THE RELATIONSHIP BETWEEN URIC ACID AND CARDIOVASCULAR DISEASE RISK MARKERS IN TYPE II DIABETES AT MULAGO NATIONAL REFERRAL HOSPITAL KAMPALA

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DECLARATION

I Sengoba Moses declare that the information given in this booklet is purely my own work. I have read the rules and regulations of plagiarism by the International Health Sciences University and honestly state that this research report has not been submitted to any other institution for any qualification. All sources quoted have been acknowledged by means of references.

Signature ……. Date……

APPROVAL

The information in this research report entitled the relationship between uric acid and cardiovascular disease risk markers has been compiled and submitted with approval of my supervisor

Signature …… Date …….

Mr. OYET CAESAR
SUPERVISOR

DEDICATION

With unquenchable love and deep respect, I dedicate this dissertation to my beloved parents Mr. Kabanda Benon and Ms. Jane Kabanda for their great and continuous support, encouragement and prayers.

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Firstly I would like to thank God for his provision and strength that enabled me complete this report. With loveand appreciation we wish to acknowledge the contributions of MulagoNational Referral Hospital, for having allowed me have my data collection from their hospital.

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LIST OF ABBREVIATIONS

CVD	Cardiovascular Disease
DM	Diabetes Mellitus
CHD	Coronary Heart Disease
CAD	Coronary Heart Disease
IDDM	Insulin Dependent Diabetes Mellitus
NIDD	Non - Insulin Dependent Diabetes
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
UA	Uric Acid
TRIG	Triglyceride
CHOL	Cholesterol
UA	Uric Acid

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ABSTRACT

Introduction: A relationship between hyperuricemia and Cardiovascular Disease (CVD) has been established since the 1900's and commonly increased uric acid levels are found in patients with cardiovascular disease and many prospective and cohort studies have demonstrated uric acid as a CVD risk factor. However a controversy exists as to whether uric acid is an independent risk factor to CVD. This study therefore aimed at determining the relationship between uric acid and CVD risk markers in type II DM patients

Materials & methods: A total number of 217type II DM participants were recruited. A prospective cross sectional study design was done. Blood samples were collected and run for lipid profile, uric acid and fasting blood sugar using the COBAS 600 Chemistry analyzer at Mulago National Referral Hospital clinical chemistry laboratory.

Results: We recruited 218 type 2 diabetic patients aged 20 – 90 years .Fifty-five (55/218; 25.2%) of the participants were males and the mean age of the participants was 49 years with 95% confidence interval of 47 – 51years. The mean fasting glucose level of the participants was 10.4 mmol/l. Most of the participants (n=111; 50.9%) had poor glycemic control with fasting glucose level greater than7.0mmol/l and15 (6.9%) of the participants had low blood glucose (lower than 3.8mmol/l).

Up to 34.4% of the participants had increased Total cholesterol level higher than 5.7mmol/l, with reduced HDL level (<0.91mmol/L).36.7% of the participants had high levels of low density lipoprotein (> 3.37) and only 19.7% had higher levels of HDL.

Only Total Cholesterol and Low Density Lipoprotein correlate with age but the rest of the variables i.e. Uric Acid, Fasting Blood Glucose, High Density Lipoprotein and Triglycerides do not have any correlation with age.

The mean uric acid level of the participants was 4.84mg/dl with a 95% confidence interval of 4.66 – 5.03mg/dl. Males had higher uric acid levels compared to females (mean of 5.07mg/dl 95% CI 4.61 – 5.56mg/dl compared to 4.72mg/dl with 95% CI of 4.57 – 4.98mg/dl in females) p-value 0.0043.

Conclusion: Despite the several population and cohort studies that demonstrated a relationship between uric acid and CVD risk markers, the present study demonstrated that there is no significant relationship between uric acid and CVD risk markers. We suggest that a population study with a bigger sample size be done to confirm the findings.

CHAPTER ONE: INTRODUCTION

1.0 Introduction

Diabetes was the leading cause of death in 2010 with 8.3% (25.1million) in the US and increased in 2012 to 9.3% and expected that 4.4% of the world's population will have DM by 2030 yet 29% of the cases were undiagnosed (Wild et al.,2000). In china, 17.2% of chines adults died of CVD due to DM

According to Mendis *et al.,* (2011); Finegold *et al*., (2012), cardiovascular disease mainly CAD has been the most common cause of death worldwide though recently it has reduced in high income countries.

Krishnan *et al*., (2013) confirmed that a considerable number of new diabetic cases can be statistically attributed to hyperuricemia (8.7%)

Uric acid is the end product of enzymatic breakdown of the purine nucleic acids (Bishop *et al.,* 2013).

Hyperuricemia (elevated levels of uric acid) is the chief and the principal peril factor of suggestive gout whose medical significance is the development of gout, metabolic disorder, CAD and type 2 Diabetes. It may occur because of decreased excretion by the kidney, over production or both. Under excretion is the most cause of Hyperuricemia than over production (Bishop *et al.,* 2013).

Hyperuricemia is mostly asymptomatic and in the general population its prevalence is estimated at 2 - 13%. Uric acid levels range between 2.4-6.0 mg/dl in females and 3.4-7.0 mg/dl in males. Hyperuricemia is a common finding and in general, it's notably associated with type 2 DM independent of the Body Mass Index, Hyperlipidemia and blood pressure (Abdul, 2011)

Diabetes mellitus is a metabolic disorder in which blood glucose levels are elevated above the normal. This is due to either failure to release enough insulin or failure to adequately use insulin or even both (Bishop *et al.,* 2013)

Unlike type 1 diabetes which is insulin dependent involving total beta cell destruction and total insulin deficiency, type 2 DM is non- insulin dependent with a reduced function of the beta cells of the pancreas, malfunction of the insulin receptors or reduction in the number of receptors such that even if insulin is produced these receptors cannot respond to it (Campbell, 2011).

In 2000, the prevalence of diabetes for all age groups worldwide was estimated to be 2.8% and it's expected to increase up to 4.4% by the year 2030. The numerical value of individuals with DM is anticipated to increase from 171 million in the year 2000 to 366 million come 2030 (Wild *et al.,* 2000*)*

Quiniones *et al.,* 1995 observed that Hyperuricemia is a frequent finding in insulin resistant states.

World heart federation cardiovascular health, 2011 shows that a diabetic patient has more chances of developing CVD than that without DM and most DM patients die of CVD

Diabetes increases the risk of cardiovascular disease due to hypertension which is more than twice in people with diabetes than in those with normal blood glucose levels, abnormal blood lipids and obesity which also occur more frequently in diabetic patients.

Poorly managed diabetes can create damage to blood vessels hence becoming more susceptible to damage from atherosclerosis and hypertension. People with diabetes develop atherosclerosis at a younger age and more severely than people without diabetes

Cardiovascular disease: is a group of diseases that involve the heart, blood vessels i.e. veins arteries and capillaries or both (Maton *et al.,* 1993).The causes are many though atherosclerosis and hypertension share the biggest percentage and then aging that comes with so many morphological and physiological changes which may alter the function of the cardiovascular system increasing the risk of developing the disease even in health individuals (Dantas *et al.,* 2012)

According to Mendis*et al.,* (2011); Finegold*et al*., (2012), cardiovascular disease mainly CAD has been the most common cause of death worldwide though recently it has reduced in high income countries. Cardiovascular disease includes CAD, hypertension, pulmonary artery disease, cardiomyopathy, heart failure, cardiac dysrhythmias, inflammatory heart disease, valvular heart disease, peripheral arterial disease, stroke and cerebrovascular disease. There are several risk factors to cardiovascular disease which include: Age which is estimated that 87% of death due to coronary artery disease is after 60 years and the risk to only stroke doubles after age 55. Another factor is sex where males are having a higher risk compared to pre-menopausal females but after menopause the risk is the same for men and women (Ash, 2011).

Hypertension, radiation therapy, smoking, diabetes, unhealthy eating and others are other factors exposing individuals to cardiovascular disease risk (Christian, 2014).

A relationship between Hyperuricemia and CV disease has been established since the 1900's and commonly elevated uric acid levels are found in patients with cardiovascular disease and many prospective and cohort studies have demonstrated uric acid as a CVD risk factor. However a controversy exists on the independence of uric acid as a predictor of CVD or not as many studies have demonstrated that Hyperuricemia is just linked with CVD because of confounding factors such as obesity, high blood pressure, use of diuretics, dyslipidemia and insulin resistance (Nieto *et al.,* 2000;Culleton *et al.,* 2001).

1.1 Statement of the problem

Cardiovascular Disease mainly, CAD has remained the principal cause of mortality world wide despite recent considerable declines (Mendis *et al.*, 2011)

About 600000 people in the United States die from Cardio artery disease yearly that is 1 in 4 deaths (25%) and every year 715000 Americans have a heart attack, 15% of which die (Maier *et al*., 2014)

The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030.

The number of people suffering from Diabetes is expected to increase from 171 million to 366 million by the year 2030 (Wild *et al.,* 2000). A study in china showed that 17.2% Chinese adults were dying of cardiovascular disease due to diabetes (Dong feng *et al.*, 2014)

The National diabetes statistics report, 2014 showed that in 2003 – 2006, CVD death rates were about 1.7 times higher among adults 18yrs and above with diagnosed DM than among those without diagnosed DM. It was also found that adults 20yrs and above with DM had a heart attack 1.8 times and stroke 1.5 times than those without DM in 2010

Bishop *et al*., (2013) demonstrated that individuals with type 2 DM have higher chances of developing macro and micro vascular diseases or complications.

Hyperuricemia predisposes to diabetes (Davis et al., 2013) which diabetes especially type 2 predisposes to cardiovascular disease. Uric acid has been found increased in patients with cardiovascular disease but it’s not considered among the CVD risk markers. This has led to increased death due to CVD especially among Diabetic patients because the available

cardiovascular disease risk markers that is lipid profile and C - reactive protein are expensive to perform in low setting like Uganda (about Ug.shs 80,000). There are a few Ugandans who can afford to pay such money for check up to determine the risk to cardiovascular disease so by the time they go to the hospital, they have already developed CVD.

Therefore uric acid which is cost friendly (about Ug.shs. 10,000) as compared to lipid profile if established as a risk marker by this research, will probably be adopted by the ministry of health to reduce on mortality rates due to cardiovascular diseases

1.2 Objectives

1.20 General objective

To determine the relationship between uric acid and cardiovascular disease risk markers in type 2 diabetic patients

1.2.1 Specific objectives

To determine the levels of uric acid in type 2 diabetic patients

To determine the levels of cardiovascular disease risk markers in type 2 diabetic patients

To assess the distribution of Fasting Blood Sugar and Uric Acid with age and gender

1.2.2 Research questions

What are the levels of uric acid in type 2 diabetes?

What are the levels of cardiovascular risk markers in type 2 diabetic patients?

What are the levels of uric acid and fasting blood sugar and their distribution with age and gender?

1.3 Justification

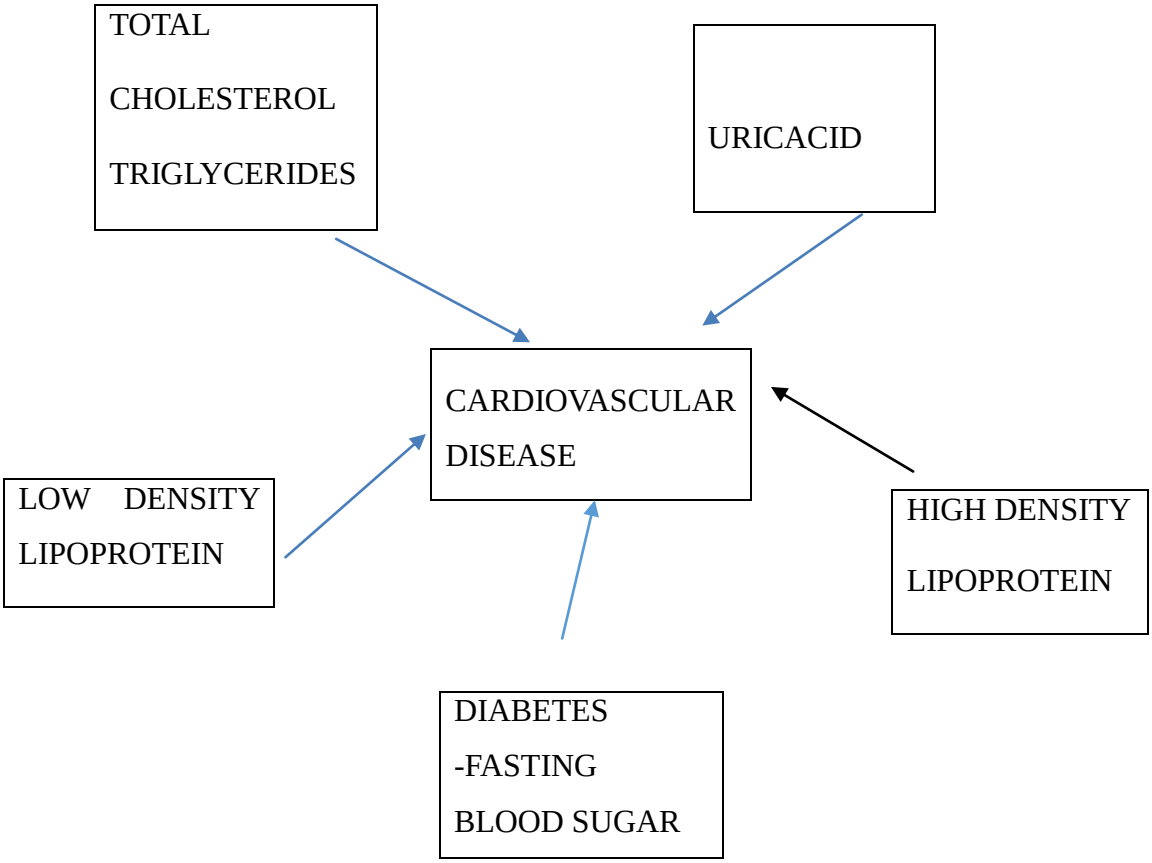
This study shall inform policy makers especially at ministry of health to include uric acid as a marker for the risk to cardiovascular disease which will lead to early diagnosis and help even the low income earners to access the services and reduce on the number of people who die due to CVD.

1.4 Hypothesis

Null hypothesis: there is no relationship between uric acid and CVD risk markers in II DM patients

Alternative hypothesis: there is a relationship between uric acid and CVD risk markers in type II DM patients.

1.4 Conceptual framework



CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This chapter includes information found by other researchers on uric acid, diabetes, cardiovascular disease and the relationship between uric acid and cardiovascular disease

2.1 Uric acid

Uric acid is the major product of nucleoprotein catabolism in humans and higher primates.

Uric acid is the product of breakdown of the purine nucleic acids (Bishop *et al.*, 2013).

It is formed endogenously or exogenously (dietary) but most of it is derived from the later from DNA and RNA (nucleic acids), cell breakdown and replacement (Robert *et al*., 2011)

It is filtered by the glomerulus of the kidney and secreted by the distal convoluted tubules into the urine though most of it is re-absorbed in the proximal convoluted tubule and reused (Bishop *et al*., 2013). 70% of uric acid is excreted in urine and the remainder passes into the gastrointestinal tract where it is degraded by bacterial enzymes (Robert *et al.*, 2013: Schoeff *et al*., 2013)

Uric acid is relatively insoluble in plasma and at high concentration it can be deposited in the joints and tissues leading to painful inflammation. Hyperuricemia i.e. high levels of uric acid is defined as serum or plasma uric acid levels greater than 7.0mg/dl in men and greater than 6.0mg/dl in women.

2.11 Causes of high levels of uric acid (Hyperuricemia)

The causes of Hyperuricemia are divided into four main categories i.e. increased dietary intake; overproduction, under-excretion and specific enzyme defects though it's mostly caused by a combination of overproduction and under excretion of uric acid (Linda *et al*., 2011)

1. Increased dietary intake

Some familiar foods contain precursors of uric acid which once ingested in higher amounts may lead to increased uric acid formation hence Hyperuricemia. Examples of foods include liver, kidney, sweet breads, salmon fish, sardines, haddock, scallops, beers, etc. (Merck manual, 1997)

2. Overproduction of uric acid

This can be due to increased breakdown of cell nuclei (DNA and RNA), as seen in patients on chemotherapy for proliferative diseases such as leukemia, lymphoma, multiple myeloma, and

polycythemia leading increased cell destruction hence elevated plasma uric acid concentration (Bishop *et al*., 2013). Patients suffering from hemolytic or megaloblastic anemia may also have elevated uric acid concentration due to increased hemolysis or breakdown of blood cells which contain the nucleic acids that are precursors of uric acid (Bishop *et al*., 2013). Starvation can also cause hyperuricemia as a result of increased tissue catabolism due to inadequate dietary intake. Harrison´s online 2012

3. Under excretion of uric acid

Uric acid excretion is a function of the kidney which excretes 70% of it in urine and therefore kidney disease e.g. glomerulonephritis, chronic renal disease impair the kidney´s ability to eliminate uric acid by interfering with filtration by glomerulus and secretion by distal convoluted tubules (Merck manual, 1997 and Bishop *et al*., 2013).

Hyperuricemia due to under excretion is a common feature of pre-eclampsia and lactic acidosis probably as a result of completion for binding sites in the renal tubules (Schoeff *et al*., 2013). Nephrolithiasis which is formation of kidney stones may also occur due to under excretion of uric acid.

2.2 Diabetes mellitus

Is metabolic disorder characterised by hyperglycaemia i.e. an increase in plasma glucose levels due to impaired insulin secretion, action or both. Glucose is the only form of sugar found in the blood. Other sugars like fructose and galactose once absorbed from the gut, they are quickly converted to glucose which is used by the body. When glucose becomes too much in the body, many tissues are damaged and if it's little in blood, body functions are impaired because its food for the cells (Campbell, 2011). In 2010, diabetes was the leading cause of death in the US and 234051 death certificates listed DM as the underlying cause of death which is a very big number.

The prevalence of undiagnosed DM is very high in that of 29.1million, only 21.0million were diagnosed and the rest (8.1million) were undiagnosed (Scheen *et al*., 1996).

The pancreas, liver and other endocrine hormones all participate in controlling blood sugar concentrations within a narrow range. During a brief fast, glucose is supplied to the extracellular fluid from the liver through glycogenolysis. When the fasting period is extended, glucose is synthesized from other sources through gluconeogenesis (formation of glucose 6-phosphate from non - carbohydrate sources).

Regulation of blood sugar is by two major hormones i.e. insulin which is a hypoglycemic agent and glucagon a hyperglycemic agent both of which are produced by the pancreas and antagonize each other (Bishop *et al*., 2013). In 2012, 9.3% of the American population, had diabetes compared to 2010 that had 25.8million or 8.3%.

2.2.1 Insulin

Is a hormone released from the pancreas and is the principal substance responsible for maintaining appropriate blood sugar levels through allowing glucose to be transported into cells so that they produce energy and store the glucose until its needed (Merck manual, 1997).Once the beta cells of islets of Langerhans responsible for insulin production in the pancreas detect elevated levels of glucose, they release hormone insulin that brings about an increased transfer of glucose from tissue fluids into the cells and hence increased metabolism that brings it to the normal (Schoeff *et al*., 2013)

Most cell membranes are impervious to glucose therefore insulin is required to stimulate and open the cell membrane bound gate so that glucose can go through the gate from the tissue fluid to the cytosol of the cells reducing the amount in the blood and tissue fluid. This necessity of transporting glucose into the cells explains why people with type 1 insulin dependent diabetes require some insulin in their blood almost always (Campbell, 2011)

It also regulates glucose by increasing glycogenesis (conversion of glucose to glycogen for storage), lipogenesis (conversion of carbohydrate to fatty acids) and glycolysis (metabolism of glucose molecule to pyruvate or lactate for energy production) and inhibiting glycogenolysis (glycogen breakdown to glucose for use as energy) (Fody *et al*., 2013)

2.2.2 Glucagon

Is the primary hormone responsible for increasing glucose levels. It's synthesized by alpha cells of islets of Langerhans in the pancreas and released during stress and fasting states. When the cells detect a decrease in plasma glucose, they release glucagon that acts by increasing plasma glucose levels by glycogenolysis (breakdown of glycogen to glucose for use) in the liver and an increase in gluconeogenesis (Schoeff *et al*., 2013)

Currently there are 2 forms of diabetes i.e. types 1 and type 2

2.2.3 Type 1 DM

According to Bishop *et al*., (2013), type 1 Diabetes mellitus is characterized by inappropriate hyperglycemia primarily a result of cellular mediated autoimmune destruction of pancreatic islet beta cells causing an absolute deficiency of insulin secretion. It constitutes only 10 – 20% of all cases of Diabetes mellitus and commonly occurs in childhood and adolescence.

Merck manual, 1997 and Fody *et al*., (2013) stress that it's initiated by an environmental factor, viral infection, and nutritional factor in childhood or early adulthood in individuals with a genetic predisposition causing the autoimmune destruction of beta cells of the pancreas leading to a decreased production of insulin.

More than 90% of the beta cells in this type are permanently destroyed and in order for a person to survive such severe insulin deficiency, regular insulin injection is required (Merck manual, 1997)

Another evidence to support the autoimmune nature of this type of DM comes from autoantibodies that have been found in the blood of 90% of newly diagnosed patients. Further studies have recently demonstrated the presence of autoantibodies in children during the 1st few years of life who develop type 1 diabetes mellitus several years later. Observations have also been made that beta cell survival is prolonged if patients are given immunosuppressant drugs which is another support for the autoimmune explanation of the type 1 Diabetes Mellitus (Campbell, 2011)

2.2.4 Type 2 Diabetes Mellitus

Is metabolic disorder characterized by hyperglycemia as a result of an individual´s resistance to insulin with an insulin secretory defect resulting in a relative insulin deficiency (Fody *et al*., 2013). It develops during the middle life (adult onset) and constitutes the majority of diabetic cases with environmental and genetic factors being the underlying cause. (Campbell, 2011)

Non-insulin-dependent diabetes mellitus is a common metabolic disorder that afflicts 2% to 5% of the adult population of most Western countries, though there is a variation internationally (King *et al*., 1993). The predisposing factors to this type is obesity (80% of NIDD in UK are obese), abdominal fat, increase in age and lack of physical exercise and most cases go undiagnosed (Schoeff *et al*., 2013). Type 2 DM is a leading cause of disability and death in developed and developing nations (Scheen *et al*., 1996).

The pathophysiology of type 2 DM begins when insulin receptors on liver and skeletal muscle cells become worn-out due to overuse and also intracellular secondary messenger systems that are activated by the insulin receptor complex are ineffective due to partly genetic reasons which contributes to insulin resistance. In the early stages of developing NIDD, glucose levels begin to rise and insulin production is increased by the beta cells to normalize the levels for a given period of time but over a long period of time, beta cell over stimulation causes them to decline in function and mass dropping down insulin levels in blood which in-turn leads to increased glucose levels and hence DM (Campbell, 2011)

2.3 Uric acid and type 2 diabetes

Hyperuricemia is a common finding in non - insulin dependent diabetes or type 2 DM (Kelley *et al*., 2001). A study by Abdul *et al*., (2004) showed that serum uric acid has a positive significant correlation with type 2 diabetes in general and obese patients independent of BMI, hyperlipidemia and hypertension.

The exact mechanism of hyperuricemia found in diabetic patients is unclear though compensatory hyperinsulinemia observed in insulin resistant individuals is thought to cause an ant-uricosuric effect on the kidneys (Clausen *et al*., 1998).

According to Edwards *et al*., (2002), the relationship between type 2 Diabetes and hyperuricemia is also linked to genetic predisposition.

Different theories have been presented to explain the exact mechanism of hyperuricemia related of found in type 2 diabetic patients.

Based on Quiniones *et al*., (1995) study, insulin (which is high in diabetes) induces change in fractional uric acid and sodium excretion together with hyperinsulinemia which reduce uric acid and sodium elimination in such patients. This retention increases uric acid levels in blood.

Muscelii *et al*., (1996) also observed that increased levels of insulin (hyperinsulinemia) caused a significant decrease in urinary excretion of uric acid leading to its increase in blood.

2.4 Diabetes and cardiovascular disease

The national diabetes statistics report, 2014 showed that in 2009-2012, 71% of adults 18 years and above with diagnosed diabetes mellitus had BP $\geq$ 140/90 mmHg. It added that in 2003 – 2006, CVD death rates were about 1.7 times higher among adults 18yrs and above with

diagnosed DM than among those without diagnosed DM. It was also found that adults 20yrs and above with DM had a heart attack 1.8 times and stroke 1.5 times than those without DM in 2010

Bishop *et al*., (2013) demonstrated that individuals with type 2 DM are at a higher risk of developing macro and micro vascular diseases or complications.

Macro vascular complications affect the large arteries and suggestions have been made that atheroma formation (patchy thickening in inner lining of arteries due to fat laden macrocyte accumulation (Merck manual, 1997) in DM is evoked by overtime rise in glucose levels making it migrate into lining of arterial walls attracting LDL that adhere leading to fatty accumulation in vessel lumen. These fat deposits bring about fibrous collagen deposition and hence atheromatous plague formation that narrows the arteries especially coronary arteries causing CAD which is the leading cause of morbidity in people with DM accounting for 70% death.

Microvascular complications affect small vessels i.e. arterioles and capillaries where thickening and increase in base membrane rigidity occurs narrowing the lumen of these vessels and creating elasticity loss leading to tissue ischemia and hypoxia (Campbell, 2011)

A relationship between uric acid (UA) levels and cardiovascular diseases has been previously reported though its importance as a risk factor is still controversial.

2.5 Uric acid and cardiovascular disease

Since 1900's, a correlation between hyperuricemia and CV disease has been demonstrated. Commonly hyperuricemia is found in patients with CVD as several prospective and cohort studies have demonstrated uric acid as a CVD risk factor. However a controversy exists as to whether uric acid independently predicts CVD or it's just due to confounding factors such as hypertension, obesity, use of diuretics, insulin resistance and dyslipidemia. (Nieto *et al*., 2000; Culleton *et al*., 2001)

Davis et al., 2013 supported the concept that Hyperuricemia can be a significant and independent cardiovascular risk factor, not only for cardiovascular and cerebrovascular diseases, but also for renal failure, hypertension and type 2 Diabetes. Hyperuricemia is commonly found in individuals at higher risk of developing cardiovascular disease including men, post-menopausal women since oestrogen favors uric acid renal excretion, obesity and hypertension.

A study by Li *et al*., (2014) demonstrated that elevated serum uric acid levels were associated with CVD, independent of conventional CVD risk factors and the presence of metabolic syndrome. Also it was observed that the risk to CVD increased in individuals with Hyperuricemia.

Several studies by Neogi *et al*. (2009); Bos *et al*., (2006); Krishnan *et al*., (2006); Ito *et al*., (2011) Juraschek *et al*., (2014), have showed a strong relationship between serum uric acid and coronary heart disease and proposed that uric acid be an independent risk factor of CVD. A meta-analysis by Kim *et al*., 2002 demonstrated that increased uric acid levels (hyperuricemia) can shoot up the risk to coronary heart disease independent of the established CHD risk markers.

A hypothesis of how Hyperuricemia can expose an individual to CVD is that neutrophil and platelet activation which is known predisposing factor to thrombosis, together with increased C-reactive protein.

A study by Brand *et al*., (1985) showed that serum uric acid values correlated with systolic and diastolic blood pressure in both men and women and the relationship was stronger in women than in men and for systolic than for diastolic pressure. According to (Jiang *et al*., 2006; Haffner *et al*., 1992; Isomaa *et al*., 2001; Lakka *et al*., 2002), metabolic syndrome (MetS) which is regarded as the clustering of cardiovascular risk factors has been found to have an association with development of cardiovascular disease and increasing the risk of death to cardiovascular disease.

Not all epidemiological studies regard uric acid as an independent marker for cardiovascular disease. Framingham heart study demonstrated that though uric acid had a significant relationship with the risk to cardiovascular disease, it´s significance after correcting some variables like hypertension, BMI, diuretic use and others was not substantial (Culleton *et al*., 1999)

Bickel *et al*., (2002) shows that patients with angiographically confirmed coronary artery disease with serum uric acid levels >7mg/dl had five times more chances of death as compared to those with uric acid levels <5.4mg/dl, and that a 1mg/dl increase in serum uric acid could increase death by 26% comparable to the 20% to 25% increase in MI associated with a 10- to 12-mm Hg increase in systolic blood pressure. Hypertensive patients normally have Hyperuricemia which

may be due to a defect in urate excretion by the kidneys and there is a 3-5 fold increased risk of developing coronary artery disease in patients with hypertension and Hyperuricemia than in patients with normal uric acid levels (Tykarski *et al*., 1991; Breckenridge, 1966)

Alderman *et al*., 1998 demonstrated that in average a 1 mg/dl in-treatment of serum uric acid was related to a 32% increase in cardiovascular events and that despite reasonable blood pressure control, the association of Hyperuricemia with CVD continued. A graded relationship exists between serum uric acid levels and mortality in heart failure patients. Heart failure patients with serum uric acid levels of > 800 µmol/L had a relative risk of mortality that was 18-times higher than that in patients with uric acid levels £ 400 µmol/L (Alderman *et al*., 2004).

CHAPTER THREE: RESEARCH METHODOLOGY

3.0 Introduction

This chapter included in details the methods of data collection, study area, study population, sample selection criteria, data processing and data analysis.

3.1 Study area

The research was conducted from Mulago National Referral Hospital, located in central division Kampala district whose vision is to be the foremost center of health care delivery in the whole of Africa. It was founded in the year 1913 and in the year 1962 it was expanded by constructing lower Mulago to offer better services and facilities to patients.

It serves as a National referral Hospital for the whole of Uganda and as a general hospital as well as a health Centre IV and III for the whole of Kampala metropolitan. It serves as a National Referral for the entire country and a general hospital as well as Health Centre IV, III for the Kampala metropolitan. This Hospital has a bed capacity of 1790 and has been the center of excellence for patient care, training of health workers and research.

3.2 Study design

The study was a qualitative research in which the researcher used a cross sectional study design.

3.3Target population

The population targeted by this study was the Type 2 diabetic patients.

3.4 Study Population

The study population in this research was Type 2 Diabetic Patients attending Mulago National Referral Hospital Diabetic Clinic on Wednesdays

3.5 Sampling and sample size determination

A random sampling method was employed by the study to determine specific group of people for the participation. The study used the Kish and Leslie (1965) technique to determine the least

sample size denoted n.

$$n = \frac{Z^2 p\,(1 - p)}{d^2}$$

Where n = the required sample size

z = the statistical certainty chosen at 95% confidence interval and is 1.96

P = prevalence of uric acid approximated at 17.2% (Alikor *et al.,* 2013)

d= the deviation error set at 0.05

Therefore; $n = z^2\, p\, (1\text{-}p)/d^2 = 1.96^2 * (0.172 * 0.828)/0.05^2 = 218.84$

Therefore; sample size n = 218

3.6 Variable selection

3.60 Dependent variable

Cardiovascular disease

3.61 Independent variables

Diabetes – fasting blood sugar

Uric acid

Cholesterol levels

Triglyceride levels

Low density lipoprotein

High density lipoprotein

3.7 Inclusion criteria

Type 2 diabetic patients at Mulago hospital diabetic clinic with no history of kidney disease who were available at the time of data collection and consented to participate in the study.

Note. Kidney disease patients were excluded because uric acid also increases in patients with kidney disease especially nephritis because filtration and excretion is impaired so it accumulates in blood.

3.8 Exclusion criteria

Type 1 diabetic patients and those with kidney disease plus whoever that did not sign the consent form

3.9 Data collection procedure

The researcher explained the topic and the research objectives to type 2 diabetic patients (selection was done by the nurses in case a patient never knew his/her type) that attended the diabetic clinic and then a consent form was issued to them. All type 2 Diabetic patients on a particular Wednesday that understood and accepted to participate were required to sign the form and then were recruited. A patient was recruited once on a given Wednesday (day of Diabetic clinic) and then different patients on the subsequent Wednesdays.

5ml of venous blood were collected from each patient, separated (centrifuged) and then run for lipid profile i.e. Total cholesterol, Triglyceride, Low and High Density Lipoproteins, then Uric Acid, and Fasting blood sugar by the automated chemistry analyzer

3.10 Data Management and Analysis

All data collected were recorded in a book and later in a computer. Analysis was done after all the data had been collected and involved the production and interpretation of tables, graphs using SPSS, ANOVA, multi-variate regression and correlation analysis.

3.11 Quality control

The instruments were pre-tested among a fifth of the total sample size in a different area that is Butabika Hospital and this helped the researcher to assess its clarity, accuracy and reliability to enable the collection of appropriate data. Calibration of instrument (chemistry analyzer) was done before any samples were run together with running controls with the test samples to ensure accuracy and reliability plus Ensuring analysis of fasting serum samples

3.12 Ethical procedures

After approval of the research proposal by the supervisor, an introduction letter from the university was issued to the researcher who presented it to the Institution Review Board of Mulago National Referral Hospital Kampala for permission to carry out the study and after it

was granted, data collection began.

An informed consent was obtained from participants prior to sample collection. The consent forms clearly explained the study topic, objectives, the benefits and risks involved. It also made it clear that participation was voluntary and no one would be compelled to participate if the study was perceived to cause any kind of discomfort or unease.

The consent form also clarified that the participant had the right to pull out of the study at any given time. Considering the sensitive nature of the study population, the consent form included a confidentiality clause where the researcher assured the participants that their information shall be kept as a secret.

Participants who knew English were given the consent forms to read through, some asked the researcher questions which were answered and those who consented signed the form and were immediately recruited. For the participants who could not understand English, the interviewer/researcher explained the consent statement to the participant in Luganda and asked those who had consented to sign or thumbprint as they wished. Names and addresses of respondents were recorded because the only benefit for the participants was to receive their results so that they could know the function of their heart, the effect Diabetes has caused and prevent heart disease.

CHAPTER FOUR: RESULTS

4.0 Demographic characteristics

We recruited 218 type 2 diabetic patients aged 20 – 90 years. Fifty five 55/218;25.2%) of the participants were males and the mean age of the participants was 49 years with 95% confidence interval of 47 – 51 years. The distribution of the age of the participants is as shown in figure4.1.

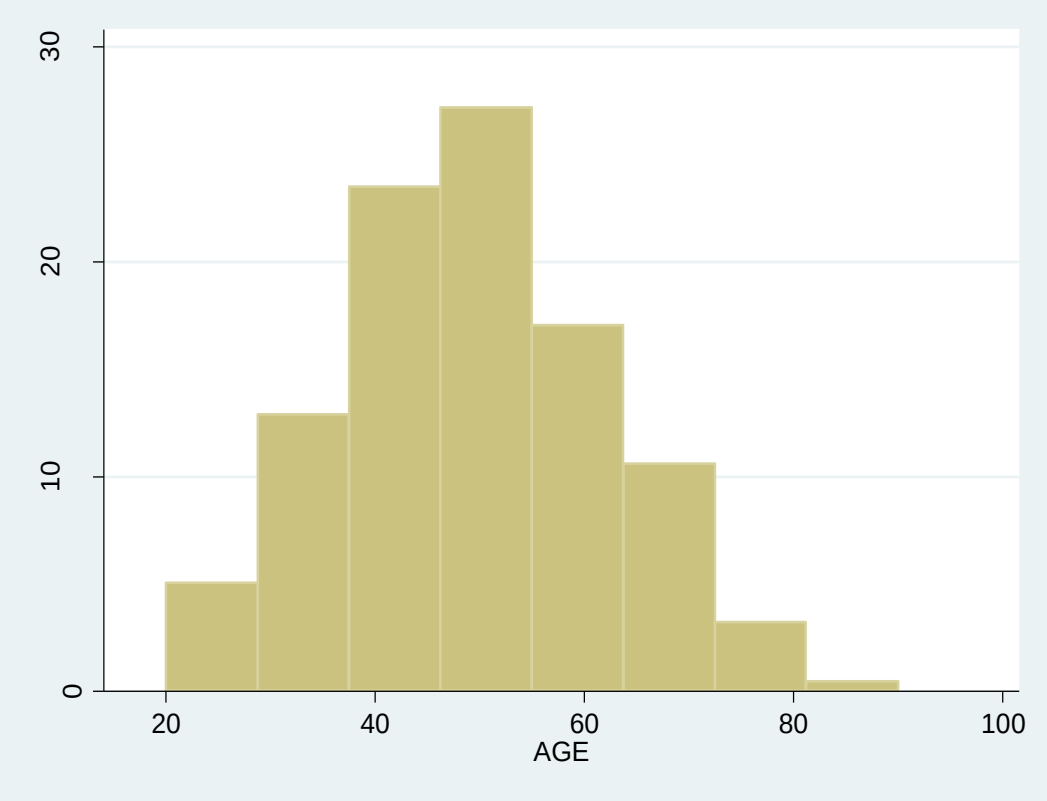


Figure 1 **: Age (years) distribution of the participants (n=218)**

4.2 The distribution of fasting blood glucose among participants

The mean fasting glucose level of the participants was 10.4mmol/L with the distribution as shown in table 4.1. Most of the participants (n=111; 50.9%) had poor glycemic control with fasting glucose level greater than 7.0mmol/L and 15 (6.9%) of the participants had low fasting blood glucose (lower than 3.8mmol/L). There was no correlation between fasting blood glucose with age and gender.

Table 1 : **Distribution of fasting glucose level among the participants (n=218)**

	Fasting Glucose range	Frequency	Percent
	<3.8mmol/L	15	6.9
	3.8 – 7.0mmol/L	92	42.2
	>7.0mmol/L	111	50.9
	Total	218	100.0

Figure 2 : **Graph of fasting blood sugar with age**

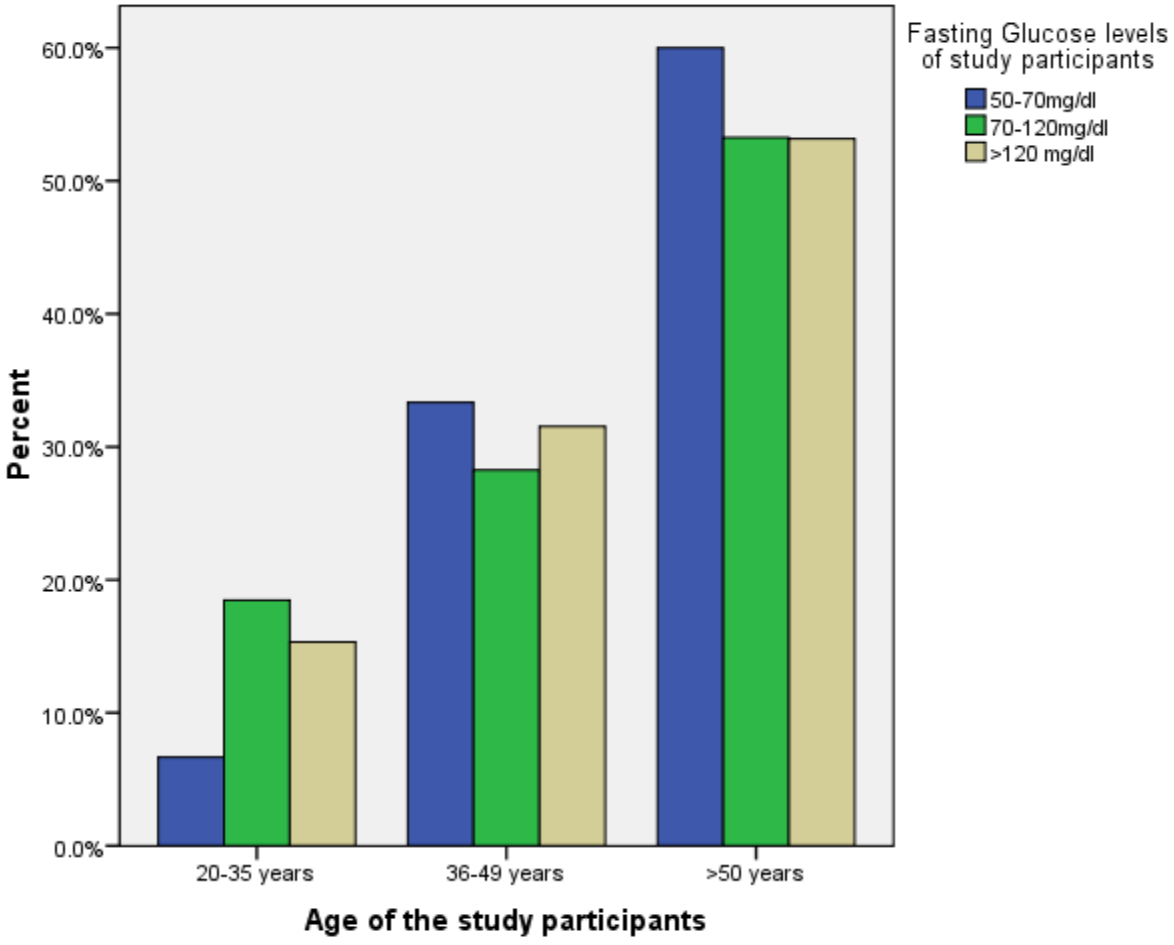


Table 2 : **Correlation of variables with age**

Variable	**Mean square**	**F**	**P-Value**
Uric acid	0.131	1.681	0.18
Total cholesterol	4.429	10.579	0.000
Fasting glucose	0.021	0.055	0.946
High density lipoprotein	0.386	0.953	0.387
Triglyceride	0.273	1.429	0.242
Low density lipoprotein	0.860	3.781	0.024

Only Total Cholesterol and Low Density Lipoprotein correlate with age but the rest of the variables i.e. Uric Acid, Fasting Blood Glucose, High Density Lipoprotein and Triglycerides do not have any correlation with age.

4.3 Levels of uric acids

The mean uric acid level of the participants was 4.84mg/dl with a 95% confidence interval of 4.66 – 5.03mg/dl. Males had higher uric acid levels compared to females (mean of 5.07mg/dl 95% CI 4.61 – 5.56mg/dl compared to 4.72mg/dl with 95% CI of 4.57 – 4.98mg/dl in females) p-value 0.0043.

4.4 Cardiovascular disease risk markers in diabetic patients

Up to 34.4% of the participants had increased Total cholesterol level higher than 5.7mmol/l, with reduced HDL level (<0.91mmol/l). 36.7% of the participants had high levels of low density lipoprotein (> 3.37) and only 19.7% had higher levels of HDL. The distributions of the cardiovascular markers are as shown in table 4.3.

Table 3 : **The distribution of the markers of cardiovascular disease**

Variable	Concentration (mmol/l)	Frequency	Percent
High density lipoprotein	<0.9	45	20.6
	0.9-1.45	130	59.6
	>1.45	43	19.7
Low density lipoprotein	<3.37	138	63.3
	>3.37	80	36.7
Total cholesterol	<3.69	30	13.8
	3.7 – 5.7	113	51.8
	>5.7	75	34.4

4.5 Correlation of uric acid with CVD risk markers

Only High Density Lipoprotein as an independent factor correlates with Uric Acid but the others that is Triglycerides, Total cholesterol and LDL were not significantly correlated with high uric acid levels with significant *p*-values (>0.05). A combination of Cholesterol and Triglycerides and then HDL, LDL and CHOL, plus HDL, LDL and TRIG gave a correlation with Uric Acid

Table 4 **: correlation of uric acid and CVD risk markers**

Corrected Model	2.524[a]	25	.101	1.340	.139
Intercept	30.867	1	30.867	409.534	.000
CHOL	.086	3	.029	.379	.768
HDL	.497	2	.248	3.296	.039
TRIG	.212	1	.212	2.807	.095
LDL	.132	1	.132	1.753	.187
CHOL * HDL	.064	4	.016	.211	.932
CHOL * TRIG	.567	2	.284	3.762	.025
CHOL * LDL	.096	1	.096	1.278	.260
HDL * TRIG	.175	2	.087	1.160	.316
HDL * LDL	.050	2	.025	.333	.717
TRIG * LDL	.208	1	.208	2.764	.098
CHOL * HDL * TRIG	.768	2	.384	5.093	.007
CHOL * HDL * LDL	.330	1	.330	4.383	.038
CHOL * TRIG * LDL	.000	0	.	.	.
HDL * TRIG * LDL	.404	1	.404	5.355	.022
CHOL * HDL * TRIG * LDL	.000	0	.	.	.
Error	14.471	192	.075		
Total	237.000	218			
Corrected Total	16.995	217			

a. R Squared = .149 (Adjusted R Squared = .038)

CHAPTER FIVE: DISCUSSION

5.0 Introduction

In this study, a patient was considered to have poor glycemic control if he had fasting blood glucose levels > 7.0mm0l/l. risk to CVD was taken to be high with total cholesterol values > 5.7mmol/l, LDL > 3.37mmol/l and HDL < 0.9mmol/l.

5.1 Demographic characteristics

The mean age of the participants was 49 years with 95% of 47 – 51 years. Age is an important factor to consider because type II DM usually develops or begins after age 30 though sometimes can occur in children and adolescents and the risk increases with age in addition to other predisposing factors like obesity, lack of physical exercise, abdominal fat and others with genetic and environment factors being the primary cause (Merck manual, 1997). Only 25.2% of the participants were males indicating that females have a higher risk of developing type II DM than males.

On the contrary, some previous studies have reported a trend-shift where more men are diagnosed with type II DM than women. This has been claimed to be due to the sedentary life style that men live which leads to increased obesity and that men have more abdominal and visceral fat which is associated with poor insulin sensitivity compared to their counterparts the females who have more peripheral and subcutaneous fat that is associated with increase in the sensitivity of insulin. This offers women protection against type II DM (Diapedia, 2014). This research however has shown that females have a higher risk of developing type II DM.

5.2 Distribution of fasting blood glucose among participants

The mean fasting glucose levelofthe participantswas10.4mmol/L with most of them (n=111; 50.9%) having fasting glucose level greater than7.0mmol/L. This implies that there was a poor control of the blood sugar which could either be due to failure of the patients to adhere to the treatment, sedentary lifestyle and poor management by the clinicians. This was evidenced when patients could come past the appointment day and then, they would be given another appointment without any medications yet some were complaining that they were from far places

meaning they could not afford reporting the next week hence more time spent without medication.

5.3 Distribution of uric acid among participants

The mean uric acid level of the participants was 4.84mg/dl with a 95% confidence interval of 4.66 – 5.03mg/dl. Males had higher uric acid levels compared to females (mean of 5.07mg/dl 95% CI 4.61 – 5.56mg/dl compared to 4.72mg/dl with 95% CI of 4.57 – 4.98mg/dl in females) p-value 0.0043. Serum uric acid levels differ individually depending on the diet, kidney function, factors related to metabolism and genetic background. Females tend to have low levels of uric acid compared to males of a similar age. This may be explained as due to the lower post secretory re-absorption of uric acid by the renal tubules. Some studies have demonstrated an effect of plasma estrogen on the low uric acid levels probably by increasing the renal clearance of uric acid in women, reduced re-absorption hence low levels in blood (Garcia *et al*., 2008). However Antón *et al.,* 2014 concluded that plasma estrogen does not have any effect on the kidney handling of uric acid.

5.4 Cardiovascular disease risk markers in diabetic patients

Up to 34.4% of the participants had increased Total cholesterol level higher than 5.7mmol/l with reduced HDL level (<0.91mmol/l). Elevated cholesterol levels increase a person's risk to developing CHD so it's measured to assess the risk status of the patient. LDL is bad cholesterol because it transports cholesterol from the tissues to blood increasing its levels therefore any increase in blood offers a greater risk to CVD. HDL is good cholesterol because it increases utilization of cholesterol by transporting it from blood to tissues so high levels are a benefit and low levels are a disadvantage. According to this study, there is a high risk of developing CVD among these DM patients due to the high levels of Total Cholesterol, LDL and low levels of HDL in relation to the poor glycemic control.

5.5 Uric acid and cardiovascular disease risk markers

Many previous studies have shown a strong relationship between serum uric acid levels and CVD (LiQin *et al,* 2014: Neogi *et al.,* 2009). Other studies have gone ahead to suggest that uric acid be used as an independent risk factor for cardiovascular disease (Eswar *et al.,* 2011).

Another study by Zhen Yang *et al.,* 2014 demonstrated that increased serum uric acid level is

associated with CVD, autonomous of traditional CVD risk factors. This study gave more evidence that the risk of CVD increased in participants with Hyperuricemia. On the contrary, in this present study we found out that there was no significant relationship between uric acid and cardiovascular disease risk markers. Only HDL showed a relationship with uric acid of which increased levels of HDL reduce the risk to CVD. This study is supported by Ghei *et al.,* (2002) who strongly urged basing on the genetic relations of Hyperuricemia and CVD risk factors and demonstrated that uric acid is not a fundamental cause or agent in CVD.

5.6 Recommendations

I recommend that bigger prospective population studies be done in Uganda to confirm the non - existence of the relationship between uric acid and CVD so that the attempt to include uric acid among the CVD risk markers is stopped.

5.7 Limitations

Although most of the confounders were keenly controlled, some participants might have had many chronic illnesses and were on medications that could have an effect on the current findings. The sample size was small in relation to the population of Ugandans with Diabetes

5.8 Conclusion

In conclusion, this study has demonstrated no relationship between uric acid and CVD risk markers.

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APPENDIX I: INFORMED CONSENT FORM

STUDY TOPIC: RELATIONSHIP BETWEEN URIC ACID AND CARDIOVASCULAR DISEASE RISK MARKERS IN TYPE II DIABETIC PATIENTS

Rationale of the study: The study is intended to assess the relationship between uric acid & cardiovascular disease risk markers so that if it's proven that a correlation exists, then uric acid which is cheaper can be used to determine the risk to cardiovascular disease thereby reducing death due to cardiovascular disease in Diabetic patients is reduced.

Investigator: Mr. SENGOBA MOSES.
STUDENT AT INTERNATIONAL HEALTH SCIENCES UNIVERSITY
BACHELORS MEDICAL LABORATORY SCIENCE

Why type II Diabetic patients

Type II diabetic patients are more prone or at high risk of developing cardiovascular diseases. In fact 80% of death in Diabetic patients is due to cardiovascular diseases

Objectives of the study

General objective

To determine the correlation between uric acid and cardiovascular disease risk markers in type II diabetic patients

Specific objectives

To assess the level of uric acid in type 2 diabetic patients
To determine the levels of cardiovascular risk markers i.e. triglycerides, cholesterol and C-reactive protein in type II DM patients
To determine the levels of HbA1C in diabetic patients and it's correlation with uric acid levels

Procedure

5ml of venous blood shall be collected from each patient and then put in red and grey topped Vacutainer tubes 3ml and 2ml each respectively.

Schedules of specimen collection

Specimens shall be collected only on Wednesdays from 3rd. 06. 2015 up to when 218 samples are obtained

Risks involved

There are no risks involved in collecting blood samples from patients

Benefits of participation

There are no direct benefits in terms of treatment for diabetes but participants shall be given results for free and in case a relationship between uric acid and cardiovascular disease is established, uric acid will probably be considered as a risk marker to cardiovascular disease. This will therefore help to reduce on the global increase in the number of death in diabetic patients due to cardiovascular disease since uric acid is cheaper as compared to the available CVD risk markers i.e. lipid profile. Participants will be given the results

Compensation for injury

In case of any needle prick injury during blood collection, the researcher shall provide the necessary treatment to the subject in question until total recovery

Compensation for participation

There will be no reward in terms of money given to participants because it is a student's academic research that has no money to give to every participant (218) for the award of a Bachelor's degree in medical laboratory science

Supervisor

Mr. Oyet Caesar
Lecturer IHSU
0703621141
caesaroyet@yahoo.com

Contact person on Research and Ethics committee

Dr. Nakwagala Nelson Frederick

Chairperson Mulago research & ethics committee

0772325869

Confidentiality

The information that shall be provided by the participants shall be kept confidential only known by the principal investigator. Initials shall be used instead of names to identify the participants

Statement of consent

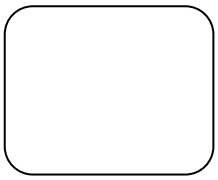
I have read and understood the nature of the study and its aim and that am free to withdraw at any time once it starts if I feel uncomfortable with the study, and the information provided will be excluded. I therefore consent to participate in the study without any kind of coerciment.

Name ……

Sign ……

Date ……

Thumb print



Investigator

Name ……

Sign ……

Date ……

Contacts; 0788874274/ 0757199250

Email; sengobamoses@gmail.com

APPENDIX II: INTRODUCTION LETTER

IHSU INTERNATIONAL HEALTH SCIENCES UNIVERSITY
sanitas per scientiam
PART OF INTERNATIONAL MEDICAL GROUP

making a difference to health care

Dean's Office-Institute of Allied Health Sciences

Kampala, 13th March 2015

THE CHAIRPERSON
MULAGO RESEARCH AND
ETHICS COMMITTEE

Dear Sir/Madam,

RE: ASSISTANCE FOR RESEARCH

Greetings from International Health Sciences University.

This is to introduce to you **Sengoba Moses**, Reg. No. **2011-BMLS-PT-009** who is a student of our University. As part of the requirements for the award of a Bachelors of Medical Laboratory Science of our University, the student is required to carry out research in partial fulfillment of his award.

His topic of research is: **The relationship between Uric Acid and Cardiovascular Disease Risk Markers in Type II diabetic patients. A case of Mulago National Referral Hospital Diabetic Clinic – Kampala District.**

This therefore is to kindly request you to render the student assistance as may be necessary for his research.

and indeed the entire University are grateful in advance for all assistance that will be accorded to our student.

Sincerely Yours,

13 MAR 2015

Okiria John Charles
Senior Lecturer / Dean, Institute of Allied Health Sciences

The International Health Sciences University
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APPENDIX III: RESEARCH APPROVAL LETTER

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IN ANY CORRESPONDENCE ON THIS SUBJECT PLEASE QUOTES NO...

THE REPUBLIC OF UGANDA

MULAGO NATIONAL REFERRAL HOSPITAL
P.O. Box 7051
KAMPALA, UGANDA

9th July, 2015.

Mr. Sengoba Moses
Principal Investigator
Institute of Allied Health Sciences
International Health Sciences University.

Dear Sengoba,

Re: Approval of Protocol MREC: 795: "The Relationship Between Uric Acid and Cardiovascular Disease Risk Markers in Type II Diabetic Patients; A Case of Mulago National Referral Diabetic Clinic Kampala District".

The Mulago Hospital Research and Ethics Committee reviewed your proposal referenced above and hereby grant approval for the conduct of this study for a period of (1) year from 9th July, 2015 to 8th July, 2016.

This approval covers the protocol and the accompanying documents listed below;

- Consent form.
- Laboratory procedures form

This approval is subjected to the following conditions:

1. That the study site may be monitored by the Mulago research and ethics committee at any time.
2. That you will be abide by the regulations governing research in the country as set by the Ugandan National Council for Science and Technology including abiding to all reporting requirements for serious adverse events, unanticipated events and protocol violations.
3. That no changes to the protocol and study documents will be implemented until they are reviewed and approved by the Mulago Research and Ethics Committee.
4. That you provide annual progressive reports and request for renewal of approval at least 60 days before expiry of the current approval.
5. That you provide an end of study report upon completion of the study including a summary of the results and any publications.
6. That you will include Mulago hospital in your acknowledgements in all your publications.

APPROVED
APPROVAL DATE EXPIRY DATE
09 JUL 2015 08 JUL 2016
MULAGO RESEARCH ETHICS COMMITTEE
IRC

I wish you the best in this Endeavour.

DR.NAKWAGALA FREDERICK NELSON
CHAIRMAN- MULAGO RESEARCH & ETHICS COMMITTEE.

Vision: "To be the leading centre of Health Care Services"

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APPENDIX IV: LABORATORY PROCEDURES

Total Cholesterol

Elevated levels of cholesterol increase the risk for coronary heart disease (CHD). Cholesterol is measured to help assess the patient's risk status and to follow the progress of patient's treatment to lower serum cholesterol concentrations.

Desirable cholesterol levels are considered to be those below 200 mg/dl in adults and below 170 mg/dl in children.

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration.

Reactions

Cholesteryl ester hydrolase

Cholesteryl ester + H_2O ----------->cholesterol + fatty acid

Cholesterol oxidase

Cholesterol + O_2 ----------> cholest-4-en-3-one + H_2O_2

Peroxidase

$2H_2O_2$ + 4-aminophenazone + phenol ----------> 4-(p-benzoquinone-monoimino)-phenazone + 4 H_2O

Reference range

According to the risk of developing CVD (WHO)

100 – 200 mg/dl	Desirable
200 – 240 mg/dl	Borderline high
240 – 400 mg /dl	Increased risk
> 400 mg/dl	High risk

Triglycerides

High levels of serum triglycerides help mark conditions that are associated with increased risk for CHD and peripheral atherosclerosis. High triglycerides are associated with increased risk for CAD in patients with other risk factors, such as low HDL-cholesterol, some patient groups with elevated Apo lipoprotein B concentrations, and patients with forms of LDL that may be particularly atherogenic.

Desirable fasting triglyceride levels are considered to be those below 200 mg/dl,
Very high triglycerides can result in pancreatitis and should be promptly evaluated and treated. Triglycerides are also measured because the value is used to calculate low density lipoprotein (LDL)-cholesterol concentrations

Principle of test method

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

Lipase

Triglycerides + $3H_2O$ ----------> glycerol + fatty acids

Glycerokinase

Glycerol + ATP ----------> glycerol-3-phosphate + ADP

Glycerophosphate oxidase

Glycerol-3-phosphate + O_2 ----------> dihydroxyacetone phosphate + H_2O_2

Peroxidase

H_2O_2 + 4-aminophenazone + 4-chlorophenol ----------> 4-(p-benzoquinone-monoimino) -phenazone + $2H_2O$ + HCL.

Further classification

Borderline	200-400 mg/dl
High	400-1,000 mg/dl
Very High	(> 1000 mg/dl)

High density lipoprotein (HDL) cholesterol

Low serum concentrations of HDL-cholesterol are associated with increased risk for CHD. Coronary risk increases markedly as the HDL concentration decreases from 40- to 30 mg/dl. A low HDL-cholesterol concentration is considered to be a value below 35 mg/dl for females and 30mg/dl in males and high HDL, >65 mg/dl in males and >70mg/dl in females.

HDL-cholesterol values are also used in the calculation of LDL-cholesterol

Samples

Serum

Plasma

Principle of the test method

The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-cholesterol is detected under the assay conditions.

The method uses sulfated alpha-cyclodextrin in the presence of Mg^{+2}, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement.

Reactions

ApoB containing lipoproteins + α -cyclodextrin + Mg^{+2} + dextran SO_4 ---> soluble non-reactive complexes with apoB-containing lipoproteins

PEG-cholesteryl esterase

HDL-cholesteryl esters..... > HDL-unesterified cholesterol + fatty acid

PEG-cholesterol oxidase

Unesterifiedchol + O_2 .....>cholestenone + H_2O_2

H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine + H_2O + H^+ peroxidase >qunoneimine dye + H_2O

LDL-cholesterol

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL.

(Total cholesterol) = [(VLDL-cholesterol) + (LDL-chol) + [HDL-chol]

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

[LDL-chol] = [total chol] - [HDL-chol] – [TG]/5, where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dl.

Uric acid

It's measured to confirm diagnosis and monitor treatment of gout, to prevent uric acid nephropathy

during chemotherapeutic treatment, to assess inherited disorders of purine metabolism, to detect kidney dysfunction and to assist in the diagnosis of renal calculi

Test principle

Uric acid is oxidized by uricase to produce allantoin and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and 3.5-dichloro-2-hydroxybenzene sulfonate in a reaction catalyzed by peroxidase to produce a colored product. Absorbance at 520 nm is measured and the change in absorbance is directly proportional to the concentration of uric acid in the sample.

Reactions

Uric acid + O_2 + H_20 $\xrightarrow{\text{Uricase}}$ Allantoin + CO_2 + H_2O_2

H_2O_2 $\xrightarrow{\text{Peroxidase}}$ H_2O_2 + O_2

O_2 + 4-aminoantipyrine (4-AAP) + Phenol $\longrightarrow$ colored compound

Reference range

0.5–12 mg/dl

Preparation of the patient for lipid profile

The patient shall be advised to be on suitable diet for 2 – 3 weeks prior to testing

The patients will be requested to fast for at least 8 – 12 hours

Smoking and alcohol consumption shall be discouraged for such a period.

Some drugs like statin, corticosteroids, oral contraceptives that inhibit malonyl CoA making the results obtained to be for those from the diet interfering with the actual results. Therefore all these shall be discouraged.

General procedure for processing the samples

Fasting for uric acid is not required

A minimum of 0.6 ml serum will be required to run any test.

Blood samples will be collected in plain tubes for lipid profile and uric acid and purple top for glycated hemoglobin, labeled properly and accessioned using both hard and soft copy

Then the samples will be centrifuged to obtain serum apart from EDTA blood

0.6ml of the sample will be pipetted into the sample caps placed in the sample racks and then loaded in the machine that will process them and give results that will be reviewed by the site supervisor before they are printed out.