# CORRELATION BETWEEN APOLIPOPROTEIN B AND LIPID PROFILE AS MARKERS OF CARDIOVASCULAR RISK IN PATIENTS WITH TYPE 2 DIABETES AT KENYATTA NATIONAL HOSPITAL, NAIROBI.

A dissertation submitted to the University of Nairobi in part fulfillment of the degree of Master of Medicine in Human Pathology

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## Student's declaration

I, Dr. Wangari Wambugu declare that this is my original work, and that as far as I am aware, it has not been presented to any other university or forum

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AMORIS | Apolipoprotein-related Mortality Risk Study |
| Apo B | Apolipoprotein B |
| ATP III | Adult Treatment Panel III |
| CAD | Coronary Artery Disease |
| CETP | Cholesteryl Ester Transfer Protein |
| CHD | Coronary Heart Disease |
| CVA | Cerebrovascular Accident |
| DM | Diabetes Mellitus |
| GAD 65 | Glutamic Acid Decarboxylase |
| HDL-C | High Density Lipoprotein cholesterol |
| HUQAS | Human Quality Assurance Scheme |
| IDF | International Diabetes Federation |
| IDL | Intermediate Density Lipoprotein |
| IFCC | International Federation of Clinical Chemistry |
| IL-6 | Interleukin-6 |
| KNH | Kenyatta National Hospital |
| LDL-C | Low Density Lipoprotein cholesterol |
| Lpa | Lipoprotein a |
| MI | Myocardial Infarction |
| NCEP | National Cholesterol Education Program |

| | |
|---|---|
| NEFA | Non-Esterified Fatty Acids |
| OGTT | Oral Glucose Tolerance Test |
| OHA | Oral Hypoglycaemics |
| PI | Principal Investigator |
| PVD | Peripheral Vascular Disease |
| TC | Total Cholesterol |
| TG | Triglycerides |
| TNF-α | Tumour Necrosis factor alpha |
| UKPDS | United Kingdom Prospective Diabetes Study |
| UoN | University of Nairobi |
| VLDL | Very Low Density lipoprotein |
| WHO | World Health Organisation |
| Yr | Year |

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## ABSTRACT

**Background:** Diabetes Mellitus (DM) is associated with significant mortality and morbidity which are far worse in the developing countries than elsewhere. Vascular diseases in DM account for the majority of deaths in diabetics. Dyslipidaemia is a major potential modifiable risk factor for the macrovascular complications in diabetics. In DM, abnormalities in lipid and lipoprotein metabolism occur mainly due to insulin resistance and/or insulin deficiency. Diabetic dyslipidaemia consists of a characteristic pattern characterized by high plasma triglycerides, low high density lipoprotein cholesterol (HDL-C) and increased concentrations of small dense low density lipoprotein cholesterol (LDL-C) particles which are all atherogenic. A routine lipid profile does not accurately assess the presence of small dense low density cholesterol particles. Apolipoprotein B (apo B) is the principal lipoprotein moiety of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), lipoprotein a (Lpa) and both large buoyant and small dense LDL and therefore more accurately assess the cardiovascular disease risk. This study aimed at assessing the correlation between apo B and the routine lipid profile as markers of cardiovascular risk in type 2 diabetic patients at outpatient diabetic clinic of Kenyatta National Hospital.

**Study objectives**: The main objective of the study was to assess the correlation between apolipoprotein B and lipid profile as markers of cardiovascular disease risk in type 2 diabetic patients attending the outpatient diabetic clinic of Kenyatta National Hospital.

**Methods and materials**: The study was a descriptive cross-sectional study carried out in the diabetic clinic of Kenyatta National Hospital in Nairobi, Kenya. The study population consisted of type 2 diabetic patients. Ninety six type 2 diabetic patients not on lipid-lowering drugs were examined and the following parameters were taken: blood pressure, weight and height. Body mass index and waist to hip ratio were calculated. Metabolic syndrome was defined by the presence of three of the following parameters: diabetes, hypertension, dyslipidaemia and central obesity. Blood samples were then drawn and analyzed for total cholesterol, LDL-C, HDL-C and triglycerides and apolipoprotein B using Humastar 600® Biochemical analyser. The final concentration of the analytes was determined by measurement of the absorbance of the final product after an enzymatic reaction.

**Data management and analysis**: Demographic, medical history, physical examination and laboratory analysis data was collected using a structured questionnaire. The data was coded, entered in SPSS work sheet cleaned for confounders and analysed using SPSS version 18.0. The 5% level of significance (95% confidence interval) with p-values of <0.05 was considered statistically significant

**Results**: Of the 96 patients were studied, 60.7% were females. The age range was 33-88yr with mean of 59.5yr and a median of 60yr. Eighty percent(80%) of the patients studied had low HDL-C (<1.35mmol/L), 74.5% had high total cholesterol (> 4.38mmol/L), 71.3% had high triglycerides (> 1.05mmol/L), 69.1% had high LDL-C (> 2.49mmol/L), 78.7% had high non-HDL-C (>3.03mmol/L) and 61.7% had high apo B (>0.65g/L). The most frequent lipid disorder was low HDL-C with the least frequent being high apo B. There was a strong positive correlation between apo B and non-HDL-C (p value < 0.001). In addition, 20.7% of patients with normal total cholesterol had high apo B and 22.4% of patients with normal triglycerides had high apo B. Further, 27.6% of patients with normal LDL-C had high apo B. The mean body mass index ($kg/m^2$) of females was 29.7 and 27.7 for males. The mean waist to hip ratio for males was 0.99 and that of females 0.91. Seventy four percent (74%) of the patients studied had metabolic syndrome.

**Conclusion and Recommendations:** A significant proportion of the patients studied had dyslipidaemia cutting across all the parameters. Apo B has helped identify additional dyslipidaemia phenotypes in patients with normal total cholesterol and normal LDL-C. Non-HDL-C should routinely be calculated for type 2 diabetic patients to aid in cardiovascular risk assessment since it measures total atherogenic potential that may be missed by LDL-C.

## INTRODUCTION

Diabetes Mellitus (DM) is associated with significant morbidity and mortality despite improving standards of health care. This is worse in the developing countries where resources are scarce(1). Macrovascular complications that include coronary heart disease (CHD), cerebrovascular accidents (CVA), and peripheral vascular disease (PVD) which are different facets of the same vascular disease account for more than 70% of all deaths in diabetic patients(2). The incidence of coronary heart disease is about 2-4 fold greater in diabetics than non-diabetics, occurs at a younger age and has a much greater case fatality. In fact, diabetic patients with no history of vascular disease have the same risk of having a myocardial infarction or dying from a vascular disease as non-diabetic patients with prior history of coronary heart disease(3,4).

Potential modifiable risk factors for the macrovascular complications in type 2 diabetic patients include persistent hyperglycaemia, hypertension, dyslipidaemia and smoking(4). Therefore, for successful management of type 2 DM, there needs to be an aggressive integrated approach that aims to control blood glucose while also targeting the associated factors.

In Diabetes Mellitus, there are abnormalities in lipid and lipoprotein metabolism but dyslipidaemia may also herald future diabetes. A spectrum of diabetic dyslipidaemia can include all phenotypes identified in the general population however; one phenotype is particularly common in DM and is attributed mainly to insulin resistance and insulin deficiency(5). It has a characteristic pattern consisting of high plasma triglycerides (TG), low HDL cholesterol (HDL-C), and increased concentration of small dense LDL cholesterol (LDL-C) particles that may be more susceptible to oxidation(5,6). Chronic hyperglycaemia promotes glycation of LDL-C and this together with the small dense lipoprotein particles increase the atherogenicity of LDL-C(6).

Typically, in the assessment of cardio-vascular risk in diabetic dyslipidaemia a fasting lipid profile is done. This consists of total cholesterol (TC), triglycerides, HDL-C and LDL-C. Some facilities also include TC/HDL-C ratio. Ideally this should be done at the time of diagnosis of diabetes mellitus and then as clinically indicated.

However, Apolipoprotein B (apo B) has been shown to be a better risk marker than LDL-C for cardio-vascular disease in patients with high cardiometabolic risk such as diabetes mellitus and

metabolic syndrome(7). There is one apo B for each molecule of LDL, VLDL and IDL which are all atherogenic. Therefore, total apo B levels correspond to the total number of atherogenic particles.

Patients at high risk of cardiovascular disease such as in diabetes mellitus and metabolic syndrome require accurate risk assessment and focused treatment. Measurement of apo B as part of routine lipid profile could play a pivotal role in optimal cardiovascular disease risk reduction.

# LITERATURE REVIEW

## Diabetes Mellitus

Diabetes Mellitus (DM) was defined by WHO (1999) as a group of metabolic disorders of multiple aetiology characterized by chronic hyperglycaemia with disturbances in carbohydrate, fat and protein metabolism(8). This results from defects in insulin secretion, insulin action or both.

DM is now becoming a worldwide pandemic and its effects are particularly severe in low and middle income countries. Global estimates of the prevalence of Diabetes for 2010 and 2030 produced for International Diabetes Federation (IDF) estimated the worldwide prevalence of diabetes among adults (aged 20-79yr) for 2010 to be 285 million among adults, 6.4% of the population. This will increase to 7.7%, 439 million people by 2030 if no interventions are put in place. Between 2010 and 2030 there will be an increase of 69% in number of adult patients with diabetes in developing countries and 20% increase in the developed countries. This indicates a growing burden of diabetes particularly in the developing countries(9).

Sub-Saharan Africa has not been spared either where diabetes was once considered a rare disease. Its prevalence is rising rapidly due to rapid urbanisation, ageing population, obesity and sedentary lifestyles(1,10). In 2010, 12.1 million adults were estimated to have diabetes though only about 15% had been diagnosed. This means that more tend to present late when they have already developed complications. By 2030, 23.9million adults in sub-Saharan Africa will have diabetes. Just like the rest of the world, type 2 diabetes accounts for more than 90% of diabetes even in Sub-Saharan Africa(11).

The Kenya Diabetes National Strategy (2010-1015) estimates that about 1.2 million (3.3% of the population) people have diabetes(12). This may be an underestimation since an estimated two thirds of diabetics have not been diagnosed. If this trend continues this is expected to rise to 1.5million (4.5%) by 2025 (12,13).

Several pathogenetic mechanisms are involved in the development of diabetes. They range from autoimmune destruction of the β-pancreatic cells with consequent insulin deficiency to

abnormalities that result in resistance to insulin action. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue response to insulin action.

Diabetes Mellitus is classified into aetiopathological categories- type 1, type 2, gestational and other specific types of diabetes(8). In type 1 DM there is β- cell destruction in the pancreas leading to absolute insulin deficiency. In most cases, this is due to an autoimmune process where there are autoantibodies to either islet cells, insulin, GAD65 or to tyrosine phosphatases 1A-2 and 1A-2β. In others, cases the cause is idiopathic. Type 2 DM which account for 90-95% cases of all cases of diabetes is characterized by resistance to insulin action and an inadequate compensatory insulin secretory response. Specific aetiologies are not known but are usually associated with obesity or visceral fat which causes insulin resistance. In this category hyperglycaemia may persist for long without causing clinical symptoms but causing pathological and functional damage in various target organs(8).

Patients with diabetes mellitus present with characteristic symptoms of polydipsia, polyuria, weight loss and sometimes polyphagia and blurred vision. They are also susceptible to certain infections. Acute life-threatening consequences of diabetes include hypoglycaemia, hyperglycaemia with ketoacidosis and non-ketotic hyperosmolar coma.

Chronic hyperglycaemia is associated with long-term complications of diabetes that are classified into either microvascular or macrovascular complications. Microvascular include diabetic nephropathy, neuropathy and retinopathy. Macrovascular complications include coronary artery disease, peripheral artery disease and cerebrovascular accidents. It's important to understand the relationship between diabetes and vascular disease to aid primary and secondary prevention of these complications.

Diagnosis of DM is based on a criteria revised in 2006 by WHO/IDF expert committee on the Diagnosis and Classification of Diabetes Mellitus (14). Use of Glycated haemoglobin was endorsed by WHO in 2011(15,16). Criteria for the diagnosis of diabetes:

1. Glycated haemoglobin ≥6.5%. OR

2. Fasting blood sugar ≥7.0mmol/l (126 mg/dl). OR

3. Two hour (2-hr) plasma glucose ≥11.1mmol/l (200 mg/dl) during an OGTT (75g loading glucose). OR

4. In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ≥11.1mmol/l (200 mg/dl).

## Diabetes and Macrovascular complications

The long-term complications of diabetes- coronary artery disease (CAD), peripheral artery disease (PAD) and cerebrovascular accident (CVA) are all different facets of the same vascular disease and account for more than 70% of all deaths in diabetics(2). The central pathogenetic mechanism is atherosclerosis which leads to widespread narrowing of arterial walls throughout the body(17).

Atherosclerosis is thought to result from chronic inflammation and endothelial injury of the arterial walls. In response to endothelial injury and inflammation, oxidized lipids from LDL particles accumulate in the endothelial wall of arteries. Monocytes then infiltrate the arterial wall and differentiate into macrophages which accumulate oxidized lipids to form foam cells. Once foam cells are formed they stimulate macrophage proliferation and attraction of T-lymphocytes. T-lymphocytes induce smooth muscle proliferation in the arterial walls and collagen accumulation. The net result is formation of lipid-rich atherosclerotic lesion with a fibrous cap. Progressive increase of this lesion leads to narrowing of the arteries or it may be embolized to occlude a vessel at another site.

In addition, in type 2 diabetes there is increased coagulability and impaired fibrinolysis which further increases the risk of vascular occlusion and cardiovascular events.

United Kingdom Prospective Diabetes Study (UKPDS) identified major modifiable cardiovascular risk factors in type 2 diabetics as hyperglycaemia, hypertension, increased LDL-C, decreased HDL-C and smoking(4). Therefore, for proper management of diabetes an aggressive management targeting all the risk factors is required.

Among the long-term complications of diabetes, CAD is most commonly associated with diabetes. Studies have shown that the risk of myocardial infarction (MI) in diabetics is equivalent

to the risk in non-diabetics with previous history of MI(3). Diabetes has also been shown to be a strong independent risk factor for development of ischemic heart disease, stroke and death(18).

## Diabetic Dyslipidaemia

Diabetic patients tend to have abnormalities in lipoprotein and lipid metabolism. They have a characteristic pattern termed diabetic dyslipidaemia which consists of increased triglycerides mainly triglyceride-rich VLDL particularly post-prandially (post-prandial lipaemia), low HDL-C and increased concentrations of small dense LDL-C(19,20). The main defect resulting in the above characteristic triad is hepatic overproduction of VLDL particles, particularly $VLDL_1$(21). This pattern is frequently seen in type 2 DM and not in type 1 diabetes and is a treatable risk factor of cardiovascular disease.

In type 2 diabetes, the obesity/insulin resistant state is the basis of the abnormalities in lipid and lipoprotein metabolism which have been shown to occur independently of hyperglycaemia. In addition, this characteristic phenotype of diabetic dyslipidaemia is also found predominantly in patients with insulin resistance but normal plasma glucose levels(22).

The dyslipidaemia is easily amenable to life-style modification, lipid lowering drugs and in low HDL-C levels niacin is beneficial(20).

Lipid abnormalities are quite a frequent finding among diabetic patients. In a study done of lipid profiles in ambulatory patients with type 2 diabetes at KNH; 43.8% of females and 57.6% of males had LDL-C of more than 2.6mmol/l which is above optimal among the diabetics(23).

### Lipid abnormalities in type 2 diabetes mellitus

1. Hypertriglyceridaemia

   In type 2 diabetes, reduced action of insulin on adipocytes results in decreased lipolysis, decreased suppression of stored triglycerides and so greater release of non-esterified fatty acids (NEFA). The resulting increased delivery of NEFA to liver increases hepatic production of triglycerides which serve to drive VLDL production (6,19,20). VLDL is the major triglyceride-carrying lipoprotein and production of triglyceride-rich particle (termed VLDL 1) is suppressed by insulin.

   In the post-prandial state, high circulating insulin concentrations usually suppress VLDL production but when there's reduced action of insulin at hepatocyte level there is failure to

suppress VLDL production and therefore post-prandial lipaemia. Hence, in insulin resistant states VLDL is increased by both greater NEFA delivery to the liver and by reduced insulin mediated suppression. In addition, in both fasting and post-prandial states, there's reduced action of insulin on lipoprotein lipase which results in reduced clearance of triglyceride-rich lipoproteins, VLDL and chylomicrons. Insulin usually stimulates lipoprotein lipase and so stimulates hydrolysis of lipoprotein associated triglyceride(20). In the insulin-resistant state there is reduced triglyceride hydrolysis and prolonged clearance from the circulation of triglyceride-rich lipoproteins, VLDL and chylomicrons contributing to both fasting and post-prandial lipaemia.

2. Low HDL-C concentrations

   There are several mechanisms that have been put forth to explain low HDL-C levels in type 2 diabetes. When there are increased concentrations of plasma VLDL in hypertriglyceridaemia, this drives VLDL-transported triglyceride to be exchanged for HDL-transported cholesteryl ester mediated by cholesteryl ester transfer protein (CETP). HDL-triglyceride is rapidly hydrolysed by hepatic lipase or lipoprotein lipase to small HDL particles that are rapidly cleared from the plasma(20).

   In patients who don't have increased VLDL/hypertriglyceridaemia, there may be inability to upregulate apolipoprotein A-1 production (main HDL-C lipoprotein moiety) owing to insulin resistance.

   In addition, alteration of other enzymes involved in HDL metabolism may be altered in insulin resistance.

3. Increased small dense LDL-C

   Increased levels of VLDL-transported triglyceride enable CETP to promote further transfer of triglyceride to LDL in exchange for LDL-transported cholesteryl esters. The triglyceride-rich LDL undergoes hydrolysis by hepatic or lipoprotein lipase which results in lipid-depleted small dense LDL particles. Large buoyant LDL particles are cleared rapidly by LDL receptors while small dense particles do not bind readily to LDL receptor and thus persist longer in the circulation. They are also readily modified by oxidation and particularly in type 2 diabetes by glycation and become more atherogenic(6,20).

### Lipid markers of cardiovascular disease risk in type 2 diabetes

Typically, assessment of cardiovascular risk in both diabetic and non-diabetic patients requires a fasting lipid profile that consists of total cholesterol (TC), HDL-C, LDL-C and triglycerides. TC/HDL-C may also be done. If a non-fasting blood sample is used then only TC and HDL-C will be useful(24).

The third report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) identified the risk categories for the cholesterol levels. It identifies the LDL-C as the main target of lipid-lowering drugs. Diabetes is categorized as high risk and is equivalent to coronary heart disease. The goal for the LDL therapy should be less than 2.59mmol/L(24).

Triglycerides below 1.5mmol/L have been shown to be optimal and not associated with lipid abnormalities present in hypertriglyceridaemia(25).

## Apolipoprotein B

### Structure and function

Lipoproteins are made up of an insoluble lipid core surrounded by a coat of phospholipids, free cholesterol and apolipoproteins. Each class of lipid is associated with a distinctive apolipoprotein as shown in the table below(26). Apolipoproteins have collectively three major functions: (1) modulating activity of enzymes that act on the lipoproteins, (2) maintaining the structural integrity of the lipoprotein complex and (3) facilitating the uptake of lipoproteins by acting as ligands for specific cell-surface receptors(27).

Table 1: Major Apolipoprotein components in human plasma lipoproteins (26)

| Lipoprotein | Major Apolipoprotein component |
|---|---|
| Chylomicron | B-48, C, E |
| VLDL | B-100, C, E |
| IDL | B-100, E |
| LDL | B-100 |
| HDL | A-I, A-II, C |

Apolipoprotein B (apo B) is a large amphipathic glycoprotein with two isoforms: Apo B-48 and apo B-100(26,27). Apo B-48 is synthesized in the small intestine and is a structural component of chylomicrons and is responsible for their formation and secretion. Apo B-100 is synthesized in the liver and is present in VLDL, IDL, lipoprotein a (Lpa) and in both large buoyant LDL and small dense LDL particles. There is only one molecule of apo B-100 for each lipoprotein molecule and therefore it reflects the total number of potentially atherogenic particles(25,26,28).

Apo B-100 is the dominating Apo B in the plasma compared to apo B-48 even in the post-prandial state which comprises less than one percent. Thus usually apo B measurement usually refers to apo B-100 levels(29).

More than 90% of all apo B in blood is found in LDL. Therefore, in cases where LDL-C is normal or in the lower range, high apo B may indicate an increased number of small dense LDL particles that are more atherogenic.

**Rationale for using Apolipoprotein B for cardiovascular disease risk assessment**

Cardiovascular disease risk is more directly related to the number and size of circulating atherogenic particles in any lipoprotein fraction(29). This determines the likelihood of each member of their class entering and lodging in an arterial wall. Conventional lipid indices equate the risk due to a lipoprotein fraction to the plasma lipid concentration of that fraction. Thus LDL-C estimates the risk due to LDL. However, lipid concentration of the principal atherogenic particle differs substantially in different individuals and lipid levels do not automatically equate to lipoprotein particles(29). In contrast, each molecule of VLDL, IDL, LDL and Lpa contains one molecule of apo B. Therefore, total apo B levels represent the total number of atherogenic particles.

In high cardiometabolic states such as diabetes and metabolic syndrome, LDL-C is usually present as small dense particles and therefore conventional estimate of LDL-C may not give a correct estimate of total atherogenic LDL-C. Thus, in such cases apo B which is also present in small dense LDL-C better assesses the risk(30,31).

In addition, traditionally LDL-C was obtained by calculation from other parameters thus introducing a multitude of errors. Currently, direct assays have become available but standardization with a common reference standard has been difficult(25,26). In contrast, apo B

measurements have been standardized by IFCC/WHO with a common reference standard being available thus improving inter-laboratory reproducibility(27,32).

In addition, apo B measurements do not require fasting blood samples. The assay is readily automated though may not be widely available.

**Value of Apolipoprotein B as a cardiovascular risk marker**

Several studies have demonstrated apo B to be a better risk marker than LDL-C. The AMORIS study which was a large prospective study carried out among the Swedish population demonstrated that apo B and apo B /apo A-I were strongly and positively associated with increased risk of fatal myocardial infarction. They were even more useful in individuals who had normal or low LDL-C cholesterol(33).

A recent study done in the Korean population demonstrated that apo B is a strong independent risk marker of cardiovascular disease as assessed using Framingham Risk Score(34).

In a study done in Spain among type 2 diabetic patients, hyperapo B was found in more than half of normocholesterolemic type 2 diabetic patients and was frequently associated with low HDL-C and hypertriglyceridaemia(35). The researchers recommended that apo B should be routinely measured in type 2 diabetic patients.

In the INTERHEART study of 2008 that compared results of a large population across 52 countries, apo B was found to be a better risk predictor than total cholesterol, LDL-C and non-HDL-C(36). More so they found apo B/apo A-I to be a superior marker.

Recently, apo B has also been shown to be a more consistent goal for statin therapy in type 2 diabetic patients rather than LDL-C or non-HDL-C(34,35).

Most studies have shown that apo B measurements would play a critical role in cardiovascular risk assessment. However, it has not been adapted to routine clinical practice mainly because of difficult to change from conventional practices that one is used to. A Consensus Conference Report from the American Diabetes Association and the American College of Cardiology suggested that measurement of apo B be added to LDL-C and non-HDL-C in patients at high cardiometabolic risk. They suggested the target apo B levels at < 0.90g/L for high risk patients and < 0.80g/L for the highest risk patients(37). Highest risk patients are patients with known

cardiovascular risk or diabetes plus one of the major risk factors like hypertension, smoking or family history of premature coronary heart disease (Table 2 below).

Table 2: Suggested Treatment goals in patients with cardiometabolic risk and lipoprotein abnormalities.

| | LDL-C (mmol/L) | Non-HDL-C (mmol/L) | Apo B (g/L) |
|---|---|---|---|
| Highest risk patients including 1) known CVD 2) DM plus one of the major CVD risk | < 2.33 | <2.59 | <0.80 |
| High risk patients including 1) no DM or known clinical CVD but 2 or more additional major risks or 2) DM with no major additional risk. | <2.59 | <3.37 | <0.90 |

**Non-HDL-C**

Non-HDL-C is calculated as total cholesterol minus HDL-C and includes all lipoproteins that contain Apo B. When triglycerides are high most of the cholesterol is in the form of VLDL-C which is highly correlated with atherogenic remnants. Therefore, when non-HDL-C is combined with LDL-C cardiovascular risk prediction is enhanced (6,16). Non-HDL-C has been identified by NCEP ATP III as secondary target for statin therapy in patients with hypertriglyceridaemia (24).

## Use of population based reference values for lipids interpretation

Reference intervals vary from region to region. The ISO 15189:2003 standard recommends that biological reference intervals appropriate for the reference population and patient population be used when reporting laboratory results(38). NCEP ATP III categorization of risk was based on American population which is different from the African population which has been shown to have lower lipid values. A study done in Southern Africa, in Botswana, showed that total cholesterol thresholds for cardiovascular disease risk stratification was lower than that used in NCEP ATP III(39). A study done to establish reference ranges of fasting lipids for healthy adults in Nairobi, Kenya showed lower values of the lipid parameters(40).

In addition, in the INTERHEART study, reference values obtained from the control population in black African countries were lower compared to those obtained from other regions(36). This further indicates that lipid levels among Africans are lower and therefore reference values based on the local population should be used. Based on this local reference values were used.

## RATIONALE FOR THE STUDY

Type 2 DM is associated with cardiovascular disease which is partly due to abnormalities in lipid and lipoprotein metabolism. Diabetic dyslipidaemia comprises multiple lipoprotein disorders, typically high triglyceride concentrations (TG), low levels of HDL cholesterol (HDL-C) and normal or elevated LDL cholesterol (LDL-C) but with a predominance of small dense LDL particles.

LDL-C levels are the main therapeutic goal of both diabetic and non-diabetic dyslipidaemia but their concentrations does not represent the whole mass of lipoprotein particles that also include IDL-C and VLDL-C which have also been shown to be atherogenic. In diabetes small dense LDL particles are also not reflected in the routine LDL-C measurement.

Apolipoprotein B is the principal protein moiety of LDL (both large buoyant and small dense forms), IDL, VLDL and Lpa. Its concentration is thus a good estimate of total mass of atherogenic particles. Apo B has been associated with increased risk of cardio-vascular disease independently of LDL-C levels in type 2 diabetic patients.

This study therefore aimed at finding out the benefit of combining measurements of apo B with the routine parameters of lipid profiles as markers of cardiovascular risk in type 2 diabetic patients attending the outpatient diabetic clinic of KNH.

## RESEARCH QUESTION:

Is combination of apo B measurement with the routine parameters of lipid profile aid in providing more information for cardiovascular risk assessment than the routine parameters of lipid profile?

# STUDY OBJECTIVES

## Broad objective:

To assess the correlation between apolipoprotein B and routine parameters of lipid profile as markers of cardiovascular disease risk in type 2 diabetic patients attending the outpatient diabetic clinic of Kenyatta National Hospital.

## Specific objectives:

1. To measure apo B and the routine parameters of lipid profile (TC, LDL-C, TG, and HDL-C) in type 2 diabetic patients attending the outpatient diabetic clinic of Kenyatta National Hospital.

2. To correlate apo B levels with those of routine parameters of lipid profile (TC, LDL-C, TG, and HDL-C) in type 2 diabetic patients attending the outpatient diabetic clinic of Kenyatta National Hospital.

3. To calculate non-HDL-C and correlate with apolipoprotein B.

4. To determine the prevalence of metabolic syndrome among type 2 diabetic patients attending outpatient diabetic clinic of Kenyatta National Hospital.

# MATERIALS AND METHODS

## Study design

This was a descriptive comparative cross-sectional study carried out between November 2012 and February 2013.

## Study area

The study was carried out at the outpatient diabetic clinic of Kenyatta National Hospital. The main diabetic clinic takes place on Fridays with an average attendance of 50 patients. In addition, there are mini clinics every day of the week with attendance of about 30 patients each day. In each of these days only about 5-10 patients who present are not taking lipid lowering drugs.

## Study population

This consisted of type 2 diabetic patients who were 18 years and above. Type 2 diabetic patients were identified as documented in the patients' files.

### Inclusion criteria

1. Type 2 diabetic patients as identified through the clinic files
2. 18 years and above
3. Those who were not on lipid lowering drugs
4. Those who provided informed consent

### Exclusion criteria

1. Type 1 diabetic patients
2. Those who declined to give consent
3. Those with confounders like patients documented to have metabolic disorders associated with hyperlipidaemia.

## Sample size

Fisher's formula was used to calculate the sample size (41)

Sample size was determined as follows:

$$n = \frac{z^2 pq}{d^2}$$

Where: n = desired sample size

z = standard normal deviate - 1.96

p = proportion of the characteristic of interest – estimated prevalence of abnormal LDL-C among diabetic patients; an average of 50.7% from a previous study done at Kenyatta National Hospital is used to assess the lipid profile in type 2 diabetic patient.

q = (1 – p) =49.3%

d = the degree of accuracy was set at 0.1 due to limited resources available to the researcher.

Therefore the minimum estimated sample size is

$$= \frac{1.96^2 \times 0.507 \times 0.493}{0.1^2} = 96$$

Therefore, the sample size required was 96 type 2 diabetic patients.

## Sampling technique

All diabetic patients who met the inclusion criteria were consequently enrolled into the study daily until the desired number was achieved.

## Definition of terms

Hypertension: systolic blood pressure ≥ 140mmhg; diastolic blood pressure ≥ 90mmhg

Body mass index (Kg/M²) : <18.5 (underweight), 18.5-25.9 (normal), 26.9-29.9 (overweight) and ≥ 30.0 (obese).

Waist-to-hip ratio- Cut-off for high (central obesity): Male ≥ 0.90 and female ≥ 0.85.

Non-HDL-C was calculated as HDL-C subtracted from total cholesterol.

Dyslipidaemia: When any of the following parameters was found to be abnormal- low HDL-C <1.35mmol/L, non-HDL-C ≥ 3.03mmol/L, total cholesterol ≥4.38mmol/L, triglyceride ≥1.05mmol/L, LDL-C ≥2.49, Apo B ≥0.65g/L).

Metabolic syndrome: if any three of the following are present- diabetes, hypertension, dyslipidaemia and central obesity (42).

## Screening and Recruitment

The principal investigator with the help of the research assistant identified Type 2 diabetic patients not on lipid lowering drugs from the clinic files.

Recruitment of study participants who met the inclusion criteria was done consecutively until the desired sample size of 96 was reached. The recruited participants were subsequently moved to one of the available free rooms where one to one interviews was conducted by the principal investigator and the research assistant.

## Administration of consent form

The principal investigator explained the purpose of the study, the benefits and risks involved (Appendix I) and then informed consent was sought (Appendix II).

## Administration of the questionnaire

A screening questionnaire was used to identify eligible participants (Appendix III). Then the study questionnaire (Appendix IV) was administered by the PI and the research assistant to those who met the inclusion criteria. The patients continued with their scheduled review and results of the study were communicated to the attending physician.

## Physical examination

Physical examination details were taken from all the study participants. This included blood pressure, weight, height, waist and hip circumferences. Body mass index and waist to hip ratio were calculated.

## Laboratory Procedures

### Specimen Collection

Blood specimens from the participants were drawn after the interviews if the participant had fasted. Fasting was based on their last meal, which was at least 8 hours from the last meal. If the participant was not fasted, he/she was requested to come back while fasted and transport reimbursed. Mobile phone numbers were taken to remind the patients to return.

3 ml of blood was drawn aseptically from the participant's vein into a well labelled plain bottle during their visits at the diabetic clinics. Samples were then transported to the Paediatric Laboratory, University of Nairobi by the PI where a fully automated Humastar 600® biochemical analyser is located.

**Separation and Storage**

Blood in the labelled plain vacutainer was left to clot, then centrifuged at 3000rpm for two minutes and serum pipetted into well labelled cryovials. Half of the serum was analysed immediately by the principal investigator and laboratory technicians for total cholesterol, triglycerides, HDL-C and LDL-C. The other half was frozen at $-20^0$C in the laboratory awaiting analysis for apo B. The precipitant was discarded safely. The cryovials were inscribed with study numbers for identification.

**Specimen Analysis.**

Analysis of the specimens was done in the Paediatric Department laboratory using Humastar 600® automated biochemical analyser. Half of the specimen was analysed immediately for total cholesterol, triglycerides, HDL-C and LDL-C. The rest of the specimen was stored at $-20^0$C awaiting analysis for apo- B. Freeze and thaw was done once only, and the thawed specimen was homogenized. Apo B was analysed in three batches after all the specimens were collected over a period of three months.

**Interpretation of results**

The NCEP has defined 'desirable', borderline', and 'high levels' of blood lipids as less than $50^{th}$ percentile, between $50^{th}$-$75^{th}$ percentile and above $75^{th}$ percentiles respectively. For this study, to determine the desirable values, the cut off levels were based on the $50^{th}$ percentile values as obtained from a local study(24,43). The cut offs are given below with description of methodology of each assay.

The reference values were obtained from a local study done in 1996 among 110 healthy controls including both genders, aged 15-55years. The study participants were recruited from family planning (those attending for the first time not on contraceptives), infertility clinics (females with normal menstrual cycle and males with normozoospermia) in KNH. Other participants included health workers and students in KNH. They were investigated to rule out diabetes, liver disease

and thyroidal dysfunction. Cholesterol was analysed by CHOD-PAP method, triglycerides by GPO-PAP method, HDL-C by precipitation then CHOD-PAP method, LDL-C was calculated and apo B by immunoturbidimetry then nephelometry. These methods would allow for transferability of results to the current study(43).

**Methodology of the Assays**

(Appendix V)

**Total cholesterol**

The test was carried out using Enzymatic Colorimetric test for cholesterol with Lipid Clearing Factor according to CHOD-PAP method.

Method:

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine dye was formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and a peroxidase.

Interpretation of results:

Cut-off for total cholesterol was set as follows: low risk $\leq$ 4.38mmol/L

**Triglycerides**

The assay was carried out using enzymatic colorimetric test according to GPO-PAP method.

Method:

The triglycerides were determined after enzymatic hydrolysis with lipases. The colour indicator was quinoneimine dye formed from hydrogen peroxide, 4-amino-antipyrine and 4-chlorophenol under catalytic influence of peroxidase which was measured spectrophotometrically.

Interpretation of results:

Cut off for low risk triglycerides was set at < 1.07mmol/L

**LDL-C**

This is an enzymatic colorimetric test.

Method:

This assay combines two steps: in the first step chylomicrons, VLDL and HDL cholesterol were specifically removed by enzymatic reaction. In the second step the remaining LDL-C was determined by well established enzymatic reaction in the presence of specific surfactants for LDL.

Interpretation of results:

Cut-off for low risk LDL-C was set at < 2.49mmol/L

**HDL-C**

This was an enzymatic colorimetric test.

Method:

The assay combined two specific steps: In the first step VLDL and LDL cholesterol were specifically eliminated and destroyed by enzymatic reaction. In the second step remaining cholesterol from HDL fraction was determined by specific enzymatic reactions in the presence of HDL surfactants.

Interpretation of results:

< 1.35mmol/L high risk

≥ 1.35mmol/L low risk

**Apolipoprotein B**

This was an immunoturbidimetric test.

Method:

Apo B antigens in the sample or standard caused immunological agglutination with anti-apo B antibodies in the reagent. The extent of agglutination was proportional to the apo B concentration in the sample and was measured by turbidimetry.

Interpretation of results: Cut-off value for low risk apo B was set at ≤ 0.65g/L.

**Non-HDL-C**

This was derived subtracting HDL-C from total cholesterol. There was no added cost to perform this test.

*Non-HDL-C=Total cholesterol- HDL-C*

Interpretation of results: Cut-off for low risk non-HDL-C was set at < 3.03mmol/L

**Quality Assurance**

Measures were taken to ensure that the laboratory results were valid. They included the following:

Pre-analytical quality: The measures included

- Correct identification of the patient and correct labelling of the specimen bottles
- Elimination of interfering factors e.g. use of fasting samples
- Use of stable reagents was employed.

Analytical measures:

- The tests were carried out using well serviced and well calibrated equipment.
- Only well-labelled samples of adequate volume were analysed.
- The samples were only thawed once and analysis was done according to the manufacturer's specifications
- Commercial control materials (normal controls) i.e. Human and Serodos control materials were used (for lipid profile and apo B respectively). These control materials were included in each batch and results were only accepted if the internal quality control was within acceptable limits i.e. within two standard deviations. The assay performance characteristics were evaluated by coefficient of variations (CV) of the internal controls in

each assay and were found to be within acceptable limits. To compute CV for Apo B seven results were used. They were as follows: CVs of total cholesterol (3.5%), triglyceride (2.9%), HDL-C (11%), LDL-C (10.5%) and apo B (14.5%), recommended CVs are as follows ≤ 8.9%, ≤ 15%, ≤ 13%, ≤ 12% and ≤ 20% respectively.

Post analytical measures:

- Data interpretation was done based on the reference ranges derived from a local study
- Two people counter-checked the results to ensure that there was no transcription error.

Standard operating procedures (SOPs)

Standard operating procedures were followed from specimen collection, specimen analysis to issuance of results.

External quality assessment:

The Paediatric department Laboratory routinely participates in an external quality control program by Human Quality Assurance Scheme (HUQAS). The lipid panel consisting of triglycerides, total cholesterol, LDL-C and HDL-C are included in this external quality control program and the results have been within acceptable limits. However, apo B is not included because as it was introduced to the laboratory by the researcher.

## Data Management and Analysis

The collected data was coded and entered into Microsoft Access Database and SPSS version 18.0 was used to analyse the data. The data was cleaned for outliers to ensure high quality data. The study population was described using demographic characteristics, medical history, drug history and physical examination. Categorical variables such as gender and medical history data was summarised into proportions while continuous data such as age and physical examination data was presented as means/medians.

Laboratory results namely apo B, total cholesterol, LDL-C, TG and HDL-C was summarised into means/medians then categorised into normal and abnormal levels based on the $50^{th}$ percentile cut off values. Apo B was correlated with total cholesterol, LDL-C, TG and HDL-C using Pearson correlation coefficient (r). In addition, the categorised data of the parameters was tested for association using Chi-square test of association. Odds ratio was used to estimate the risk of

abnormal levels of apo B in type 2 diabetes. The 5% level of significance (95% confidence interval) with p-values of <0.05 being considered statistically significant. All the findings were presented in form of tables and graphs.

## Ethical Considerations

The study commenced upon approval by the Department of Human Pathology (UoN) and the KNH/UoN Scientific and Ethical Research Committee.

Pre-consent counselling involved:

i. Information and explanation on the research nature and overall goal
ii. Detailed explanation of the procedures involved, outlining their safety or lack of.
iii. Assurance that participation is voluntary and one can withdraw at any point without losing other benefits from KNH.
iv. Confidentiality and custody of patient information, specimen and results
v. Assurance on free access to their results and their medical interpretations.
vi. The benefits and unforeseen harm of participating in the study were explained in unambiguous language as contained in the Study explanations. (Appendix III).
vii. The results were communicated back to the physician to improve patient management.

## Study Limitations

The investigator collected 96 samples for evaluation of lipids and apo B. Two kits of apo B were purchased (each ~70 tests). However, during calibration more than the expected reagent was utilized because calibration had to be repeated four (4) times to ensure the right curve was obtained and every time calibration was done controls were assayed. For each calibration five dilutions were prepared which were run with controls. In addition, though the test kit indicates that 70 tests can be done, each kit could run only about 64 tests because there was a significant amount of dead volume that remained in the automated analyser used. Therefore, 94 out of 96 (in addition to three control samples) were assayed for apo B in three batches. The reagents for apo B had to be imported and due to financial and time constraint a whole kit could not be imported to analyse two samples.

## RESULTS

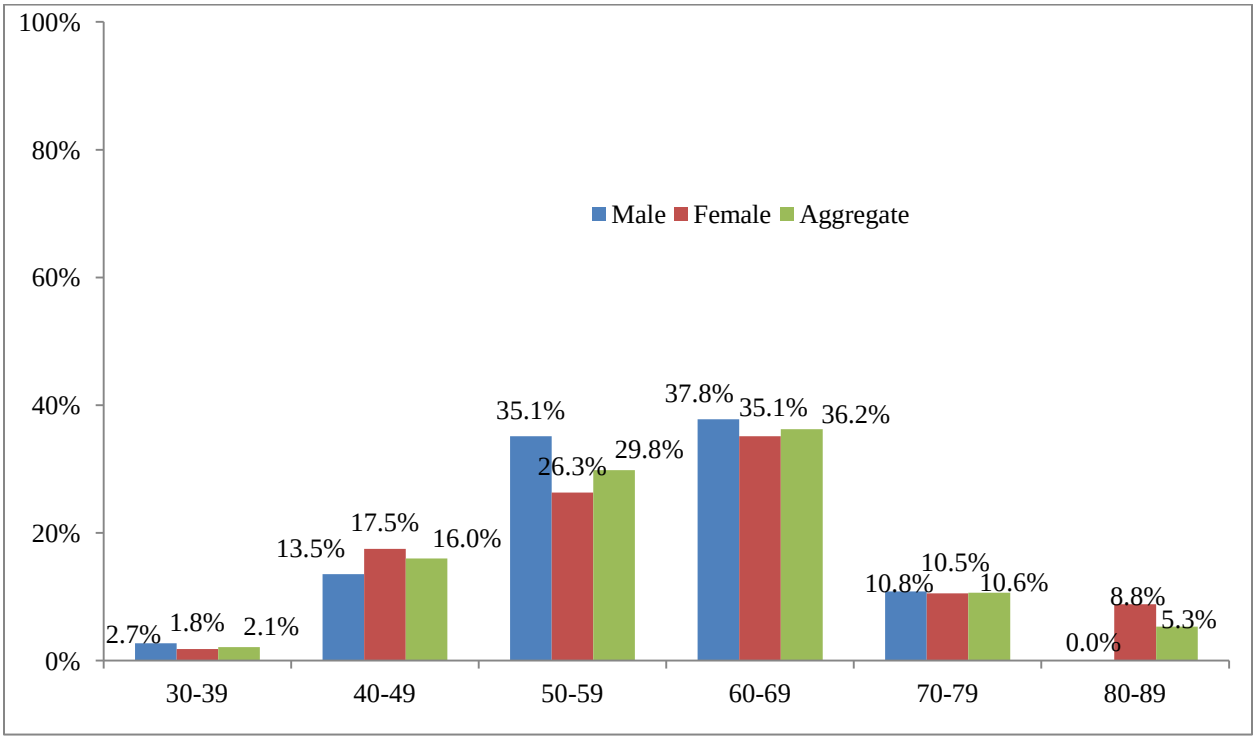
The main clinical and laboratory features are summarized and depicted in Table 1.

**Table 1: Main clinical and laboratory features.**

| Patients | Male<br>No (%) | Female<br>No (%) | Total<br>No (%) |
|---|---|---|---|
| **Physical examination** | | | |
| Number of study participants (percentage) | 38 (39.6) | 58 (60.4) | 96 (100) |
| Age(yr), mean(1SD) | 59 (9.7) | 59.9 (11.5) | 59.5 (10.8) |
| BMI (kg/m²), mean(1SD) | 27.7 (5.2) | 29.7 (6.4) | 28.8 (5.8) |
| Waist to hip ratio, mean(1SD) | 0.99 (0.09) | 0.91 (0.1) | 0.94 (0.1) |
| Systolic Bp(mmHg), mean(1SD) | 139 (21) | 139 (16) | 139 (18) |
| Diastolic Bp(mmHg), mean(1SD) | 79 (12) | 79.8 (11) | 79 (11) |
| **Laboratory results** | | | |
| Total cholesterol (mmol/L), mean(1SD) | 4.99 (1.30) | 5.68 (1.68) | 5.4 (1.6) |
| Triglycerides (mmol/L), mean(1SD) | 1.76 (1.06) | 1.9 (1.64) | 1.8 (1.4) |
| LDL-C (mmol/L), mean(1SD) | 3.06 (1.37) | 3.5 (1.42) | 3.3 (1.4) |
| HDL-C (mmol/L), mean(1SD) | 1.02 (0.42) | 1.12 (0.31) | 1.1 (0.4) |
| Apo B (g/L), mean(1SD) | 0.68 (0.22) | 0.71 (0.31) | 0.69 (0.27) |
| Non-HDL-C (mmol/L), mean (1SD) | 3.98(1.18) | 4.55 (1.48) | 4.32 (1.39) |

### Demographic characteristics of the study population

**Figure 1: Distribution of age in years of the study population (N=96)**



Majority (82%) of the patients were between 40 and 69 years. 58(60.4%) were females while 37(39.6%) were males. The mean age of both the females and males was 59years *(Figure 1 above).*

## Laboratory results

### Lipid profile of the study population

**Figure 2: Total cholesterol (mmol/L) distribution by gender (high ≥4.38mmol/L), N=96**

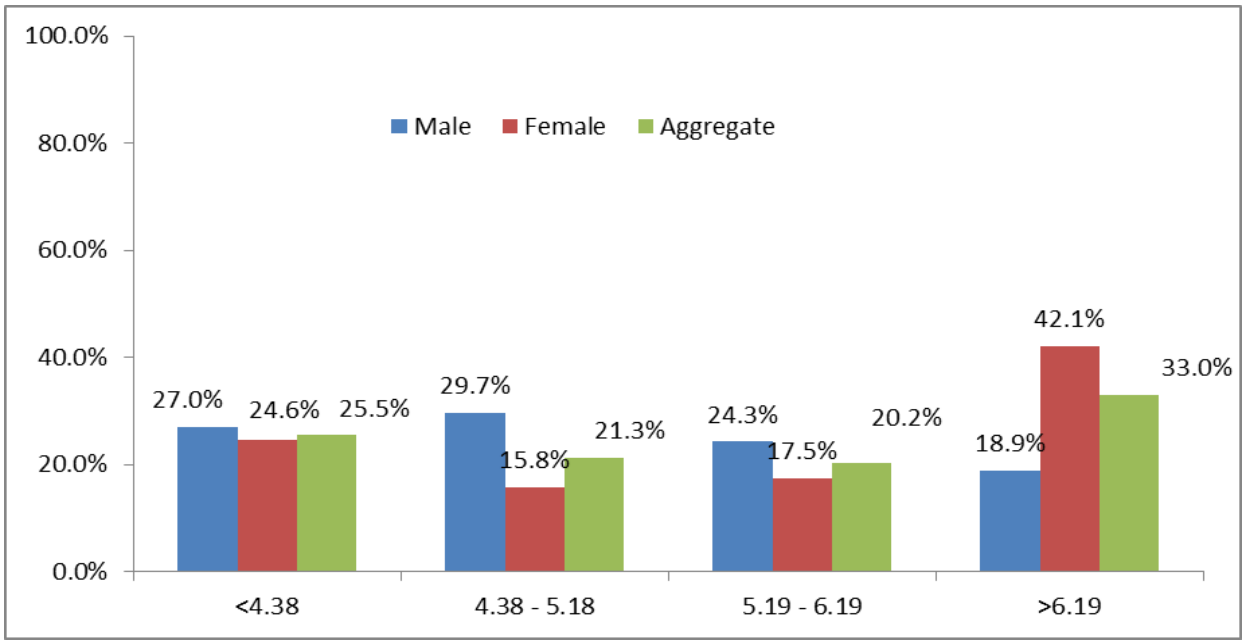



Figure 2 above shows that 74.5% of the patients had high cholesterol. The mean total cholesterol of females was higher than in males (5.68 mmol/L versus 4.99mmol/L); this was statistically significant with a p value of 0.04. Slightly more males than females had total cholesterol less than 4.38mmol/L (27.0% versus 24.6%) but this was not statistically significant.

**Figure 3: Triglycerides (mmol/L) distribution by gender (high ≥1.05mmol/L), N=96**

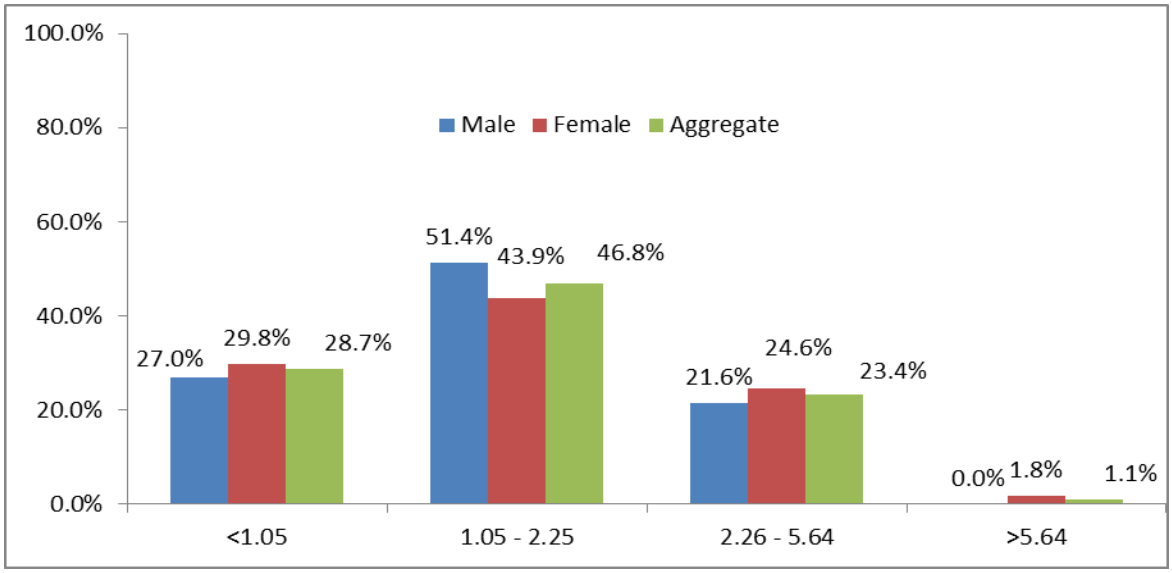
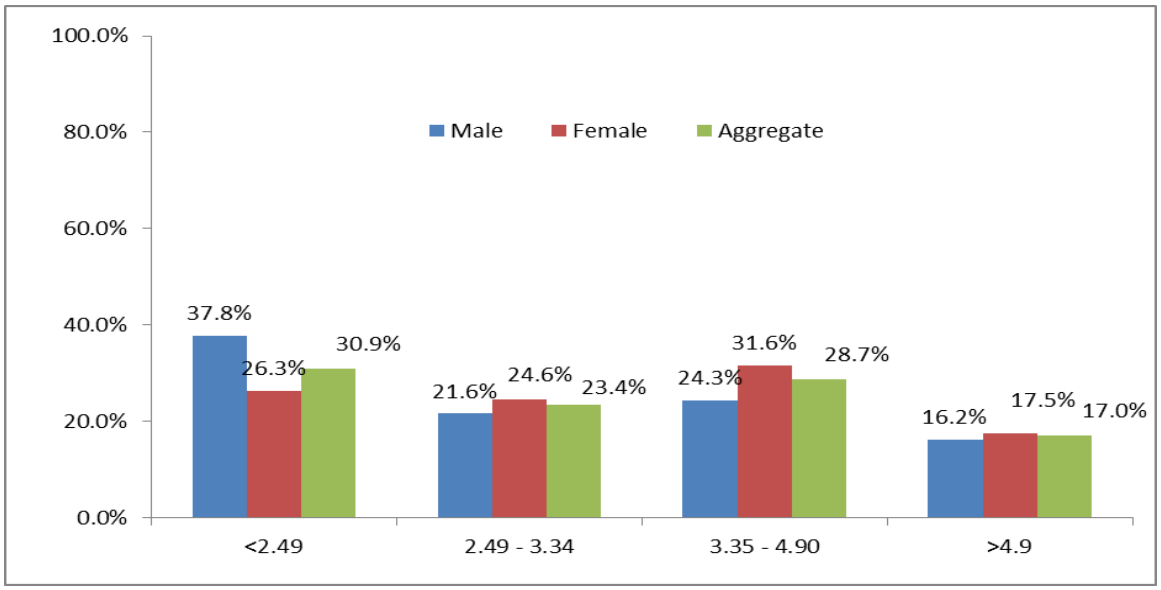


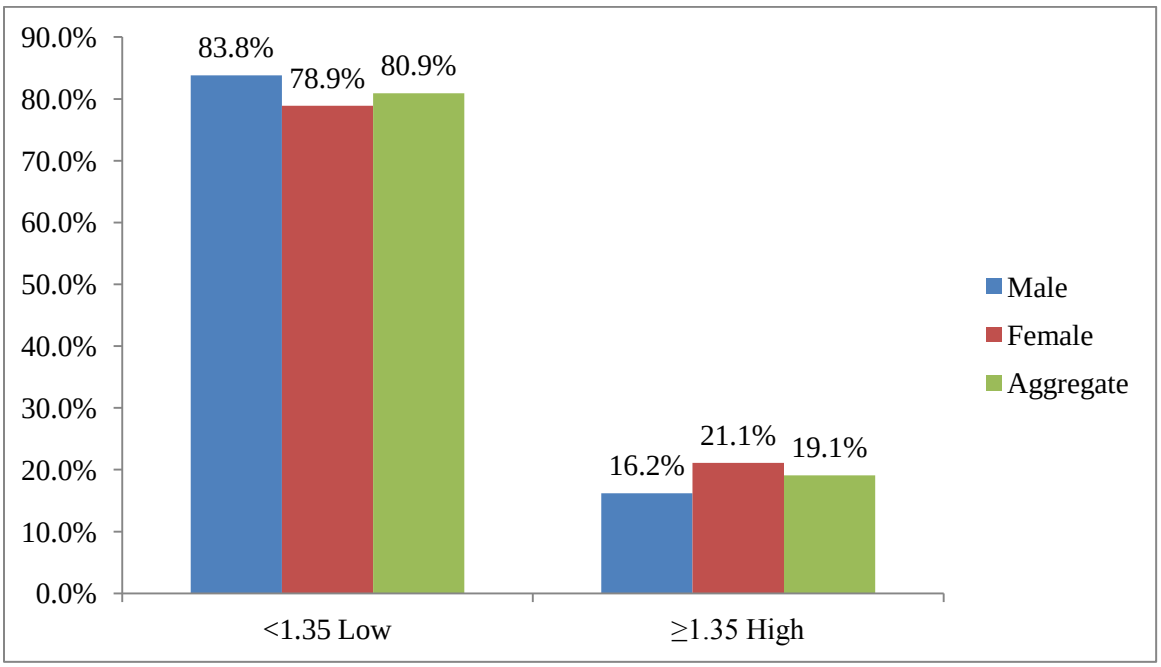

Figure 3 above shows that 71.3% of the patients had high triglyceride levels. Majority of the patients (i.e. 46.8%) with high triglycerides had levels between 1.05-2.25mmol/L. Only 28.7% of the patients had desirable levels of triglycerides. The mean triglycerides level for males was 1.76mmol/L with a range of 2.6-8.1mmol/L while that of females was higher at 1.9mmol/L with a range of 0.4 to 11.4mmol/L; the difference was not statistically significant.

**Figure 4: LDL-C (mmol/L) distribution by gender (high ≥2.49mmol/L), N=96**



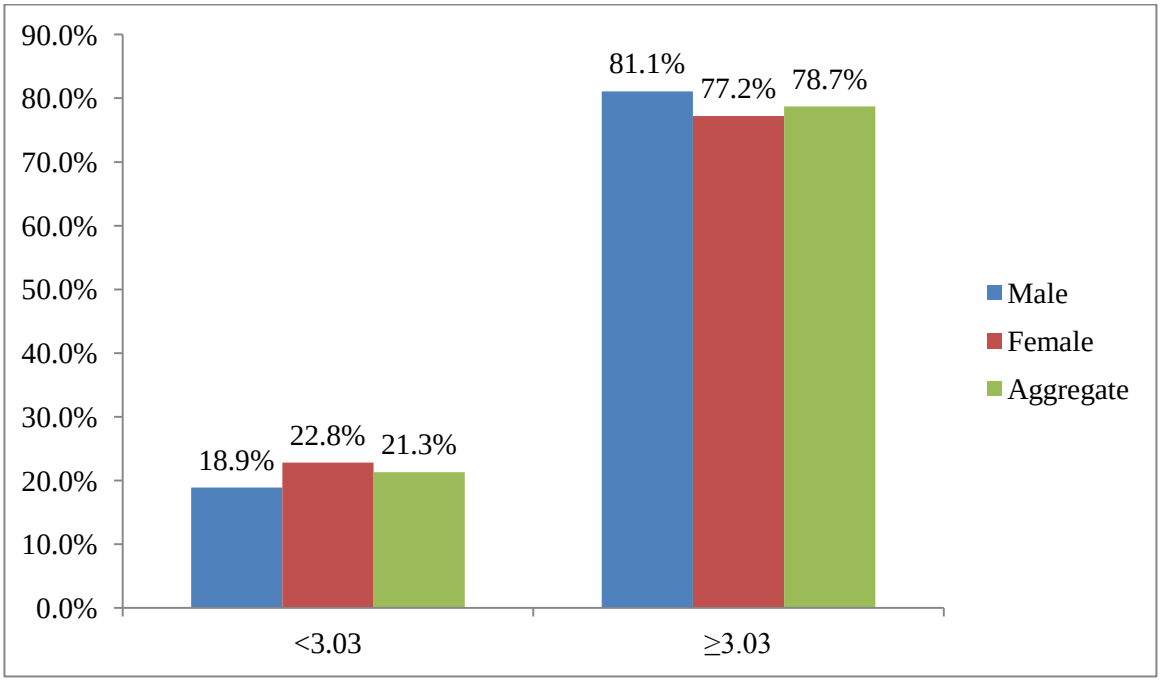
Majority of the patients had LDL-C more than 2.49mmol/L. There were more males than females with LDL-C <2.49mmol/L. The mean LDL-C for males was 3.06mmol/L with a range of 0.7-5.4mmol/L while the mean for females was higher at 3.5mmol/L but not statistically significant with a range of 1.2-7.0mmol/L *(Figure 4 above).*

**Figure 5: HDL-C (mmol/L) distribution by gender (N=96)**



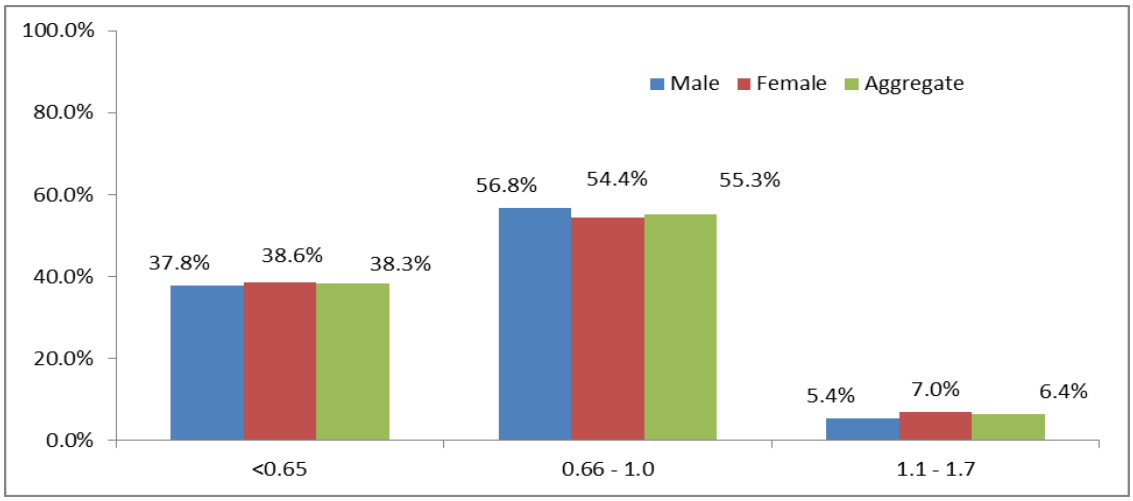

Most of the patients had low HDL-C levels with a higher percentage of males having slightly lower levels than females (83.8% versus 78.9%) but no statistical difference. The mean HDL-C for males was 1.02mmol/L with range of 0.33-2.71mmol/L while the mean among females was 1.12 with a range of 0.55-2.17mmol/L *(Figure 5 above).*

**Figure 6: Non-HDL-C (mmol/L) distribution by gender**




Majority of the patients had high non-HDL-C cholesterol with more males than females having higher levels (81.1% of males and 77% of females). The mean non-HDL-C for males was 3.98mmol/L with a range of 1.74-6.64mmol/L while for females was 4.55mmol/L with a range of 1.71-7.54mmol/L. The difference was not statistically significant *(Figure 6 above).*

**Figure 7: Apo B (g/L) distribution by gender (high > 0.65g/L), N=94.**

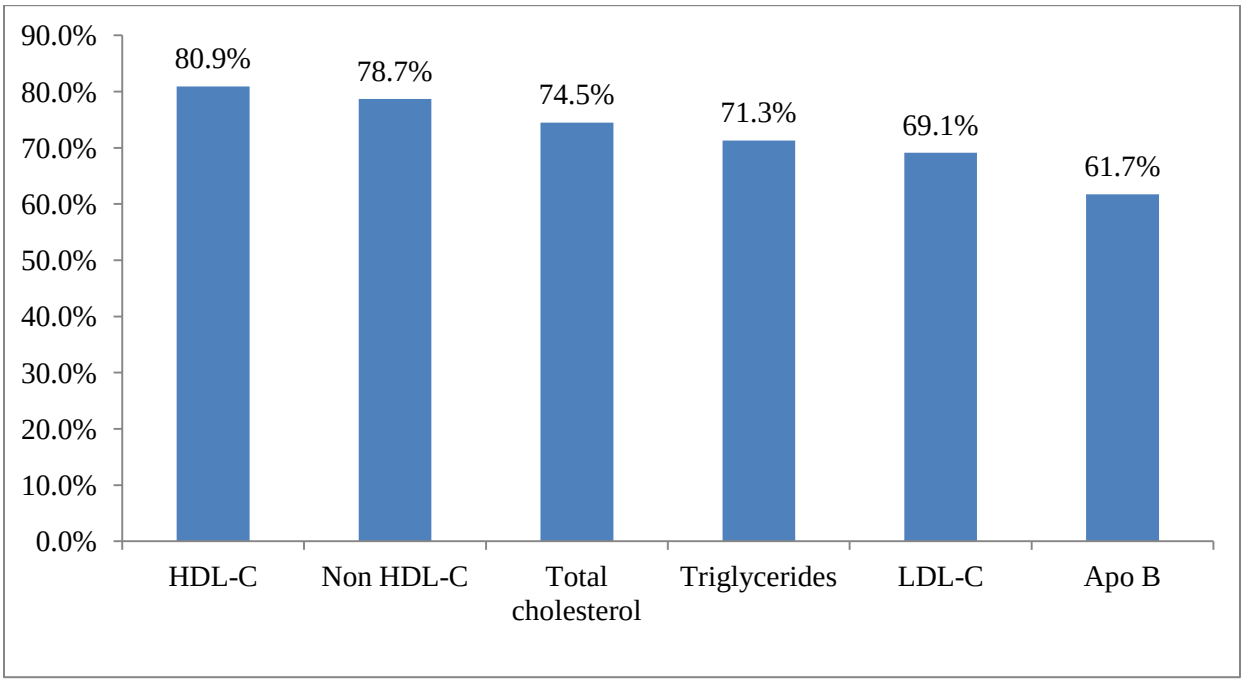



Majority of the patients had Apo B levels between 0.66-1.0g/L. slightly more females than males had levels of Apo B more than 1.1g/L. The mean Apo B levels for males was 0.68g/L with a

range of 0.21-1.36g/L while the mean for females was 0.71g/L with a range of 0.20-1.68g/L . The difference was not statistically significant *(figure 7 above).*

**Figure 8: Summary of frequency of dyslipidemias in the study population**

*(For HDL-C, non-HDL-C, total cholesterol, triglycerides and LDL-C, N=96; for Apo B, N=94).*




*(Cut-offs for low risk values HDL-C ≥1.35mmol/L, non-HDL-C <3.03mmol/L, total cholesterol <4.38mmol/L, triglyceride <1.05mmol/L, LDL-C<2.49, Apo B <0.65g/L).*

Figure 8 above summarises the frequency of dyslipidemias. The most frequent dyslipidaemia was low HDL-C (80.9%) with the least frequent being high Apo B (61.7%).

**Figure 9: Correlation of apo B with Total Cholesterol in the study population**

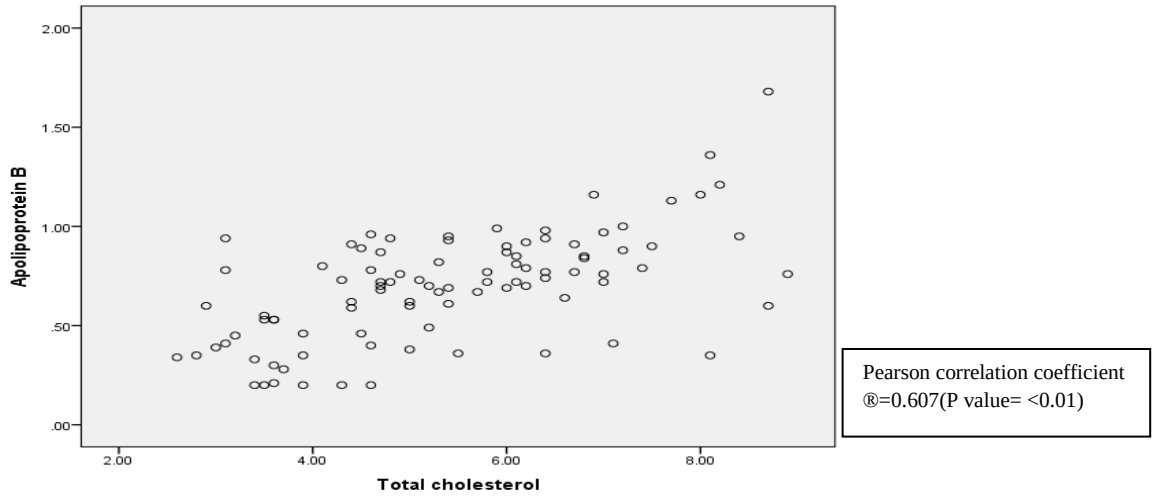



Figure 9 above shows that there is positive correlation between apo B and total cholesterol (P value of <0.01). Most of the patients with high total cholesterol were also likely to have high apo B levels.

**Figure 10: Correlation between apo B and LDL-C in the study population**

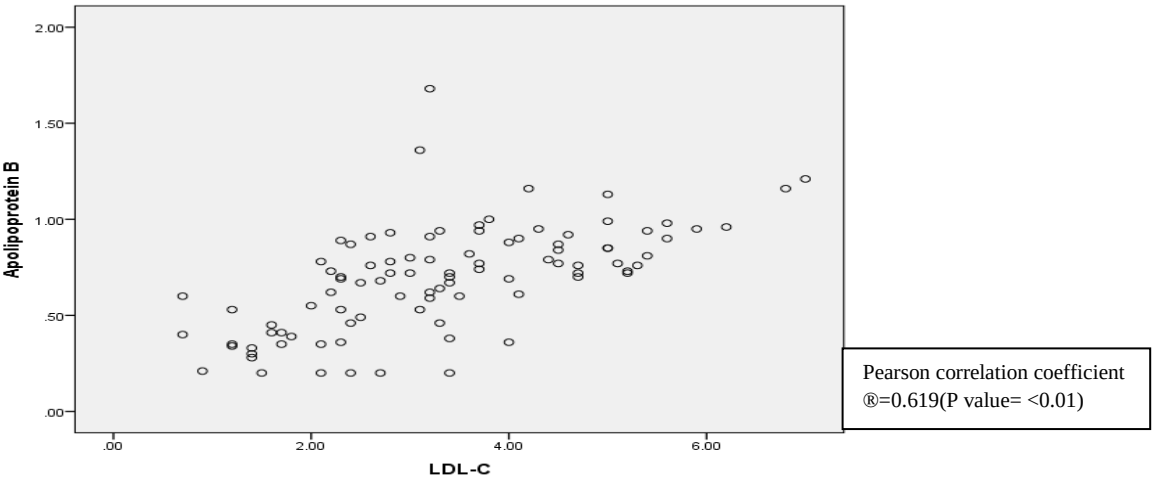



Figure 10 above shows that there is a good correlation between apo B and LDL-C with a P value of <0.01. Patients with high apo B levels were also likely to have high LDL-C levels.

## Figure 11: Correlation between apo B and triglycerides in the study population

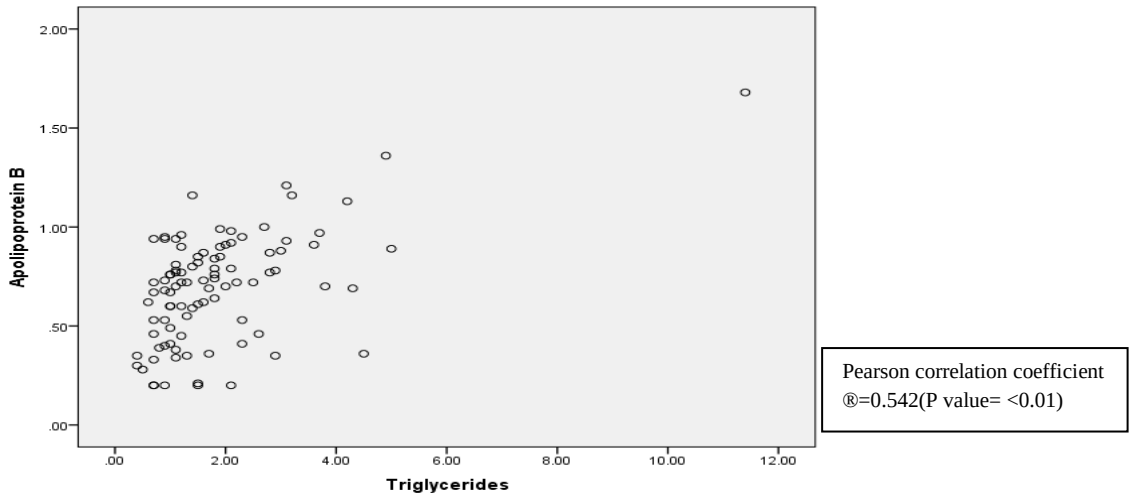
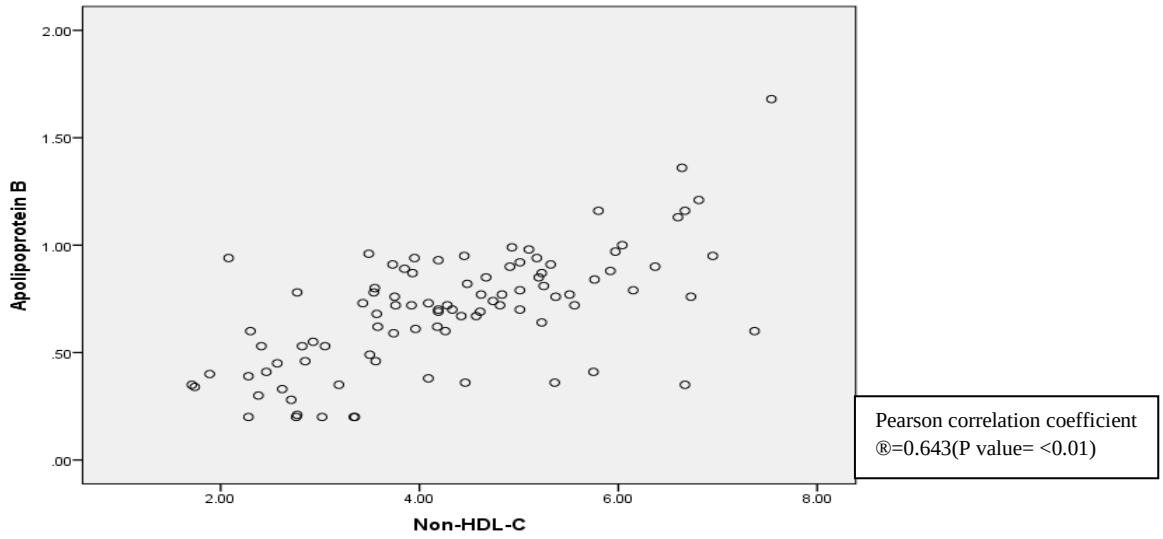



Figure 11 above shows that there is good correlation between apo B and triglycerides (P value <0.01). Most of the patients with high triglycerides were also likely to have high apo B levels.

## Figure 12: Correlation between apo B and non-HDL C in the study population




Apo B and non-HDL C show a good positive correlation *(Figure 12 above).* Most of the patients with high non-HDL-C also had high apo B levels.

**Figure 13: Correlation between apo B with HDL-C in the study population**

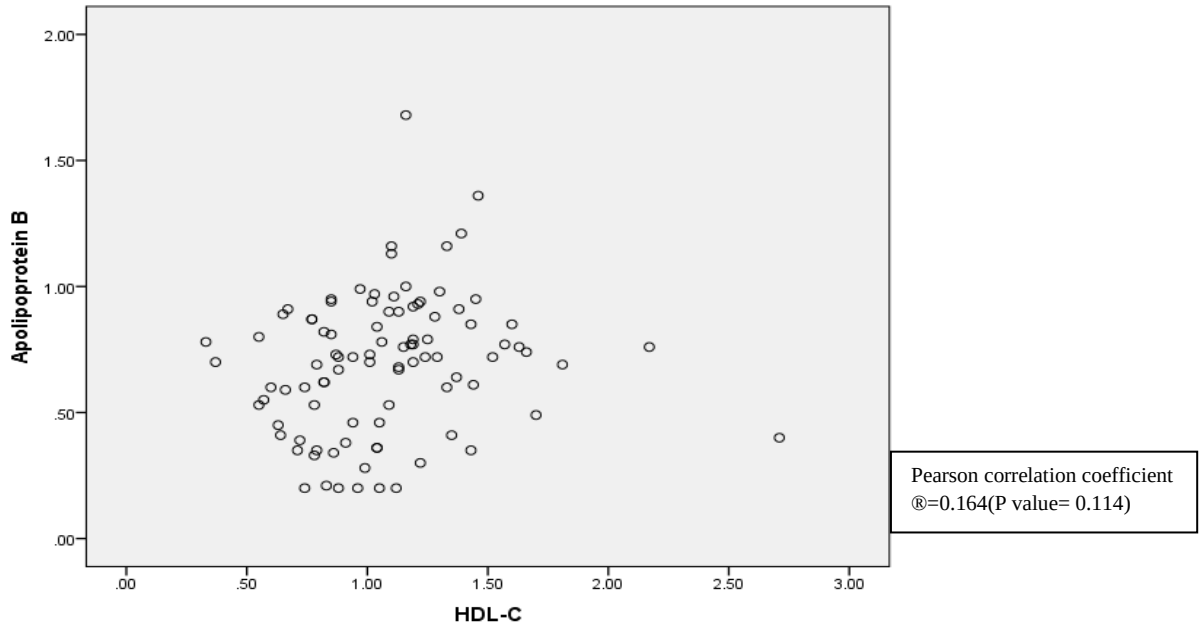



Figure 13 above shows there is no correlation between apo B and HDL-C (P value 0.114). This is because most of the patients with high apo B have low HDL-C levels.

**Table 2: Odds ratios between LDL-C and total cholesterol and triglycerides in the study population (N=94)**

| | LDL-C | | OR (95% CI) | P value |
|---|---|---|---|---|
| | **High** | **Normal** | | |
| **Total Cholesterol** | | | | |
| High | 61 (93.8) | 9 (31.0) | 33.9 (9.4-122.1) | <0.001 |
| Normal | 4 (6.2) | 20 (69.0) | 1.0 | |
| **Triglyceride** | | | | |
| High | 51 (78.5) | 16 (55.2) | 3.0 (1.2-7.6) | 0.024 |
| Normal | 14 (21.5) | 13 (44.8) | 1.0 | |

P value is significant *(P value <0.05)* in both cases between LDL-C and total cholesterol and LDL-C and triglycerides. This means that there was a high likelihood of having high LDL-C in patients with either high total cholesterol or triglycerides. In addition, 6.2% of patients with normal total cholesterol had high LDL-C while 21.5% of patients with normal triglycerides had high LDL-C *(Table 2 above).*

**Table 3: Odds ratios between apolipoprotein B and lipid profile (TC, TG, LDL-C, HDL-C, non-HDL-C) in the study population (N=94)**

| Variable | Apo B | | OR (95% CI) | P value |
|---|---|---|---|---|
| | High | Normal | | |
| **Total Cholesterol** | | | | |
| High | 46 (79.3) | 24 (66.7) | 1.9 (0.7-4.9) | 0.175 |
| Normal | 12 (20.7) | 12 (33.3) | 1.0 | |
| **Triglyceride** | | | | |
| High | 45 (77.6) | 22 (61.1) | 2.2 (0.9-5.5) | 0.089 |
| Normal | 13 (22.4) | 14 (38.9) | 1.0 | |
| **LDL-C** | | | | |
| High | 42 (72.4) | 23 (63.9) | 1.5 (0.6-3.6) | 0.384 |
| Normal | 16 (27.6) | 13 (36.1) | 1.0 | |
| **HDL-C** | | | | |
| Low | 44 (75.9) | 33 (91.7) | 0.3 (0.1-1.1) | 0.053 |
| Normal | 14 (24.1) | 3 (8.3) | 1.0 | |
| **Non-HDL-C** | | | | |
| High | 56 (96.6) | 18 (50.0) | 28 (5.9-132.5) | <0.001 |
| Normal | 2 (3.4) | 18 (50.0) | 1.0 | |

P value of <0.05 was considered statistically significant. The odds ratio of having high apo B and high non-HDL-C was statistically significant while between high apo B and other parameters was not significant. However, 20.7% of patients with normal cholesterol had high apo B and 22.4% of patients with normal triglycerides had high apo B. 27.6% of patients with normal LDL-C had high apo B while 63.9% of patients with normal apo B had high LDL-C *(Table 3 above).*

## Medical history of the study population

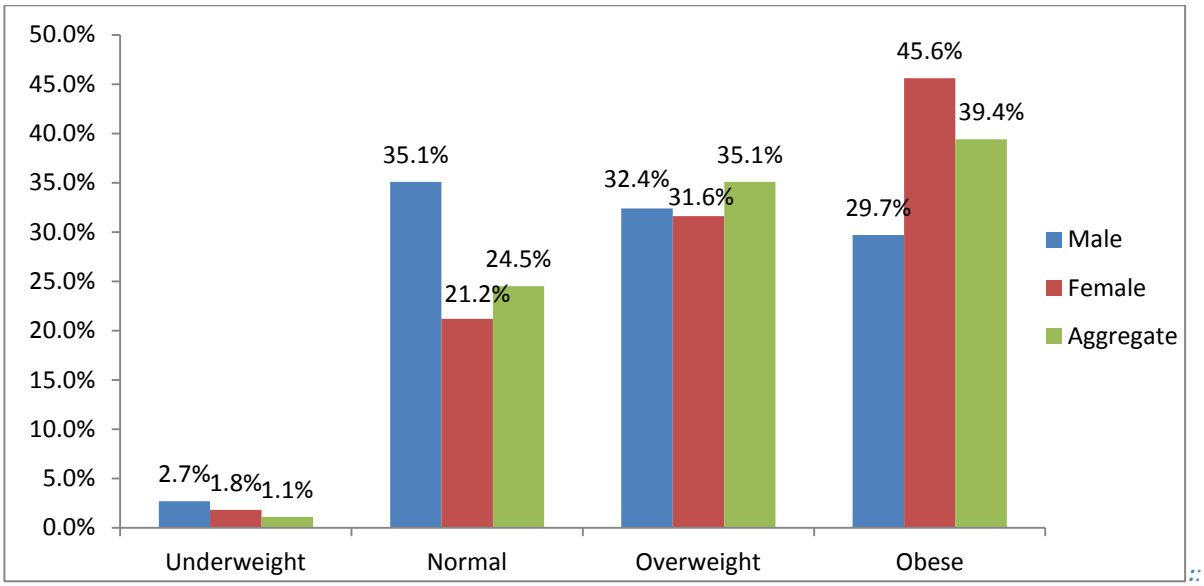
### Table 4: Main clinical history of the study population (N=96)

| Variable | Frequency (%) |
|---|---|
| **Dyslipidaemia** | |
| Yes | 9 (9.3) |
| No | 75 (78.2) |
| Not known | 12 (12.5) |
| **Hypertension** | |
| Yes | 68 (70.8) |
| No | 28 (29.2) |
| **Smoking** | |
| Yes | 6 (6.3) |
| No | 90 (93.7) |
| **OHA** | |
| Yes | 70 (72.9) |
| No | 26 (27.1) |
| **Insulin** | |
| Yes | 57 (59.4) |
| No | 39 (40.6) |
| **Combined (OHA+Insulin)** | |
| Yes | 44 (45.8) |
| No | 52 (54.2) |
| **Antihypertensives** | |
| Yes | 68 (70.8) |
| No | 28 (29.2) |

This history was elicited from the patients and from their files. 75% of the patients had no history of dyslipidaemia, 68% of the patients were hypertensive and were on antihypertensives. Patients were either on oral hypoglycaemics, insulin or combined *(Table 4 above).*

## Physical examination findings

Figure 14: BMI of the study population (N=96)




Most of the patients (74.5%) were either overweight or obese. They were more males than females (35.1% vs 21.2%) with a noraml BMI. In addition, more females than males were obese (45.6% vs 29.1% ). The mean BMI of males was 27.7 with a range of 18 to 45 while that of females was higher at 29.7 with a range of 17.5 to 50 *(figure 14 above).*

**Table 5: Waist to hip ratio and blood pressure of the study population (N=96)**

| Variable | Frequency (%) |
|---|---|
| **Waist to Hip ratio** | |
| High | 74 (77.1) |
| Normal | 22(22.9) |
| **Systolic Blood Pressure(mmHg)** | |
| Mean (SD) | 139.4 (18.2) |
| Range | 93 – 200 |
| High | 42 (44.7) |
| Normal | 52 (55.3) |
| **Diastolic Blood Pressure(mmHg)** | |
| Mean (SD) | 79.5 (11.4) |
| Range | 50 – 114 |
| High | 19 (20.2) |
| Normal | 75 (79.8) |
| **Blood pressure(mmHg)** | |
| High | 47 (50.0) |
| Normal | 47 (50.0) |

Table 5 above shows that 77% of the patients had an undesirable waist to hip ratio and half of the patients had elevated blood pressure. The mean waist to hip ratio for males was 0.99 with a range of 0.81-1.19 while the mean for females was 0.91 with a range of 0.74-1.19. The blood pressure of the study population was similar for both males and females with the mean systolic blood pressure being 139.4mmHg with a range of 93-200mmHg and mean diastolic pressure being 79.5mmHg with a range of 50-114mmHg.

**Figure 15 : Prevalence of metabolic syndrome (N=96)**

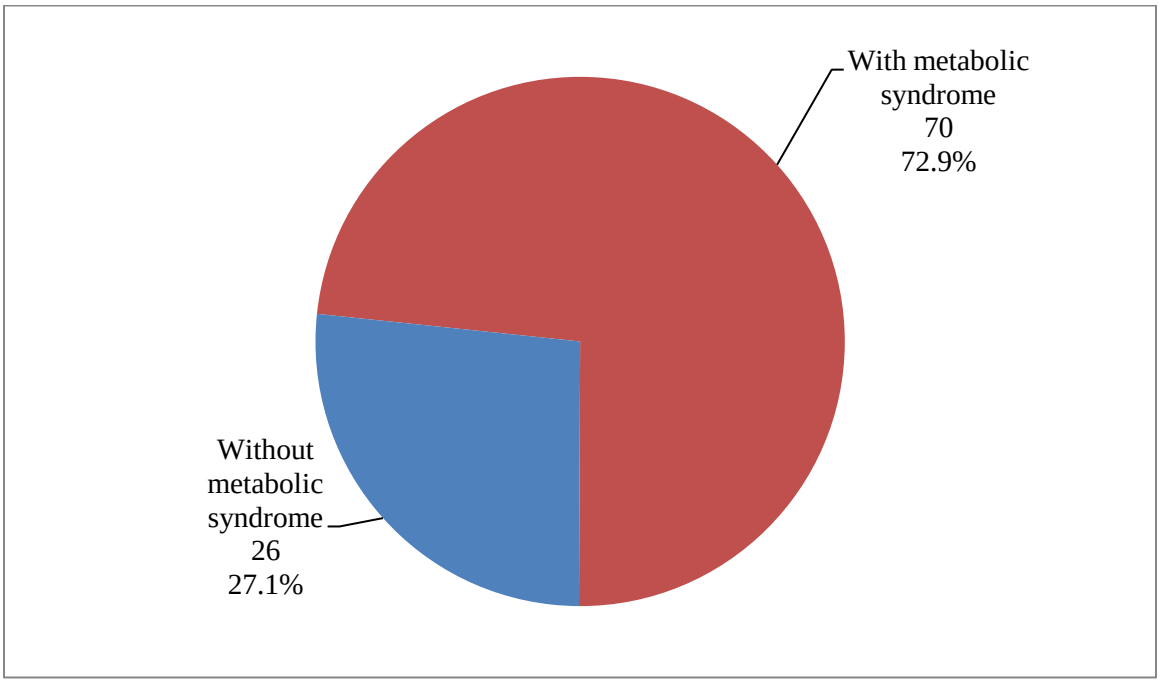



Figure 15 above shows that 72.9% of the study population had metabolic syndrome while 27.1% did not.

## DISCUSSION

This study gives an insight in to the levels and patterns of dyslipidaemias in the local type 2 diabetic population where a total of 96 patients were studied. The reference values were based on local data (personal communication by Maina)

Most of the patients were female which is consistent with a previous study done in the same population of diabetic patients where about 57% of the patients were females(23).

Majority of the patients (82%) were aged 40-69 years. This result is similar with the global estimates of diabetes where the majority of diabetic patients in developing countries are between 40 and 60 years(9). This forms the major workforce in an economy and therefore the great need for aggressive management of the diabetic patients(11). Most of the diabetic patients in developed countries tend to be older, 60yr and above(9).

In this study, various levels and patterns of dyslipidaemias were identified. The most common lipid disorder was low HDL-C followed by hypercholesterolemia and hypertriglyceridaemia with the least common being hyperbetalipoproteinaemia. The patterns of dyslipidaemia in the current study consisted of high total cholesterol, high triglycerides, high LDL-C, low HDL-C, high non-HDL-C and high Apo B. Diabetic patients tend to have a characteristic dyslipidaemia consisting of high triglycerides mainly triglyceride-rich VLDL particularly post-prandially (post-prandial lipaemia), low HDL-C and increased concentrations of small dense LDL-C. Total cholesterol and LDL-C cholesterol tend be normal or only slightly raised (19,20).

Majority of the patients (74%) had hypercholesterolemia which is consistent with a local study done at Kenyatta National Hospital where about 70% of the patients had high cholesterol(23). This shows that most of the diabetic patients in the local population either have undetected or poorly controlled high cholesterol and are therefore at risk of cardiovascular disease.

More than 70% of the patients had hypertriglyceridaemia and this is consistent with previous studies in American and European regions that showed that diabetic patients had higher triglyceride levels than non-diabetic patients(2,6). In type 2 diabetic patients there is increased free fatty-acid release from insulin-resistant fat cells, the increased flux of free fatty acids into the liver in the presence of adequate glycogen stores promotes triglyceride production(6,19). However, the high levels of triglycerides differs from a previous study that showed only about

30% of the patients had high triglycerides(23). One of the reasons for this discrepancy could be the lower cut- offs used in this study which used local data for the reference values.

About 70% of the patients had a high LDL-C while data from a previous study showed about 50% of the patients with high LDL-C(23). The difference in this data could be because of lower cut-offs used in this study. However, proportion of patients with abnormal LDL-C in both studies is much higher than most published data where LDL-C was normal or only slightly raised in type 2 diabetic patients(19,20). Diabetic patients tend to have small dense LDL particles which are not measurable with available assays of the total LDL-C(19). Data from both local studies showing a significantly high proportion of the diabetic patients having high LDL-C as opposed to other studies may reflect that this pattern of dyslipidaemia could be different from other regions.

Apolipoprotein B was raised in about 60% of the patients which is a significantly high proportion of patients but the least frequent lipid disorder in the local type 2 diabetic population. Apo B was positively correlated with all the basic lipid parameters. In a study done by Wagner et al, apo B was found to be the most frequent lipid disorder in normocholesterolaemic type 2 diabetic patients and was found to identify additional dyslipidaemic phenotypes(35). Further, Wagner found that apo B identifies dyslipidaemic type 2 diabetic patients in the normotriglyceridaemic group(44).

LDL-C has been established as an independent risk factor for atherosclerosis. Consequently, NCEP ATP III has published guidelines on lipids and identified LDL-C as the main target of therapy(24). However, it has been noted that many patients who receive treatment and achieve the recommended LDL-C target still develop atherosclerotic complications(45). One explanation for these discrepancies is the mis-match that has been described in many patients between the LDL-C concentration reported on a basic lipid panel and the number of atherogenic lipid particles, which is often expressed as low-density lipoprotein (LDL) particle number or the number of apo B-containing lipoproteins(29).

In the current study, the population had a higher frequency of abnormal LDL-C than that of abnormal apo B. However, in the normocholesterolaemic group 6.2% had high LDL-C while 20.7% had high apo B indicating that apo B identifies more dyslipidaemia in this group. In

addition, 27% of patients with normal LDL-C had high apo B, further showing the additive value of apo B.

Among the hypertriglyceridaemic group about the same proportion of the study population (~78%) had high LDL-C and high apo B levels. In the normotriglyceridaemic, there was similar pattern 21.5% had high LDL-C while 20.7% had high apo B. This is in contrast to the study by Wagner that showed that apo B identified more dyslipidaemic patients than LDL-C(44).

The current study shows that 81% of the patients had low levels of HDL-C. This is consistent with available data where type 2 diabetic patients tend to have low HDL-C due to increased concentrations of VLDL in hypertriglyceridaemia and also inability to upregulate apolipoprotein A-1 production (main HDL-C lipoprotein moiety) owing to insulin resistance(6,20).

In addition, 79% of the patients had high levels of non-HDL-C. In the current study, non-HDL-C correlated strongly with apo B and actually there was a higher percentage of patients with high non-HDL-C than with high apo B. Non-HDL-C estimates all the cholesterol in LDL-C, IDL-C and VLD-L. NCEP has recognized non-HDL-C as a secondary target of statin therapy in patients with hypertriglyceridaemia(24). Studies have shown that both apo-B and non-HDL-C are superior to LDL-C in predicting cardiovascular risk since they identify the total atherogenic risk(31,46,47). Some studies (including INTERHEART study) have shown that apo B is superior to non-HDL-C (31,36,44,46). However, recent meta-analysis studies have shown that it may not be advisable to select one over the other since each of these reflects different measures of risk (48–52). In addition, in a study among statin treated patients non-HDL-C was shown to be a stronger predictor of cardiovascular events than both LDL-C and apo B(49,51).The advantage of non-HDL-C is that there is no added cost since it is derived by subtracting HDL-C from total cholesterol and this may be more applicable in our set-up where apo B is not widely available.

In other findings in the study population, 68% of the patients had a history of hypertension and were on antihypertensives and this is consistent with the fact that diabetes and hypertension usually occur concurrently as part of the metabolic syndrome. However, most of the participants had a negative history of dyslipidaemias and this was probably because a lipid profile had not been done. Most of the patients also did not smoke. This may be because most of the patients were females and in developing countries males predominate in smoking.

A significant number of the patients (75%) were either overweight or obese and 74% had an undesirable waist-to-hip ratio (central obesity). Female subjects were more obese than their male counterparts (45% versus 39%) as was found in a previous local study(23). Obesity including central obesity is increasing in developing countries due to lifestyle changes resulting from urbanisation and physical inactivity(1,10). Central obesity increases the risk of type 2 diabetes, metabolic syndrome and cardiovascular complications(10).

More than 70% of the study population had metabolic syndrome. The high prevalence of metabolic syndrome among diabetics is comparable to other studies(10,53). People with metabolic syndrome have about five-fold risk of developing type 2 diabetes(53). Diabetic patients with metabolic syndrome are at higher risk of CVD compared to those without(54). Central obesity is associated with insulin resistance. Several adipokines, such as leptin, adiponectin, TNF-α, IL-6, resistin, visfatin, and retinol-binding protein 4, have been suggested to be associated with insulin resistance(55). Adiponectin has important anti-atherogenic, antidiabetic, and anti- inflammatory properties and is expressed abundantly in adipocytes. High adiponectin levels correlate with high insulin sensitivity(56). However, in subjects with an excess of intra-abdominal fat mass, adiponectin levels are low. This might be because there is an increase in proinflammatory cytokines such as TNF- α which inhibit production of adiponectin (55,56). This results in insulin resistance.

# CONCLUSION AND RECOMMENDATIONS

## Conclusion

1. The majority of the patients studied had low HDL-C, elevated non-HDL-C, elevated total cholesterol, elevated triglycerides, elevated LDL-C and elevated apo B.
2. Apolipoprotein B had a positive linear correlation with total cholesterol, triglycerides, LDL-C, non-HDL-C. The strongest positive correlation was with non-HDL-C.
3. Patients with low HDL-C had high apo B levels.
4. Apo B can help identify additional dyslipidaemic phenotypes in patients with normal cholesterol and normal LDL-C.
5. Most of the patients studied had metabolic syndrome.

## Recommendations

Apo B should be included among the cardiovascular risk assessment parameters.

Non-HDL-C should routinely be calculated for all type 2 diabetic patients to aid in cardiovascular risk assessment.

All diabetic patients should be evaluated for metabolic syndrome

## Dissemination of information

The findings of this study will be communicated to the stake holders involved in diabetic care in various forums. Initial dissemination is in the form of MMED dissertation book. The findings will also be communicated back to the health providers in the diabetic clinic through talks and continuous medical education sessions. The findings will also be presented in medical conferences.

In addition, the findings will be published in a peer reviewed journal.

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# APPENDICES

## Appendix I: Consent Explanation

**Correlation of Apolipoprotein B and Lipid Profile In Type 2 Diabetes Mellitus Patients at Kenyatta National Hospital, Nairobi.**

Introduction and objectives of the study:

I am Dr. Wangari Wambugu, a master student in dept of Human Pathology at the University of Nairobi and conducting a study on comparison of Apolipoprotein B and Lipid profile in type 2 diabetic patients. Type 2 diabetic patients commonly have abnormalities in lipids which put them at risk of cardiovascular complications like myocardial infarction, cerebrovascular accidents and peripheral vascular diseases. The main aim of the study is to correlate Apo B with total cholesterol, LDL-C, triglycerides and HDL-C and find out if more patients with lipid abnormalities can be picked out using Apo B.

Benefits and risk of the study to you:

Your participation will help improve care of type 2 diabetic patients. There will be no payment for participating in the study. By participating, you will benefit by having laboratory tests to detect lipid abnormalities done at no additional cost and a report of the test results will be sent to your physician.

Risk: 3ml of blood will be drawn from the antecubital vein. The prick may be painful and a haematoma may form around the prick area.

If you consent to participate, you will:

- Sign a consent form (Appendix II)

- Answer a number of questions contained in the screening and study questionnaire(Appendix III and IV)
- Undergo a physical exam
- Have blood drawn from you for laboratory tests.

Bus fare reimbursement:

Bus fare will be reimbursed at a cost of Ksh 200 to facilitate your coming back for specimen collection while fasted.

Confidentiality:

Your participation is voluntary and you can withdraw from the study at any time. Any information given to us will remain confidential and your privacy will be respected. You may ask me any questions regarding the study now or any time during the study. If you have any questions relating to the study, kindly contact:

1. Dr. Wangari Wambugu 0721-287635
2. My Supervisors: Prof C Kigondu 0733-730796, Prof CF Otieno 0722-752558 and Mr. F Maina 0720-713580
3. The Secretary to the Ethical Research Committee, KNH Tel no 272260, Extension 4410

## Appendix II : Consent Form for the Participants

**Correlation of Apolipoprotein B and Lipid Profile In Type 2 Diabetes Mellitus Patients at Kenyatta National Hospital, Nairobi.**

I.....

.....after reading and being explained to on the study purpose by Dr. Wangari Wambugu, do hereby give informed consent to participate in the study on comparison of Apolipoprotein B and lipid profile among type 2 diabetes mellitus patients.

I am aware that i can withdraw from the study without any benefits or quality of management of my medical condition being interfered with.

Signed:.....

Thumbprint:.....

.....

.....

Signature of the Principal investigator (Dr. W. Wambugu).....

Witness:.....

Date:.....

## Appendix III: Screening questionnaire

**Correlation of Apolipoprotein B and Lipid Profile In Type 2 Diabetes Mellitus Patients At Kenyatta National Hospital, Nairobi.**

| | YES | NO |
|---|---|---|
| Age ≥18years | | |

| | YES | NO |
|---|---|---|
| **Medical history**<br>1. Type 2 diabetes<br>2. Lipid lowering drugs | | |
| **Eligibility**<br>3. Are you willing to participate in this study | | |

If answers to ALL questions are YES, except no. 2, Recruit and issue a Study number.

***FOR OFFICAL USE:***

RECRUITED (encircle) YES NO

STUDY NUMBER: | | | |

Once recruited, proceed to Study Questionnaire (Appendix IV).

## Appendix IV: Study Questionnaire

**Correlation of Apolipoprotein B and Lipid Profile In Type 2 Diabetes Mellitus Patients at Kenyatta National Hospital, Nairobi.**

Date ☐☐☐☐☐☐

dd / mm / yy

**A. Demographic data**

Name.....

Study No ☐☐☐

Hospital No ☐☐☐☐☐☐☐☐

Age (Years): ☐☐

Gender: 1. Male ☐ 2. Female ☐

**B. Medical History**

1. Dyslipidaemia YES ☐ NO ☐ Not Known ☐
2. Hypertension YES ☐ NO ☐
3. Smoking YES ☐ NO ☐

**C. Medications**

1. Hypoglycaemics

   i) Oral hypoglycaemics YES ☐ NO ☐

   ii) Insulin YES ☐ NO ☐

   iii) Combined YES ☐ NO ☐

2. Antihypertensives YES ☐ NO ☐

**C. Physical Examination**

| | |
|---|---|
| Height (M) | |
| Weight (kg) | |
| BMI(Kg/M2) | |
| Waist-Hip ratio | |
| Blood pressure (mmHg)<br>Systolic<br>Diastolic | |

**Laboratory results (fasting blood sample)**

| **Analyte** | **Ref range** | **Test result** | **Classification**<br>**Normal Abnormal** |
|---|---|---|---|
| Total cholesterol (mmol/L) | | | |
| Triglyceride (mmol/L) | | | |
| LDL-c (mmol/L) | | | |
| HDL-c (mmol/L) | | | |
| Apo B (g/L) | | | |

# Appendix V: Methodology of the Assays

## Appendix V (a): Total Cholesterol

# CHOLESTEROL liquicolor

CHOD-PAP-Method

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

**Package Sizes**

| | | | |
|---|---|---|---|
| REF | 10017 | 4 x 30 ml | Complete test kit |
| | 10019 | 3 x 250 ml | Complete test kit |
| | 10028 | 4 x 100 ml | Complete test kit |
| | 10015 | 9 x 3 ml | Standard |

IVD

**Method**

The 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.

**Reaction Principle**

Cholesterolester + $H_2O$ —CHE→ cholesterol + fatty acid

Cholesterol + $O_2$ —CHO→ cholestene-3-one + $H_2O_2$

$2\ H_2O_2$ + 4-amino-phenazone + phenol —POD→ quinoneimine + $4\ H_2O$

**Contents**

| | | |
|---|---|---|
| R(GT) | **4 x 30 ml, 3 x 250 ml or 4 x 100 ml Enzyme reagent** | |
| | Phosphate buffer (pH 6.5) | 100 mmol/l |
| | 4-Aminophenazone | 0.3 mmol/l |
| | Phenol | 5 mmol/l |
| | Peroxidase | > 5 KU/l |
| | Cholesterolesterase | > 150 U/l |
| | Cholesteroloxidase | > 100 U/l |
| | Sodium azide | 0.05 % |
| STD | **3 ml Standard** | |
| | Cholesterol | 200 mg/dl or 5.17 mmol/l |

**Reagent Preparation**

The R(GT) 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, heparinized 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 clears 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**

| Pipette into cuvettes | Reagent blank | Sample or STD |
|---|---|---|
| Sample/ STD | — | 10 µl |
| R(GT) | 1000 µl | 1000 µl |
| Mix, incubate 10 min. at 20...25°C or 5 min. at 37°C. Measure the absorbance of the sample/STD against the reagent blank (ΔA) within 60 min. | | |

**Calculation of the Cholesterol Concentration**

**1. With Factor**

| Wavelength | C [mg/dl] | C [mmol/l] |
|---|---|---|
| Hg 546 nm | 840 x ΔA | 21.7 x ΔA |
| 500 nm | 553 x ΔA | 14.3 x ΔA |

**2. With Standard**

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

$$C = 200 \times \frac{\Delta A_{sample}}{\Delta A_{STD}}\ [mg/dl]$$

or

$$C = 5.17 \times \frac{\Delta A_{sample}}{\Delta A_{STD}}\ [mmol/l]$$

**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/de/at/gb/int/SU-CHOL.pdf
www.human-de.com/de/at/gb/int/SU-CHOL.pdf

**Clinical Interpretation**

| | | |
|---|---|---|
| Suspect over | 220 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 employed. We recommend to use our animal 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**


1. Schettler, G. and Nüssel, E., Arb. Med. Soz. Med. Präv. Med. 10, 25 (1975)
2. Richmond, W., Clin. Chem. 19, 1350 (1973)
3. Ribachlau, P. et al., J. Clin. Chem. Clin. Biochem. 12, 403 (1974)
4. Trinder, P., Ann. Clin. Biochem. 6, 24 (1969)
5. ISO 15223 Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied.



SU-CHOL
INF 1001701 GB
04-2002-17


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## Appendix V (b): Triglycerides

# TRIGLYCERIDES liquicolor mono

## GPO-PAP Method

### Enzymatic Colorimetric Test for Triglycerides with Lipid Clearing Factor (LCF)

| | | | |
|---|---|---|---|
| REF [1] | 10720P | 9 x 15 ml | Complete Test Kit |
| | 10724 | 4 x 100 ml | Complete Test Kit |
| | 10725 | 3 x 250 ml | Complete Test Kit |
| | 10183 | 9 x 3 ml | Standard |

IVD

**Method**

The triglycerides are determined after enzymatic hydrolysis with lipases. Indicator is quinoneimine formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase.

**Reaction Principle**

$$\text{Triglycerides} \xrightarrow{\text{Lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{quinoneimine} + HCl + H_2O$$
$$+ \text{4-chlorophenol}$$

**Contents**

| | | |
|---|---|---|
| R1 FF | **15 ml; 100 ml or 250 ml Monoreagent** | |
| | PIPES buffer (pH 7.5) | 50 mmol/l |
| | 4-chlorophenol | 5 mmol/l |
| | 4-aminoantipyrine | 0.25 mmol/l |
| | Magnesium ions | 4.5 mmol/l |
| | ATP | 2 mmol/l |
| | Lipases | ≥ 1.3 U/ml |
| | Peroxidase | ≥ 0.5 U/ml |
| | Glycerol kinase | ≥ 0.4 U/ml |
| | Glycerol-3-phosphate oxidase | ≥ 1.5 U/ml |
| STD | **3 ml Standard** | |
| | Triglycerides | 200 mg/dl or 2.28 mmol/l |

**Reagent Preparation and Stability**

R(GT) and STD are ready for use.

The reagents are stable, even after opening, up to the stated expiry date when stored at 2...8°C. At 20...25°C the R(GT) is stable for 4 weeks. **Contamination must be avoided.**
Protect from light.

**Specimen**

Serum, heparinized plasma or EDTA plasma
Stability: 3 days at 2...8°C
4 months at -20°C

Note: Lipemic specimens usually generate turbidity of the sample reagent mixture which leads to falsely elevated results. The TRIGLYCERIDES **liquicolor** mono test avoids these falsely elevated results through its built-in Lipid-Clearing Factor (LCF). The LCF clears 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 (Rb). Only one reagent blank per series is required.

**Pipetting scheme**

Please use only the HUMAN Triglycerides Standard provided with the test kits or separately available: REF 10183.

| Pipette into cuvettes | Rb | Sample or STD |
|---|---|---|
| Sample / STD | — | 10 µl |
| R(GT) | 1000 µl | 1000 µl |

Mix and incubate for 10 min. at 20...25°C or for 5 min. at 37°C. Measure the absorbance of the sample ($\Delta A_{sample}$) and the Standard ($\Delta A_{STD}$) against the reagent blank within 60 min.

**Calculation of the Triglycerides Concentration**

$$C = 200 \times \frac{\Delta A_{sample}}{\Delta A_{STD}} \text{ [mg/dl] or } C = 2.28 \times \frac{\Delta A_{sample}}{\Delta A_{STD}} \text{ [mmol/l]}$$

**Performance Characteristics**

Linearity
The test is linear up to a triglycerides concentration of 1000 mg/dl or 11.4 mmol/l. Samples with a higher concentration have to be diluted 1 + 4 with physiological saline (0.9%) and retested. Multiply the result by 5.

Typical performance data can be found in the Verification Report, accessible via
www.human.de/data/gb/vr/SU-TRIMR.pdf or
www.human-de.com/data/gb/vr/SU-TRIMR.pdf

**Clinical Interpretation for Atherosclerotic Risk**

Suspect: over 150 mg/dl or 1.71 mmol/l
Increased: over 200 mg/dl or 2.28 mmol/l

**Quality Control**

All control sera with triglycerides values determined by this method can be employed.
We recommend to use our animal serum made 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. To correct for free glycerol, subtract 10 mg/dl (0.11 mmol/l) from the triglycerides value calculated.
2. The test is not influenced by hemoglobin values up to 150 mg/dl or by bilirubin values up to 40 mg/dl. Ascorbate may give falsely low values at > 4 mg/dl.
3. The reagents contain sodium azide (0.05%) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.

**References**


1. Schettler, G., Nüssel, E., Arb. Med. Soz. Med. Präv. Med. **10**, 25 (1975)
2. Jacobs, N. J., VanDemark, P. J., Arch. Biochem. Biophys. **88**, 250-255 (1960)
3. Kodbichek, L. K., Umbreit, W. W., J. Bacteriol. **68**, 1063-1068 (1969)
4. Trinder, P., Ann. Clin. Biochem. **6**, 24-27 (1969)
5. ISO 15223 Medical devices-Symbols to be used with medical device labels, labelling and information to be supplied



SU-TRIMR
INF 1072401 08
08-2002-9


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# Appendix V (c): LDL Cholesterol

## LDL CHOLESTEROL liquicolor

System Reagent for HumaStar 600

Enzymatic Colorimetric Test

**Package Sizes**

REF 10094600 180 Tests

IVD

**Method**

The assay combines two steps: in the 1st step chylomicrons, VLDL and HDL cholesterol are specifically removed by enzymatic reactions. In the 2nd step remaining LDL-cholesterol is determined by well established enzymatic reactions, also employing specific surfactants for LDL.

**Reaction Principle**

**1st step:**

HDL, VLDL, and chylomicrons $\xrightarrow[\text{specific conditions}]{\text{CHE + CHO}}$ cholestenone + $H_2O_2$

$2\ H_2O_2 \xrightarrow{\text{Catalase}}$ $2\ H_2O + O_2$

**2nd step:**

LDL $\xrightarrow[\text{specific surfactants}]{\text{CHE + CHO}}$ cholestenone + $H_2O_2$

$H_2O_2$ + chromogen $\xrightarrow{\text{Peroxidase}}$ quinone dye

**Contents**

| | | | |
|---|---|---|---|
| ENZ | 2 x 90 tests | **Enzyme reagent** | |
| | | Good's buffer, pH 7.0 (25°C) | 50 mmol/l |
| | | Magnesium chloride | 20 mmol/l |
| | | Cholesterol esterase | 600 U/l |
| | | Cholesterol oxidase | 500 U/l |
| | | Catalase | 600 kU/l |
| | | N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS) | 2.0 mmol/l |
| | | Preservative | < 0.1 % w/v |
| SUB | 2 x 90 tests | **Substrate** | |
| | | Peroxidase | 5000 U/l |
| | | 4-Aminoantipyrin (4-AA) | 4 mmol/l |
| | | Good's buffer, pH 7.0 (25°C) | 50 mmol/l |
| | | Sodium azide | 0.05 % w/v |
| | | Detergents | 1.4 % w/v |
| | | Preservative | < 0.1 % w/v |

**Additional material recommended but not supplied with the kit**

REF **13160**

CAL 4 x for 5 ml **AUTOCAL** lyophilised calibrator for HUMAN Clinical Chemistry Systems

| | | |
|---|---|---|
| REF | **13951** | **13151** |
| CONTROL | **SERODOS** | **SERODOS plus** |
| | 6 x for 5 ml Control serum | 6 x for 5 ml Control serum |
| | normal | abnormal |

lyophilised control serum for HUMAN Clinical Chemistry Systems

**Reagent Preparation**

The reagents are ready for use and can directly be applied on the analyzer.

**Reagent Stability**

Once opened, the reagent stored on board the analyzer remain stable for at least 60 days.

Avoid contamination. Do not freeze, do not mix caps. Protect ENZ from light.

**Specimen**

Serum, plasma

Stability: We recommend to test directly after sampling, serum can be stored at 2...8°C up to 5 days.

In plasma following concentrations of the anticoagulant should not be exceeded: EDTA-2Na <1000 mg/l; Na-citrate <5000 mg/l; heparin < 750 mg/l; NaF < 2000 mg/l, Na-oxal. < 3000 mg/l.

**Testing Procedure**

Please refer to the user manual of HumaStar 600.

**Calibration**

Prior to the first run, calibration is necessary. For calibration AUTOCAL should be employed according to the procedure shown in the AUTOCAL package insert. The assigned calibration value stated in the AUTOCAL leaflet has to be entered into the analyzer.

We recommend checking the validity of calibration before every run.

Re-calibration is necessary:

- whenever controls are out of range
- after reagent lot change
- as required according to local quality control requirements
- bi-weekly

**Quality control**

For quality control use the control material recommended in "Additional material recommended but not supplied with the kit" or other suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**Performance Characteristics**

Linearity: 5 - 300 mg/dl or 0.04 - 7.8 mmol/l

Interferences: Criterion: Recovery within ± 10 % of initial value.

No significant interference for hemoglobin, bilirubin and ascorbic acid.

Lipemic interference > 10 % above 500 mg/dl intralipid.

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

www.human.de/data/gb/vr/s6-ldl.pdf or

www.human-de.com/data/gb/vr/s6-ldl.pdf

**Reference Values**

| | Male | Female |
|---|---|---|
| Reduced risk for CHD | < 50 mg/dl | < 63 mg/dl |
| Increased risk for CHD | > 172 mg/dl | > 167 mg/dl |

CHD: Coronary heart disease

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

**Reference**

Okada M. *et al.*; J. Lab. Clin. Med. **132**, 195 - 201 (1998)

S6-LDL INF 10094601 GB 02-2009-03

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## Appendix V (d): HDL-Cholesterol

# HDL CHOLESTEROL liquicolor

Direct Homogeneous Test for the Determination of HDL-Cholesterol Enzymatic Colorimetric Test

**Package Size**

| | | | |
|---|---|---|---|
| REF | 10084 | 80 ml | Complete Test Kit |
| IVD | | | |

**Intended Use**

HUMAN's HDL Cholesterol direct is a homogeneous enzymatic assay for the quantitative determination of HDL cholesterol (HDL). HDL is regarded as a protecting lipid component against coronary heart disease (CHD). Together with LDL cholesterol (calculated by Friedewald formula) it is of diagnostic importance to estimate the individual risk for CHD.

**Method**

The assay combines two specific steps: in the 1st step chylomicrons, VLDL and LDL cholesterol are specifically eliminated and destroyed by enzymatic reactions. In the 2nd step remaining cholesterol from the HDL fraction is determined by well established specific enzymatic reactions in the presence of specific surfactants for HDL.

**Reactions Principle**

**1st step:**

LDL, VLDL, and Chylomicrons —(CHE + CHO, specific surfactant)→ cholestenone + $H_2O_2$

$2\ H_2O_2$ —(Catalase)→ $2\ H_2O + O_2$

**2nd step:**

HDL —(CHE + CHO, specific surfactant)→ cholestenone + $H_2O_2$

$H_2O_2$ + chromogen —(Peroxidase)→ quinone pigment

**Contents, Reagent Composition in the Test**

| | | |
|---|---|---|
| ENZ | **1 x 60 ml Enzymes** (white cap) | |
| | Good's buffer, pH 7.0 (20°C) | 100 mmol/l |
| | Cholesterol esterase | 800 U/l |
| | Cholesterol oxidase | 360 U/l |
| | Catalase | 800 U/ml |
| | N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) | 0.42 mmol/l |
| SUB | **1 x 20 ml Substrate** (green cap) | |
| | Peroxidase | 1000 U/l |
| | 4-Aminoantipyrin (4-AA) | 1.00 mmol/l |
| | Good's buffer, pH 7.0 (20°C) | 100 mmol/l |
| | Sodium azide | 0.05 % |
| | Detergents | < 1 % |
| CAL | **1 x 4 ml Calibrator** | |
| | Cholesterol | concentration see vial label |

**Reagent Preparation and Stability**

ENZ and SUB are ready for use.

Stability: After opening the reagents are stable up to 1 month when stored at 2...8°C. Avoid contamination. Do not freeze. Do not mix caps.

CAL: Reconstitute the content of the vial with exactly 4 ml dist. germ free water, close the vial and swirl carefully to dissolve all lyophilisate. Avoid foaming. Let stand for 30 minutes before use.

Stability: 10 days at 2...8°C. If required, freshly prepared calibrator can be devided into portions and kept frozen at -20°C for maximum 30 days. Freeze and thaw only once, mix carefully after thawing.

**Specimen**

Serum, plasma

Stability: We recommend to test directly after sampling, otherwise store the serum at +20°C (up to several weeks, avoid repeated freezing and thawing).

In plasma following concentrations of the anticoagulant should not be exceeded: EDTA-2Na = 200 mg/dl; Na-citrate = 1000 mg/dl; heparin = 50 mg/dl; NaF = 2000 mg/dl.

**Assay**

| | |
|---|---|
| Wavelength: | Hg 578 nm, 693 nm, (570 to 610 nm) |
| Optical path: | 1 cm |
| Temperature: | 37°C |
| Measurement: | Against reagent blank, one blank per series is sufficient. |

**Procedure** (manual procedure)

Warm the reagents and the cuvette to 37°C. Temperature must be kept constant (± 0.5°C) for the duration of the test.

| Pipette into cuvettes | Reagent blank (RB) | CAL / sample |
|---|---|---|
| Water | 10 µl | --- |
| CAL / Sample | --- | 10 µl |
| SUB | 750 µl | 750 µl |
| Mix gently and incubate exactly for 5 min. at 37°C | | |
| ENZ | 250 µl | 250 µl |
| Mix gently, incubate at 37°C and read the absorbance ΔA of CAL and samples against RB after 5 min. | | |

**Calculation**

Calculate the concentration of the sample as follows:

$$C_{sample} = C_{CAL} \times \frac{\Delta A_{sample}}{\Delta A_{CAL}} \text{ (mg/dl)}$$

Conversion factor: C (mg/dl) x 0.02586 = C (mmol/l)

**Performance Characteristics**

Linearity: Up to 150 mg/dl HDL.

Linearity limit depends on the analyser-specific application. If the serum concentration of HDL exceeds the measuring range, dilute the sample 1 + 1 with saline (0.9%) and repeat the test. Multiply the result by 2.

Interference: No interference was observed with triglycerides up to 1200 mg/dl, hemoglobin up to 500 mg/dl, bilirubin up to 30 mg/dl, ascorbic acid up to 50 mg/dl, and slightly turbid samples. Dilute samples with triglycerides exceeding 1200 mg/dl with phys. saline (0.9%) 1 + 1 and multiply the result by 2.

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

**www** human.de/data/gb/wf/SU-HDLDD.pdf and ../SU-HDLDD710.pdf or

**www** human-ds.com/data/gb/wf/SU-HDLDD.pdf and ../SU-HDLDD717.pdf

**Reference Values** [3]

< 35 mg/dl (< 0.9 mmol/l) risk factor for CHD

> 60 mg/dl (> 1.54 mmol/l) reduced risk for CHD

This range is given for orientation only; each laboratory should establish its own reference range, as sex, diet, age, geographical location and other factors affect the expected values.

**Quality Control**

All human serum based control sera with HDL values determined by this method can be employed.

**Automation**

The test can be run in a fixed time kinetic mode on analyzers. Applications for respective instruments are available on request.

**References**


1 Gordon, T. et al., Am. J. Med. 62, 707 (1977)

2 Izawa, S. et al., J. Med. and Pharm. Sci. 37, 1385-1388 (1997)

3 ISO 15223 Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied.


SU-HDLDD
INF 10084/1 GB
2009-11

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# Appendix V (e): Apolipoprotein B

## APOLIPOPROTEIN B, APO B

System Reagent for HumaStar 600

Immunoturbidimetric Test

**Package Sizes**

| | | |
|---|---|---|
| REF | 11102600 | 70 Tests |
| IVD | | |

**Intended Use**

Apo B is the main protein component of LDL (Low Density Lipoprotein). Apo B is necessary for the reaction with LDL receptors in the liver and on cell membranes and is thus involved in transporting cholesterol from the liver to the vessel cells.

Elevated levels of Apo B are frequently found in atherosclerotic vascular changes and are a risk factor for atherosclerosis.

**Method**

Apo B antigens in sample or standard cause immunological agglutination with the anti-Apo B antibodies in the reagent. The extent of agglutination is proportional to the Apo B concentration in the sample and can be measured by turbidimetry.

**Contents**

| | | | |
|---|---|---|---|
| RGT | 1x 70 tests | **APO B Monoreagent** | |
| | | Polyclonal anti-human APO B antiserum (goat) | |
| | | in phosphate buffered saline | pH 7.4 |
| | | Polymer enhancer PEG | 2 % |
| | | Sodium azide | 0.095 % |

**Additional material recommended but not supplied with the kit**

| | | | |
|---|---|---|---|
| REF | | **16663/20** | |
| C-CLEAN | 6 x 55 ml | **CUVETTE CLEAN** | |
| | | ready for use (see also "Notes") | |
| | | NaOH | 1 % |
| | | Detergents | 5 % |
| | | Irritant. X,(R38/41) (S26, 28, 37/39, 60) | |
| REF | | **11104** | |
| STD | For 2 x 1 ml | **APO A1 / B Standard** | |
| | | Recalcified human plasma, lyophilised. | |
| | | Sodium azide | 0.095 % |
| | | APO A1 / B concentrations traceable to WHO/IFCC reference preparations are listed on the label | |

| | | |
|---|---|---|
| REF | **13951** | **13151** |
| CONTROL | **SERODOS** | **SERODOS plus** |
| | 6 x for 5 ml Control serum | 6 x for 5 ml Control serum |
| | normal | abnormal |

lyophilised control serum for HUMAN Clinical Chemistry Systems

| | | |
|---|---|---|
| REF | | **16661/01** |
| | 16 pcs | **CHIMNEY** (black or white) for RGT (see also "Notes") |

**Reagent Preparation**

RGT is ready for use and can directly be applied on the analyzer.

**Reagent Stability**

RGT and STD are stable up to the stated expiry date when stored at 2...8°C. Once opened, RGT remains stable for at least 28 days stored on board the analyzer using it with a CHIMNEY (see also "Notes").

STD is stable after reconstitution for 7 days at 2...8°C, avoid contamination. Do not re-freeze. Please refer to the STD instructions for use for further information about the reconstitution procedure.

**Specimen**

Serum

**Testing Procedure**

Please refer to the user manual of HumaStar 600.

**Calibration**

Prior to the first run calibration (5 points) is necessary. We recommend to use STD (REF 11104) for generation of calibration curve. Preparation of STD-dilution series is shown in the STD-package insert. The calculated calibrator concentrations have to be entered into the analyzer.

We recommend checking the validity of calibration before every run.

Re-calibration is necessary:

- whenever controls are out of range
- after reagent lot change
- as required according to local quality control requirements

**Quality control**

For quality control use the control material recommended in "Additional material recommended but not supplied with the kit" or other suitable control material.

The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**Performance Characteristics**

Linearity: 20 - 290 mg/dl

Interferences Criterion: Recovery within ± 10 % of initial value

No significant interference for hemoglobin, bilirubin, triglycerides and citrate.

Lipemic samples interfere > 10 % above 600 mg/dl intralipid.

Prozone Limit No prozone effect was observed up to 1100 mg/dl

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

www.human.de/data/gb/vr/s6-apob.pdf or

www.human-de.com/data/gb/vr/s6-apob.pdf

**Reference Values**

Men: 60 - 138 mg/dl (IFCC)

Women: 52 - 129 mg/dl (IFCC)

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

**Notes**

1. The reagents contain sodium azide (0.095%). Do not swallow. Avoid contact with skin and mucous membranes.
2. All sera used for the manufacture of the standard have been tested for HBsAg, HIV- and HCV-antibodies and found to be negative using FDA approved methods. However, the material should still be regarded as potentially infectious
3. Lipemic samples must be centrifuged (10 min. at 15000 g). Only the liquid below the chylomicrons should be used for the test.
4. We recommend to use the cleaning solution C-CLEAN for optimal performance of turbidimetric assays. C-CLEAN contains NaOH which is irritant. In case of contact with skin and mucous membranes wash with copious amounts of water.
5. We recommend to use RGT only with CHIMNEY for advanced on board stability. Insert the CHIMNEY into the RGT vial up to the upper rim after first opening. Avoid contamination.

**References**


1. Rifai N. *et al.*, Ann. Clin. Lab. Science **18**, 429 (1988)
2. Gordon T. *et al.*, Ann. J. Med. **62**, 707 (1977)
3. Dati F. *et al.*, Lab. Med. **13**, 87 (1989)



S6-APOB INF 11102601 GB 02-2010-04


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Ref: KNH-ERC/A/60

20th March 2012

Dr. Beatrice Wangari Wambugu
Dept. of Human Pathology
School of Medicine
University of Nairobi

Dear Dr. Wambugu

**RESEARCH PROPOSAL: "CORRELATION OF APOLIPOPROTEIN B AND LIPID PROFILE IN TYPE 2 DIABETES MELLITUS PATIENTS AT KENYATTA N.HOSPITAL" (P460/11/2011)**

This is to inform you that the KNH/UoN-Ethics & Research Committee (ERC) has reviewed and approved your above revised research proposal. The approval periods are 20th March 2012 to 19th March 2013.

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**

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