF HIGH DENSITY LIPOPROTEIN CHOLESTEROL LEVELS AS A PREDICTOR OF DIABETIC NEPHROPATHY IN TYPE 1 DIABETES (P346/06/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above proposal. The approval periods are 10th September 2014 to 9th September 2015.

This approval is subject to compliance with the following requirements:

- a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
- b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
- c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
- d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
- e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (Attach a comprehensive progress report to support the renewal).
- f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
- g) Submission of an executive summary report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website www.uonbi.ac.ke/activities/KNHUoN.
Yours sincerely

PROF. M.L. CHINDIA
SECRETARY, KNH/UoN-ERC

c.c. The Principal, College of Health Sciences, UoN
     The Deputy Director CS, KNH
     The Chair, KNH/UoN-ERC
     The Assistant Director, Health Information, KNH
     Supervisors: Dr. David Mburu, Prof. Christine Kagwenda, Dr. Nancy Ngugi
Appendix II: Consent form

CONSENT INFORMATION FOR THE ADULT PATIENTS (> 18 YRS OF AGE)

Title: Evaluation of high density lipoprotein cholesterol levels as a predictor of diabetic nephropathy in type 1 diabetes

Introduction

I am Mr. Francis M. Wanyama a student of Kenyatta University, undertaking MSc. in Medical Biochemistry.

The purpose of this form is to provide you with information about the study I am carrying out in type 1 diabetic patients. The information will help you make an informed decision whether to take part in the study or not. Your participation in this study will be voluntary and you may withdraw from the study at any point without it affecting the treatment being given to you in any way.

Purpose of the study

This study is about evaluating HDL-cholesterol levels (the good fat that mops up the bad fat from the body and carries it to the liver to be eliminated) in type 1 diabetes as a predictor of diabetic nephropathy (diabetic kidney disease). The ‘good fat’ is protective of the kidney when in right quantities, but have tendency to decline from their normal levels in type 1 diabetes setting the stage for reduced working capacity of the kidney, the organ tasked with removal of the harmful waste from the body. If not detected earlier and the proper intervention measures instituted, the kidney function rapidly deteriorates within a span of few months or years necessitating dialysis or kidney transplant (replacement).

The goal of this study is to help advance our knowledge on how we can best manage and monitor type 1 diabetes in our clinics with regard to prevention or delay onset of renal disease through improved early and sensitive laboratory detection mechanisms.

Procedure (what will be done in the study)

If you agree to participate, you will be asked to answer a questionnaire that will take about 15 minutes. There will be a series of questions that I will ask you in confidence and all your responses will be noted down. Most questions have a ‘No or Yes’ for an answer and some questions will require you to remember some things in the past. Some relevant information to the study will also be retrieved from your medical file.

The technologist or I (study nurse), will collect from you a blood sample of about 2mls (2 spoonfuls) that will be taken to the laboratory for measurement of creatinine (a marker...
renal damage) levels and body fats (total cholesterol & HDL-C). You will also be asked to provide a sample of urine measuring about 3mls (3 spoonfuls) which will be used to determine protein excretion levels in your urine.

Your samples will be stored for one week to allow repeat tests if need be, after which it will be disposed as per the practice recommendations.

Confidentiality and data safety

You will be notified by a phone call of the availability of your test results by the investigator within one week and the test results will be revealed to you by your doctor at the clinic on subsequent visit and used for your continued care. Test results shall remain confidential. The questionnaire will not have your name and the data will be stored in a safe place where other people will not access it.

**Risks to you as a patient**

There will be some discomfort from the needle prick at the site of blood sample removal (usually from the cubital area of the arm or any other appropriate site).

Rarely swelling or bleeding may occur from the puncture site but I will make sure bleeding has stopped before I leave.

**Benefits**

You will not be charged for any of the lab tests.

The findings of the laboratory tests will form part of your usual care. Copies of the test results shall be available to you and your doctor.

This study will provide an opportunity to establish early markers of kidney damage in type 1 diabetic patients attending our clinics at Kenyatta National Hospital. This will inform specific operations in our clinics in terms of proper management and monitoring the type 1 diabetes with an aim to prevent or delay onset of kidney disease in diabetics and also improve outcomes for those already with kidney disease.

**Will there be any compensation?**

You will not receive any type of payment for participating in this study.

**Right to refuse**

Your participation in this research is voluntary. You may refuse to participate or withdraw from participation at any time. Withdrawal or refusing to participate will not affect your relationship with the clinic in anyway. You are free to ask any questions and have a right to satisfactory answers before you sign the consent form.
Duration of study

This study will take duration of nine months to conclude but I shall contact you only once.

Number of participant in study

A total of 147 participants will be enrolled in the study. If you agree to participate in this study you may kindly sign on the consent form. Thank you.

Consent form

I ……………………………………………………… consent to participate in the study on assessment of correlation between HDL cholesterol, microalbumin and serum creatinine in type 1 diabetes patients as predictors of diabetic nephropathy at Kenyatta National Hospital diabetes outpatient clinic. I do this with the knowledge of the purposes of the study and the procedures thereof. The purposes of the study and procedures have been explained to me clearly by Mr. Francis Mugeni Wanyama or his assistant. I am also aware that I can withdraw from this study without losing any benefits and quality of care of my medical condition.

_________________________________  __________________
Signature or thumb print of patient    Date

_________________________________  ________________
Signature of study nurse (witness)    Date

If you have any questions during the course of the study, you may contact the following.

Mr. Francis Mugeni Wanyama
Mobile number: 0722975146

Dr. Nancy Ngugi
Mobile number: 0722788533,

The chairman of the Ethical and Review committee,

Kenyatta national hospital

P.O.BOX 20723-00202
CONSENT FORM FOR CHILDREN UNDER 18 YRS OF AGE

Parental Permission for Children Participation in Research

Title: Evaluation of high density lipoprotein cholesterol levels as a predictor of diabetic nephropathy in type 1 diabetes.

Introduction

I am Mr. Francis M. Wanyama a student of Kenyatta University, undertaking a Masters degree in Medical Biochemistry.

The purpose of this form is to provide you (as the parent/guardian of a prospective research study participant) information about the study i am carrying out in diabetic patients. The information will help you make an informed decision whether or not to let your child participate in this study. Your child’s participation in this study will be voluntary and you may withdraw him/her from the study at any point without it affecting the treatment being given to him/her in any way.

Purpose of the study

This study is about evaluating HDL-cholesterol levels (the good fat that mops up the bad fat from the body and carries it to the liver to be eliminated) in type 1 diabetes as a predictor of diabetic nephropathy (diabetic kidney disease). The ‘good fat’ is protective of the kidney when in right quantities, but have tendency to decline from their normal levels in type 1 diabetes setting the stage for decline in the working capacity of the kidney, the organ tasked with removal of the harmful waste from the body, which if not detected earlier and the proper intervention measures instituted, the kidney function rapidly deteriorates within a span of few months or years necessitating dialysis or kidney transplant (replacement).

The goal of this study is to help advance our knowledge on how we can best manage and monitor type 1 diabetes in our clinics with regard to prevention or delay onset of renal disease through improved early and sensitive laboratory detection mechanisms.

Procedure (what will be done in the study)

If you allow your child to participate in this study, you will be asked to answer a questionnaire on his/her behalf that will take about 30 minutes. There will be a series of questions that I will ask you in confidence and all your responses will be noted down. Most questions have a ‘No or Yes’ for an answer and some questions will require you to remember some things in the past. Some relevant information to the study will also be retrieved from your child’s medical file.
The technologist or I will collect from your child a blood sample of about 2mls (2 spoonfuls) that will be taken to the laboratory for measurement of creatinine (a marker or renal damage) levels and body fats (total cholesterol and HDL-C). Your child will also be asked to provide a sample of urine measuring about 3mls (3 spoonfuls) which will be used to determine protein excretion levels in his/her urine.

Your child’s samples will be stored for one week to allow repeat tests if need be, after which they will be disposed as per the practice recommendations.

Confidentiality and data safety

You will be notified by a phone call of the availability of your child’s test results by the investigator within one week and the test results will be revealed to you through your child’s doctor at the clinic on subsequent visit then used for the child’s continued care. Test results shall remain confidential. The questionnaire will not have your child’s name and the data will be stored in a safe place where other people will not access it.

Risks to your child as a patient

There will be some discomfort from the needle prick at the site of blood sample removal (usually from the cubital area of the arm or any other appropriate site)

Rarely swelling or bleeding may occur from the puncture site but I will make sure bleeding has stopped before I leave.

Benefits

You will not be charged for any of the lab tests.

The findings of the laboratory tests will form part of your child’s usual care. Copies of the test results shall be available to you and your child’s doctor.

This study will provide an opportunity to establish early markers of damage to the kidney in type 1 diabetic patients attending our clinics at Kenyatta National Hospital. This will inform specific operations in our clinics in terms of proper management and monitoring the type 1 diabetes with an aim to prevent or delay onset of kidney disease in diabetics and also improve outcomes for those already with kidney disease.

Will there be any compensation?

You will not receive any type of payment for your child’s participation in this study.

Right to refuse

Your child’s participation in this study is voluntary. You may refuse your child to participate or withdraw from participation at any time. Withdrawal or refusing to participate will not affect your child’s relationship with the clinic in anyway. You are free
to ask any questions and have a right to satisfactory answers before you sign the consent form.

**Duration of study**

This study will take duration of nine months to conclude but I shall contact your child only once.

**Number of participant in study**

A total of 147 participants will be enrolled in the study. If you agree for your child to participate in this study you may kindly sign on the consent form.

Thank you.

**Consent form**

I ………………………………………..……………………………… parent/guardian of ……………………………………………………….. consent for my child to participate in the study on assessment of correlation between HDL cholesterol, microalbumin and estimated glomerular filtration rate in type 1 diabetes patients as predictors of diabetic nephropathy at Kenyatta National Hospital diabetes outpatient clinic. I do this with the knowledge of the purposes of the study and the procedures thereof. The purposes of the study and procedures have been explained to me clearly by the investigator Mr. Francis Mugeni Wanyama or his assistant. I am also aware that my child can withdraw from this study without losing any benefits and quality of care of his/her medical condition.

_________________________________  __________________
Signature or thumb of Parent(s) or Legal Guardian  Date

_________________________________  __________________
Signature of study nurse (witness)  Date

If you have any questions during the course of the study, you may contact the following.

Mr. Francis Mugeni Wanyama

Mobile number: 0722975146

Dr. Nancy Ngugi

Mobile number: 0722788533,

The chairman of the Ethical and Review committee,
Title: Evaluation of high density lipoprotein cholesterol levels as a predictor of diabetic nephropathy in type 1 diabetes.

Introduction

I am Mr. Francis M. Wanyama a student of Kenyatta University, undertaking a Masters degree in Medical Biochemistry.

The purpose of this form is to provide you with information about the study I am carrying out in diabetic patients. The information will help you make an informed decision whether to take part in the study or not. Your participation in this study will be voluntary and you may withdraw from the study at any point without it affecting the treatment being given to you in any way.

Purpose of the study

This study is about evaluating HDL-cholesterol levels (the good fat that mops up the bad fat from the body and carries it to the liver to be eliminated) in type 1 diabetes as a predictor of diabetic nephropathy (diabetic kidney disease). The ‘good fat’ is protective of the kidney when in right quantities, but have tendency to decline from their normal levels in type 1 diabetes setting the stage for decline in the working capacity of the kidney, the organ tasked with removal of the harmful waste from the body, which if not detected earlier and the proper intervention measures instituted, the kidney function rapidly deteriorates within a span of few months or years necessitating dialysis or kidney transplant (replacement).

The goal of this study is to help advance our knowledge on how we can best manage and monitor type 1 diabetes in our clinics with regard to prevention or delay onset of renal disease through improved early and sensitive laboratory detection mechanisms.

Procedure (what will be done in the study)

I am talking to you so that you know about the study but for you to participate, your parent/guardian has to give permission by signing a consent form on your behalf. Your parent or guardian will be asked to answer a questionnaire on your behalf which will take about 30 minutes. There will be a series of questions that I will ask your parent/guardian in confidence and all the responses will be noted down. Most questions have a ‘No or
Yes’ for an answer and some questions will require him/her to remember some things in the past. Some relevant information to the study will also be retrieved from your medical file.

The technologist or I will collect from you a blood sample of about 2mls (spoonfuls) that will be taken to the laboratory for measurement of creatinine (a marker or renal damage) levels and body fats (total cholesterol & HDL-C).

You will also be asked to provide a sample of urine measuring about 3mls (3 spoonfuls) which will be used to determine protein excretion levels in your urine. Your samples will be stored for one week to allow repeat tests if need be, after which they will be disposed as per practice recommendations.

Confidentiality and data safety

Your parent/guardian will be notified by a phone call of the availability of your test results by the investigator within one week and the test results will be revealed to you parent/guardian through your doctor at the clinic on subsequent visit then used for your continued care. Test results shall remain confidential. The questionnaire will not have your name and the data will be stored in a safely where other people will not access it.

Risks to you as a patient

There will be some discomfort from the needle prick at the site of blood sample removal (usually from the cubital area or any other appropriate site)

Rarely swelling or bleeding may occur from the puncture site but I will make sure bleeding has stopped before I leave.

Benefits

Your parent or guardian will not be charged for any of the lab tests.

The findings of the laboratory tests will form part of your usual care. Copies of the test results shall be available to your parent/guardian and your doctor.

This study will provide an opportunity to establish early markers of damage to the kidney in type 1diabetic patients attending our clinics at Kenyatta National Hospital. This will inform specific operations in our clinics in terms of proper management and monitoring the type 1 diabetes with an aim to prevent or delay onset of kidney disease in diabetics and also improve outcomes for those already with kidney disease.

Will there be any compensation?

Neither you nor your parent/guardian will receive any type of payment for participating in this study.
Right to refuse

Your participation in this research is voluntary. You may refuse to participate or withdraw from participation at any time. Withdrawal or refusing to participate will not affect your relationship with the clinic in anyway. You are free to ask any questions and have a right to satisfactory answers.

Duration of study

This study will take duration of nine months to conclude but I shall contact you only once.

Number of participant in study

A total of 147 participants will be enrolled in the study. If you agree to participate in this study your parent/guardian will sign on the consent form on your behalf.

Thank you.

Child’s assent form

I ……………………………………………………………………… agree to participate in the study on assessment of correlation between HDL cholesterol, microalbumin and estimated glomerular filtration rate in type 1 diabetes patients as predictors of diabetic nephropathy at Kenyatta National Hospital diabetes outpatient clinic. I do this with the knowledge of the purposes of the study and the procedures thereof. The purposes of the study and procedures have been explained to me clearly by the investigator Mr. Francis Mugeni Wanyama or his assistant. I am also aware that i can withdraw from this study without losing any benefits and quality of care of my medical condition.

________________________________________________________________________

Signature or thumb of Parent(s) or Legal Guardian Date

________________________________________________________________________

Signature of study nurse (witness) Date

If you have any questions during the course of the study, you may contact the following.

Mr. Francis Mugeni Wanyama
Mobile number: 0722975146

Dr. Nancy Ngugi
Mobile number: 0722788533,

The chairman of the Ethical and Review committee,

Kenyatta national hospital

P.O.BOX 20723-00202

Tel 020-2726300 Ext, 44102
Appendix III: Questionnaire

STUDY PROFORMA

Study ID number……………………

Clinic………………………………

Age ………………… years

Date/year of diagnosis of diabetes ……………………………..

Tick as appropriate.

SOCIO-DEMOGRAPHICS

1. Gender                  a) Male                       b) Female
2. Race                     a) African                    b) Caucasian         c) Asian
3. Where do you usually reside?  ………………………….
4. What is your occupation?  a) Professional        b) skilled laborer
                                 c) Unskilled laborer     d) farmer       e) others (specify)
5. Level of formal education
                                 a) None        b) primary       c) secondary     d) Tertiary      e) Adult education
6. Marital status
                                 a) Single       b) Married       c) Divorced     d) Widowed      e) separated

PAST MEDICAL HISTORY

7. Have you ever been told by a health worker that you have hypertension?
                                 a) Yes               b) No

If yes, in which year? ……………………….

8. Have you suffered from any stroke (weakness of one side of the body), myocardial
   infarction (heart attack) or amputation due to non-traumatic reasons? (This will also be
   checked from patient’s files)
                                 a) Stroke             b) Myocardial infarction   c) Amputation        d) None
9. Have you had any dialysis session?
   a) Yes                  b) No

**FAMILY HISTORY**
10. Did any of your relatives suffer from kidney disease?
    a) Yes             b) No
    if yes:
        1=1 parent     2= both parents     3= sibling     4= others (specify)

**SMOKING HABITS**
11. What is your current smoking status? (tick one)
    a) Never been a smoker
    b) Former smoker
    c) Current smoker
12. If the patient has a history of smoking?
    a) When did you start smoking? …………………
    b) When did you stop smoking? …………………
    c) Approximately how many cigarettes did or do you smoke per day? ……

**CURRENT MEDICATIONS**
13. Oral hypoglycaemic agents?
    a) Sulphonyl urea     b) Metformin     c) NSAIDs     d) Insulin     e) Paracetamol
    f) Alternative medicine e.g herbal medicine     g) Others (specify)
16. Lipid lowering agent (drug and dose information from the file)
    a) Statin,     b) nicotinic acid     c) fibrac acid

**LOWER URINARY TRACT SYMPTOMS (LUTS)**
17. Do you suffer from any of the following symptoms?
    A) Frequency [yes] [No],     b) Nocturia [yes] [No],
c) Urgency [yes] [No],  
d) Feeling of incomplete emptying [yes] [No],  
e) Intermittency [yes] [No],  
f) Straining [yes] [No],  
g) Weak stream [yes] [No],

**PHYSICAL EXAMINATION**

Height (m) 

Weight (m) 

BMI (kg/m²) 

<table>
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<tr>
<th>Supine blood pressure (mmHg)</th>
<th>Systolic</th>
<th>Diastolic</th>
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<td>1&lt;sup&gt;st&lt;/sup&gt; reading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; reading</td>
<td></td>
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<tr>
<td>Average BP (mmHg)</td>
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**LAB DATA**

<table>
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<tr>
<th>ANALYTES</th>
<th>RESULTS</th>
<th>COMMENT (L, N, H)</th>
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<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (mls/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UACR (mg/g)</td>
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<td></td>
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<tr>
<td>Nephropathy classification</td>
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</table>
Appendix IV: Test protocol for total cholesterol

**CHOLESTEROL liquicolor**

**CHO-D-PAP-Method**

Enzymatic Colorimetric Test for Cholesterol with Lipid Clearing Factor (LCF)

**Package Sizes**

<table>
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<th>10017</th>
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<td>Complete test kit</td>
</tr>
<tr>
<td></td>
<td>10015</td>
<td>9 x 3 ml</td>
<td>Standard</td>
</tr>
</tbody>
</table>

**Method**

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-amino-phenazone in the presence of phenol and peroxidase.

**Reaction Principle**

\[
\text{Cholesterol} + \text{H}_2\text{O}_2 \rightarrow \text{cholesterol + fatty acid} + \text{H}_2\text{O} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-amino-phenazone} + \text{phenol}
\]

**Contents**

<table>
<thead>
<tr>
<th>RGT</th>
<th>4 x 30 ml, 3 x 250 ml or 4 x 100 ml Enzyme reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate buffer (pH 6.5) 100 mmol/l</td>
</tr>
<tr>
<td></td>
<td>4-Aminophenazone 0.3 mmol/l</td>
</tr>
<tr>
<td></td>
<td>Phenol 5 mmol/l</td>
</tr>
<tr>
<td></td>
<td>Peroxidase &gt; 0.1 U/l</td>
</tr>
<tr>
<td></td>
<td>Cholesterol esterase &gt; 150 U/l</td>
</tr>
<tr>
<td></td>
<td>Cholesterol oxidase &gt; 100 U/l</td>
</tr>
<tr>
<td></td>
<td>Sodium azide 0.05 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STD</th>
<th>3 ml Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol 200 mg/dl or 5.17 mmol/l</td>
</tr>
</tbody>
</table>

**Reagent Preparation**

The (RGT) and the (STD) are ready for use.

**Reagent Stability**

The reagents are stable up to the given expiry date, even after opening, when stored at 2...8°C. The opened reagent is stable for 2 weeks at 15...25°C. Contamination must be avoided.

**Specimen**

Serum, heparinised or EDTA-plasma.

**Note:** Lipemic specimens usually generate turbidity of the sample/reagent mixture which leads to falsely elevated results. The **CHOLESTEROL liquicolor** test avoids these falsely elevated results through its built-in Lipid Clearing Factor (LCF). The LCF cleaves up totally a turbidity caused by lipemic specimens.

**Assay**

Wavelength: 500 nm, Hg 546 nm

Optical path: 1 cm

Temperature: 20...25°C or 37°C

Measurement: Against reagent blank. Only one reagent blank per series is required.

**Pipetting Scheme**

<table>
<thead>
<tr>
<th>Pipette into cuvettes</th>
<th>Reagent blank</th>
<th>Sample or [STD]</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGT</td>
<td>10 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>STD</td>
<td>10 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mix, incubate 10 min. at 20...25°C or 5 min. at 37°C. Measure the absorbance of the sample against the reagent blank (\(\Delta A\)) within 60 min.

**Calculation of the Cholesterol Concentration**

1. **With Factor**

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>C (mg/dl)</th>
<th>C (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>546 nm</td>
<td>840 x (\Delta A)</td>
<td>21.7 x (\Delta A)</td>
</tr>
<tr>
<td>500 nm</td>
<td>553 x (\Delta A)</td>
<td>14.3 x (\Delta A)</td>
</tr>
</tbody>
</table>

\[
C = \frac{\Delta A_{\text{sample}} - \Delta A_{\text{ST}}}{\Delta A_{\text{ST}}} \times C_{\text{ST}}
\]

2. **With Standard**

Only the standard recommended by HUMAN (enclosed in kit or separately available, REF 10015) should be used.

\[
C = \frac{\Delta A_{\text{sample}} - \Delta A_{\text{ST}}}{\Delta A_{\text{ST}}} \times C_{\text{ST}}
\]

**Performance Characteristics**

Linearity:

The test is linear up to a cholesterol concentration of 750 mg/dl (19.3 mmol/l). Dilute samples with a higher cholesterol concentration 1:2 with physiological saline (0.9%) and repeat the determination. Multiply the result by 3.

Typical performance data can be found in the Verification Report, accessible via:

- www.human.de/data/gbvr/su-chol.pdf

**Clinical Interpretation**

Suspect over 200 mg/dl or 5.7 mmol/l

Elevated over 260 mg/dl or 6.7 mmol/l

The European Atherosclerosis Society recommends to decrease the cholesterol level to approximately 180 mg/dl for adults up to 30 years and to approximately 200 mg/dl for adults over 30 years.

**Quality Control**

All control sera with values determined by this method may be supplied. We recommend to use our own serum based HUMATROL or our human serum based SERODOS quality control sera.

**Automation**

Proposals to apply the reagents on analysers are available on request. Each laboratory has to validate the application in its own responsibility.

**Notes**

1. The test is not influenced by hemoglobin values up to 200 mg/dl or by bilirubin values up to 5 mg/dl.
2. The reagents contain sodium azide as preservative (0.05%). Do not swallow. Avoid contact with skin and mucous membranes.

**References**

2. Richmond, W., Clin. Chem. 19, 1350 (1973)
Appendix V: Test protocol for HDL Cholesterol

**Appendix:**

**HDL CHOLESTEROL**

Precipitant and Standard, for Use with CHOLESTEROL liqulicor Test Kit

**Package Size**
- 10018
- 4 x 80 ml Precipitant
- 3 x 3 ml Standard

**Method**
The chylokinic, VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated by addition of phosphotungstic acid and magnesium chloride. After centrifugation the supernatant fluid contains the HDL (high density lipoprotein) fraction, which is assayed for HDL Cholesterol with the CHOLESTEROL liqulicor test kit.

**Contents, Reagent Composition**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphotungstic acid</td>
<td>0.55 mmol/l</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>25.60 mmol/l</td>
</tr>
</tbody>
</table>

**HDL**

1 x 3 ml Standard

**Cholesterol**

50 mg/dl or 1.29 mmol/l

**Reagent Stability**

**Stable** is ready for use and can directly be employed in the test. No precipitation is required! The factor in the calculation formula comprises the dilution ratio.

**Specimen**

Serum, heparinized or EDTA-plasma.

**Assay**

See CHOLESTEROL Liquicolor.

**Precipitation**

Pipette into centrifuge tubes:

<table>
<thead>
<tr>
<th>Macro</th>
<th>Semi-micro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>500 µl</td>
</tr>
<tr>
<td><strong>neutral</strong></td>
<td>1000 µl</td>
</tr>
<tr>
<td></td>
<td>200 µl</td>
</tr>
<tr>
<td></td>
<td>500 µl</td>
</tr>
</tbody>
</table>

(Repeat 4 parts of the bottle with 1 part distilled water (x=1))

We will incubate for 10 minutes at room temperature. Centrifuge for 30 minutes at 10000 g, alternatively for 10 minutes at 4000 g.

After centrifugation separate the clear supernatant from the precipitate within 1 hour and determine the cholesterol concentration using CHOLESTEROL liqulicor reagent.

**Cholesterol Determination**

Pipette into cuvettes:

<table>
<thead>
<tr>
<th>Dist Water</th>
<th>HDL supernatant</th>
<th>Reagent</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>1000 µl</td>
<td>3000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Incubate for 5 minutes at 37°C or 10 minutes at 22.8°C. Measure the absorbance of the sample and the **STD** respectively, against the reagent blank within 60 minutes (AA).

**Calculation of the HDL Cholesterol Concentration with [EIT]**

1. **Macro Method**

\[
C = 150 \times \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{std}}}
\]

2. **Semi-micro Method**

\[
C = 175 \times \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{std}}}
\]

\[
C = \frac{A_{\text{sample}}}{A_{\text{std}}}
\]

**Calculation of the LDL Cholesterol Concentration**

The LDL Cholesterol concentration (LDL-C) is calculated from the total Cholesterol concentration (TC), the HDL Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald et al.

\[
LDL-C = TC - HDL-C - \frac{TG}{5}
\]

or

\[
LDL-C = TC - HDL-C - \frac{TG}{2.2}
\]

**Clinical Interpretation**

<table>
<thead>
<tr>
<th>HDL Cholesterol</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>[mg/dl]</td>
<td>[mmol/l]</td>
<td>[mg/dl]</td>
</tr>
<tr>
<td>Prognostically favorable</td>
<td>&gt; 55</td>
<td>&gt; 1.42</td>
</tr>
<tr>
<td>Standard risk level</td>
<td>35 - 55</td>
<td>0.9 - 1.42</td>
</tr>
<tr>
<td>Risk indicator</td>
<td>&lt; 35</td>
<td>&lt; 0.9</td>
</tr>
</tbody>
</table>

**Performance Characteristics**

Typical performance data can be found in the Verification Report, accessible via:


**Quality Control**

All blank sera with values for HDL-Cholesterol determined by this method can be employed.

We recommend the use of our HUMASTROL quality control serum based on animal serum or our S40000S based on human serum.

**Notes**

1. If the supernatant is not clear (high triglycerides level), dilute the sample before the precipitation with 0.9% saline (multiply result by 2).
2. High concentrations of ascorbic acid (> 2.5 mg/dl) will give lower results.
3. Hemoglobins levels higher than 100 mg/dl and bilirubin levels higher than 30 mg/dl interfere with the test.

**References**

2. Friedewald, W.T. et al., Clin. Chem. 18, 499 (1972)
Appendix VI: Test protocol for creatinine

**CREATININE liquicolor**

Jaffe-Reaction
Photometric Colorimetric Test for Kinetic Measurements. Method without Deproteinisation

**Packaging**

- 100 ml Complete kit
- 200 ml

**Method**

Creatinine forms in alkaline solution an orange-red coloured complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample.

**Principle**

Creatinine + Picric acid → Creatinine-picrate complex

**Contents, Reagent Composition**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picric Acid</td>
<td>26 mmol/l</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>1.6 mmol/l</td>
</tr>
<tr>
<td>Creatinine</td>
<td>2 mg/dl or 176.8 μmol/l</td>
</tr>
</tbody>
</table>

**Reagent Preparation**

- For measurement at 25°C: dilute [**PP**] with dist. water in the ratio 1:4.
- For measurement at 37°C: dilute [**PP**] with dist. water in the ratio 1:7.

Store the solution in a plastic bottle.

**MIX** and diluted [**PP**] for the working reagent in the ratio 1:1.

The [**PP**] is ready for use.

**Reagent Stability**

The reagents / diluted sodium hydroxide are stable, even after opening, up to the stated expiry date when stored at 15...25°C.

Contamination must be avoided.

**Warning:** The working reagent, protected from light, is stable for 4 weeks at 15...25°C.

**Specimen**

- Serum, heparinised plasma or urine.
- Avoid hemolysed.
- Stable: 24 hours at 2...8°C
- Dilute urine 1:41 with dist. water.

**Assay**

- Wavelength: 592 nm (490...510 nm)
- Optical path: 1 cm
- Temperature: 25°C / 37°C
- Measurements: against an increasing absorbance
- Warm the reagents and cuvettes to the desired temperature and keep constant (±0.5°C) for the duration of the test.

**Pipetting Scheme**

<table>
<thead>
<tr>
<th>Pipette into cuvettes</th>
<th>Semi-micro</th>
<th>Macro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample / [PP]</td>
<td>100 μl</td>
<td>200 μl</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1000 μl</td>
<td>2000 μl</td>
</tr>
</tbody>
</table>

Mix and start the stopwatch. After 30 sec. read the absorbance A₀. Read the absorbance A₂ exactly after 2 min. A₂ - A₀ = ΔA₃₄₀₉₀ or ΔA₃₄₀₈₀

**Calculation**

1. Serum / Plasma

Please use only the standard supplied with the kit.

\[
\begin{align*}
C &= 2.0 \times \frac{\Delta A_{\text{impl}}}{\text{[mg/dl]}} \\
C &= 176.8 \times \frac{\Delta A_{\text{impl}}}{\text{[μmol/l]}}
\end{align*}
\]

2. Urine

\[
\begin{align*}
C &= 100 \times \frac{\Delta A_{\text{impl}}}{\text{[mg/dl]}} \\
C &= \text{mg/dlitr urine}/24 \text{ h} \times 0.00884 \times 24 \text{ h}
\end{align*}
\]

Creatinine concentration in 24 h urine:

\[
\begin{align*}
C &= \frac{\text{mg/dlitr urine}/24 \text{ h} \times 0.00884 \times 24 \text{ h}}{\mu\text{mol/l}}
\end{align*}
\]

Creatinine clearance:

\[
\begin{align*}
\text{ml/min} &= \frac{\mu\text{mol/l}}{0.0113 \times \text{mg/dl}}
\end{align*}
\]

**Conversion of [mg/dl] into [μmol/l] and vice versa:**

\[
\begin{align*}
\text{[mg/dl]} &= 88.402 \times \text{[μmol/l]}
\end{align*}
\]

**Performance Characteristics**

**Linearity**

The test is linear up to a creatinine concentration in serum of 13 mg/dl or 1130 μmol/l, in urine of 500 mg/dl or 44,200 μmol/l.

Dilute samples with a higher concentration in serum, plasma or diluted urine 1:5 with physiological saline (0.9%) and repeat the assay. Multiply the result by 6.

Typical performance date can be found in the Verification Report, accessible via:


**Reference Values**

<table>
<thead>
<tr>
<th>Serum</th>
<th>[mg/dl]</th>
<th>[μmol/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>6.6 – 11</td>
<td>53 – 97</td>
</tr>
<tr>
<td>Women</td>
<td>5.5 – 9</td>
<td>44 – 80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine</th>
<th>[mg/dl]</th>
<th>[μmol/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>1000 – 1500</td>
<td>24 hours</td>
</tr>
<tr>
<td>Women</td>
<td>95 – 150 ml/min</td>
<td></td>
</tr>
</tbody>
</table>

**Quality Control**

All control sera with creatinine values determined by this method can be ordered. We recommend the use of our animal serum based Huma*Test* or our human serum based SERODOS quality control sera.

**Automation**

Proposals to apply the reagents on analysers are available on request. Each laboratory has to validate the application in its own responsibility.

**Notes**

1. The reaction is highly sensitive to temperature. The chosen reaction temperature must be kept constant.
2. PIC is harmful when inhaled, swallowed or in contact with the skin. If PIC comes into contact with the skin or mucous membranes wash with plenty of water. In case of sickness, contact a doctor.
3. The assay can be affected by the presence of reducing compounds. The interference can be partially eliminated by boiling the urine for a short time.
4. A slight precipitate in the sodium hydroxide solution is insignificant.

**References**

3. Schiemer J et al., Dtsch. med. Wochschr. 89, 3018 and S640 (1964)
4. Same H, Neurokroshabers, Thiem Verl, Stuttgart, 1959
Appendix VII: Test protocol for urinary albumin-creatinine excretion
Appendix VIII: Patients biochemical, clinical and demographic data

<table>
<thead>
<tr>
<th>Study No.</th>
<th>UACR mmol/l</th>
<th>HDL mmol/l</th>
<th>TC mmol/l</th>
<th>eGFR ml/min/1.73 m²</th>
<th>BMI kg/m²</th>
<th>AG yrs</th>
<th>Dura - tion yrs</th>
<th>sBP mm Hg</th>
<th>dBP mm Hg</th>
<th>Gender</th>
<th>FHo KD</th>
<th>MS</th>
<th>ED U</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>1.47</td>
<td>4.64</td>
<td>197.6</td>
<td>22.8</td>
<td>18</td>
<td>6</td>
<td>107</td>
<td>54</td>
<td>M</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>1.48</td>
<td>4.91</td>
<td>83.6</td>
<td>19</td>
<td>14</td>
<td>6</td>
<td>98</td>
<td>64</td>
<td>M</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1.53</td>
<td>3.51</td>
<td>110.1</td>
<td>31.6</td>
<td>31</td>
<td>6</td>
<td>117</td>
<td>79</td>
<td>M</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>1.1</td>
<td>4.21</td>
<td>101</td>
<td>20.9</td>
<td>25</td>
<td>21</td>
<td>144</td>
<td>82</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>0.45</td>
<td>3.97</td>
<td>56.8</td>
<td>22.2</td>
<td>29</td>
<td>17</td>
<td>176</td>
<td>75</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>1.3</td>
<td>3.5</td>
<td>110.1</td>
<td>31.6</td>
<td>31</td>
<td>6</td>
<td>117</td>
<td>79</td>
<td>M</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>1.58</td>
<td>5.05</td>
<td>156.5</td>
<td>18.7</td>
<td>24</td>
<td>5</td>
<td>128</td>
<td>85</td>
<td>F</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>1.75</td>
<td>4.51</td>
<td>137.3</td>
<td>24.8</td>
<td>35</td>
<td>7</td>
<td>85</td>
<td>62</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>2.01</td>
<td>5.05</td>
<td>83.5</td>
<td>18.9</td>
<td>16</td>
<td>6</td>
<td>113</td>
<td>55</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>160</td>
<td>1.52</td>
<td>4.88</td>
<td>92.3</td>
<td>21.9</td>
<td>16</td>
<td>6</td>
<td>110</td>
<td>70</td>
<td>F</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>1.55</td>
<td>4.98</td>
<td>123.4</td>
<td>20.6</td>
<td>29</td>
<td>7</td>
<td>93</td>
<td>66</td>
<td>F</td>
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<td>2</td>
<td>3</td>
</tr>
<tr>
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<td>15</td>
<td>1.44</td>
<td>4.91</td>
<td>116.3</td>
<td>24.8</td>
<td>31</td>
<td>6</td>
<td>115</td>
<td>81</td>
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<td>1</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>300</td>
<td>1.04</td>
<td>5.33</td>
<td>102.7</td>
<td>24.1</td>
<td>24</td>
<td>14</td>
<td>124</td>
<td>71</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>75</td>
<td>1.36</td>
<td>3.34</td>
<td>110.5</td>
<td>22</td>
<td>21</td>
<td>6</td>
<td>111</td>
<td>74</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>75</td>
<td>1.27</td>
<td>3.19</td>
<td>240.5</td>
<td>16.9</td>
<td>18</td>
<td>7</td>
<td>133</td>
<td>83</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>150</td>
<td>1.39</td>
<td>5.9</td>
<td>91</td>
<td>28.6</td>
<td>36</td>
<td>16</td>
<td>160</td>
<td>90</td>
<td>F</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td>1.13</td>
<td>3.88</td>
<td>172</td>
<td>25.1</td>
<td>20</td>
<td>6</td>
<td>126</td>
<td>63</td>
<td>F</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>60</td>
<td>1.48</td>
<td>3.1</td>
<td>99.9</td>
<td>28.6</td>
<td>36</td>
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<td>89</td>
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<td>1</td>
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<td>2</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>1.57</td>
<td>4.13</td>
<td>128.6</td>
<td>31.6</td>
<td>31</td>
<td>7</td>
<td>132</td>
<td>76</td>
<td>M</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>1.75</td>
<td>2.9</td>
<td>125.4</td>
<td>21</td>
<td>21</td>
<td>5</td>
<td>150</td>
<td>95</td>
<td>F</td>
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<td>1</td>
<td>1</td>
</tr>
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UACR – urinary albumin-creatinine excretion ratio; HDLC – higher density lipoprotein cholesterol; TC – total cholesterol; BMI – body mass index; sBP – systolic blood pressure; dBP – diastolic blood pressure; FHoKD – Family history of kidney disease, 1 (no history), 2 (with history); MS - Marital status; Gender - F (female), M (male); EDU - Education level, 1 (primary), 2 (secondary), 3 (tertiary)