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**Title: Cross-Sectional Study on the Assessment of Dyslipidaemia using classical lipid profile and Apolipoprotein in Type 2 diabetic patients at the Diabetic Clinic of Tikur Anbessa Specialized Teaching Hospital**

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**on the Assessment of  
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## **Operational Definition**

**Apolipoprotein A1:** main protein component of high density lipoprotein in plasma, which promotes cholesterol efflux from tissues to the liver for excretion.

**Apolipoprotein B100:** protein synthesized in the liver that is the major component in lipoproteins of endogenous origin—LDL, VLDL, IDL which is responsible for carrying fat molecules (lipids), including cholesterol to cells within all tissues.

**Atherogenesis:** formation of abnormal fatty deposit in arteries.

**Cardiovascular diseases:** class of diseases that involve the heart or blood vessels. It includes coronary artery diseases (CAD) such as angina and myocardial infarction commonly known as a heart attack.

**Coronary artery disease:** also known as ischemic heart disease (IHD) it is the most common type of cardiovascular diseases that includes angina, myocardial infarction, and sudden cardiac death.

**Diabetic Dyslipidemia:** mild to marked elevation of triglyceride-rich lipoproteins (VLDL) that often precede the onset of Type 2DM for many years. In addition, LDL particles are converted to smaller, more atherogenic lipoproteins termed small-dense LDLs and result in abnormal lipid values, TC > 200 mg/dl, LDL > 100 mg/dl, HDL < 40 mg/dl and TG > 150 mg/dl as defined by WHO.

**Hyperlipidemia:** It is the most common form of dyslipidemia characterized by abnormally elevated levels of TC, TG and LDL in the blood.

**Type 2 Diabetic mellitus:** is a long term metabolic disorder that is characterized by high blood sugar, insulin resistance, and lack of insulin.

## Abbreviation

ACC/AHA	American College of Cardiology and the American Heart Association
ADA/ACC	American Diabetes Association/American College of Cardiology
Apo A	Apolipoprotein A
Apo B	Apolipoprotein B
BLSH	Black lion Specialized hospital
BMI	Body mass index
BP	Blood Pressure
CAD	Coronary artery disease
CDC	Centre for disease control
CHD	Coronary heart disease
CVD	Cardiovascular disease
DM	Diabetic mellitus
DREC	Department of research and ethical committee
EQA	External Quality Assurance
HDL	High density lipoprotein
HBA1C	Haemoglobin A1C
ICL	International clinical laboratory
IDL	Intermediate density lipoprotein

IQC	Internal quality control
LCAT	Lecithin Cholesterol Acyltransferase
LDL	Low density lipoprotein
LP A	Lipoprotein A
MUFA	Monounsaturated fatty acid
PRIME	Prospective Epidemiological Study of Myocardial Infarction
PUFA	Poly unsaturated fatty acid
RDL	Remnant density lipoprotein
SFA	Saturated fatty acid
TC	Total cholesterol
TFA	Trans fatty acid
TG	Triglyceride
VLDL	Very low density lipoprotein
WHO	World health organization

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## **Abstract**

**Background:** Type2 diabetes mellitus and its complication are becoming more prevalent in Ethiopia. It is estimated that 2-3 % of the population is living with diabetic. No Study is available in correlation of Apolipoprotein and lipid profile that is relevant in diagnosis and monitoring of diabetic dyslipidaemia in our local set up. Therefore the current study assessed the correlation of Apolipoprotein with lipid profile.

**Objective:** Assessment of Dyslipidaemia using lipidprofile, lipid ratios and Apolipoprotein value in type 2 diabetic patients who are apparently healthy attending diabetic clinic of the Tikur Anbessa Specialized Teaching Hospital, Addis Ababa.

**Method:** A Crossectional study was done during the study period from 15/10/2014 to 24/10/2016. Demographic variables were measured and Dyslipidaemia was assessed from overnight fasting serum sample by measuring Apolipoproteins and lipid profile. Degree of association and/or correlation between Apolipoprotein and classical lipid profile was evaluated using Spearman, Pearson correlation, independent sample t test , Man Whitney test and Chi square appropriately. SPSS version 20 software was used and histogram, Box Plot and Scatter plot was used to display results.

**Result:** A total of 70 type 2 Diabetic patients were participated in the study. Median age of the participants was 47(31-64). Dyslipidemia Observed in participants were high TC, low HDL, high LDL , high TG, low Apo A1, high Apo B100 ,high non HDL and high lipid ratios. Apo B 100 and Apo A1 are positively associated with LDL and HDL respectively. In addition

lipid ratios had significant positive association with atherogenic lipid parameters and negative association with anti-atherogenic lipid parameters.

**Conclusion:** Apolipoprotein A1 and B100 are associated with classical lipid profiles that help in diagnosis of dyslipidaemia in type 2 Diabetic patients

## 1. Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Type 2 diabetes, which accounts for 90–95% of diabetics, is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter category, a degree of hyperglycaemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms present for a long period of time before Type 2 diabetes is detected [1].

Dyslipidaemia is a disorder of lipid metabolism which includes lipid overproduction and/or deficiency which is characterized by change in blood lipid concentration that is low high density lipoprotein (HDL) and high triglyceride (TG) which is known to be major risk factor for cardiovascular disease in developed and developing world. Type 2 Diabetic mellitus (DM) which is the result of insulin resistance and islet of B cell dysfunction cause oxidation of low density lipoprotein (LDL) cholesterol and non-enzymatic glycation that can be internalized by macrophage is known risk factor for the development of atherosclerosis and complication of cardiovascular disease (CVD). The association between type 2 DM and dyslipidaemia also aggravate the condition and account for increased mortality of people [2].

Dyslipidaemia is the major risk factor for cardiovascular disease which is the number one cause of death in United States; Ischemic heart disease specially causes 1.4 million deaths in developed world and 5.7 million deaths in developing world. According to Center for disease control(CDC),preceding cancer and chronic lower respiratory disease, heart attack is leading cause of death in consecutive 3 years from 2009-2011 in USA[3].

Africa is a continent that has been affected by many communicable diseases for many years but recent data emphasize the alarming burden of non-communicable disease. Though there is a shortage of generalized studies in the continent, the prevalence of non-communicable disease in Cameroon was 43% in 2002[4].Likewise in Ethiopia, studies on the cardiovascular risk factors and complications of diabetes are not enough. Though Patient attendance rates and medical admissions in major hospitals are rising [5].

According to The American College of Cardiology and the American Heart Association (ACC/AHA) guideline that focuses on defining groups for whom LDL lowering is proven to be most beneficial, it recommends moderate- or high-intensity statin therapy for these four groups: Patients who have cardiovascular disease, Patients with an LDL, or “bad” cholesterol level of 190 mg/dl or higher, Patients with Type 2 diabetes who are between 40 and 75 years of age; and Patients with an estimated 10-year risk of cardiovascular disease of 7.5 % or higher who are between 40 and 75 years of age[6].The American Diabetes Association/American College of Cardiology (ADA/ACC) consensus report commend treatment goal of LDL-C 70 mg/dl and non-HDL-C goal of 100 mg/dl in addition to an Apo B goal of 80 mg/dl for patients with either established cardiovascular disease or diabetes with one risk factor[7].

Lipids are compounds that are soluble in organic solvent. They are complex alcohols that combine with fatty acid to form an ester. Upon classification of clinically important lipids cholesterol lies in sterol derivatives which have been found in almost all cells of animals. It is high molecular weight molecule containing 27 carbon atoms and starting point for metabolic pathways like steroid hormone synthesis, vitamin D synthesis, and bile acid metabolism, it also serve as a component of lipoprotein. Cholesterol from dietary product is esterified and converted to unesterified form so as to be readily absorbed and it can be converted in the liver to primary bile acids which promote fat absorption in the intestine by acting as detergents [8].

The use of saturated and trans fatty acid is associated with the development of CVD, Saturated fatty acids (SFA) are synthesized by the body and are not required in the diet; when taken in high amount in our diet SFA increase LDL concentration. Similarly Trans fatty acids (TFA) which are neither synthesized nor required by the body also increase the risk. It has been recommended that consumption of monounsaturated fatty acid and polyunsaturated fats derived from fish, olive oil, sunflower oil and nuts decrease the risk of CVD [9]. Dietary fat results in increased very low density lipoprotein secretion which causes high hepatic uptake of free fatty acid and occurrence of dyslipidemia [10]. Alcohol drinking especially ethyl consumption increase Apolipoproteins A (Apo A) and HDL-C concentration by initiating hepatic release of Apo A1 and Apo A11 which is the precursor of HDL particle and enhanced insulin sensitivity of tissues [11].

Lipids are carried in the bloodstream by complexes known as lipoproteins. Since lipids are not soluble in the plasma water, they travel in ultramicroscopic micelle-like complexes composed of phospholipids and protein on the outside with cholesterol, cholesterol esters, and triglycerides on the inside [12].

Lipoproteins are protein bound lipids which contain amphipathic cholesterol and phospholipid molecules are found on the surface of lipoproteins as a single monolayer, whereas the hydrophobic and neutral triglyceride and cholesteryl ester molecules are found in the central,

which are the main component transported by lipoprotein. They are classified based on their density by ultracentrifugation as chylomicron, VLDL, LDL and HDL in which the larger particle size, cholesteryl ester and TG content corresponds to lighter density. Low density lipoprotein are rich in cholesterol and deliver cholesterol to peripheral tissue and liver after triglyceride have been removed, High density lipoprotein which is produced by the liver and intestine transport excess cholesterol back to the liver by reverse cholesterol transport pathway. It can influence glucose metabolism directly and possibly improve insulin resistance by altering plasma membrane composition through enhanced cholesterol efflux [13].

Apo are protein portion of lipoprotein that serve as carrier for lipid which are plasma insoluble, Apo's maintain the structural integrity of lipoproteins and also serve as ligands for cell receptors, activators and inhibitors of different enzyme that change lipoprotein particle. Apo A1 is the principal protein of HDL and increase in accordance with plasma HDL level [14] it has crucial role as a cofactor of lecithin cholesterol Acyltransferase (LCAT) which is important in removing excess cholesterol from tissues and incorporating it into HDL for transport of cholesterol back to the liver it also assist excess cholesterol in peripheral cell to be externalized to HDL [15].

Apo B is high molecular weight protein which is found in LDL, Chylomicron, VLDL, Apo B 100 is the major one that help the uptake of LDL by the cell. The reason for Apolipoprotein B to be best marker for Cardiovascular disease like atherosclerosis is because it includes the cholesterol content of all atherogenic lipoproteins including LDL, IDL (intermediate density lipoprotein), RL (Remnant lipoprotein), VLDL and LP a (lipoprotein a), Therefore recently recommended to be included in lipid profile.

The Prospective Epidemiological Study of Myocardial Infarction (PRIME) study examined the association between the incidence of CHD (coronary heart disease) and several HDL related parameters, including HDL-C itself, Apo A-I, HDL A-I, and HDL A-I : A-II, All four parameters were related to CHD risk, however, Apo A-I was the strongest predictor

[16]. Plasma concentration of Apolipoproteins mainly depends on dietary intake, physical activity, age and sex [17]. Beyond measurement of lipid profile and Apolipoprotein, Non-HDL Cholesterol level is easily available to the clinician with every lipid profile ordered, thus eliminating any additional costs. Also, its derivation does not require a lipid profile to be done in the fasting state, and it avoids the potential inaccuracy caused by the inherent intra individual variability of the triglyceride measurements and plays an important role in atherogenesis of diabetics [18].

Atherogenicity index, TC/HDL and LDL/HDL ratio are the two most important indicators of vascular risk and possess higher predictive value than isolated parameters [19]. As TC/HDL ratio is considered more sensitive and specific index of cardiovascular risk than total cholesterol, the Canadian working group has chosen this lipid ratio as a secondary goal of therapy [20]. The LDL/HDL cholesterol ratio appears to be as useful as the TC/HDL ratio. Since two thirds of plasma cholesterol is found in LDL, TC and LDL are closely related. Like the TC/HDL ratio LDL/HDL ratio has more predictive power if triglyceridemia is taken into account [21]. The ApoB/ApoA-I ratio was stronger than the TC/HDL ratio and LDL/HDL ratio in predicting risk. The greater the ApoB/ApoA-I ratio, the larger the amount of cholesterol from atherogenic lipoproteins circulating through the plasma compartment and likely to induce endothelial dysfunction and trigger the Atherogenicity process [22].

It has been firmly established that VLDL concentration assessed by fasting TG levels is a major determinant of LDL size. Nevertheless postprandial hypertriglyceridemia, increased cholesteryl ester transfer protein activity, and decrease in hepatic lipase are the mechanisms that induce a decrease in HDL in type 2 diabetes [23].

Dyslipidaemia is the commonest complication of diabetes mellitus and it predisposes premature atherosclerosis and macro vascular complications. Common lipid abnormalities in diabetes are high TG, LDL-C, TC and low HDL. Since many studies in Ethiopia haven't address the relevance of Apolipoprotein in Type 2 diabetic mellitus patients that have greater

value in indicating risk of CVD, This paper tried to assess Apolipoprotein value and its correlation with classical lipid profile that serve as a baseline for physician decision. It also proposes the relationship between Age, gender, BMI, BP and lipid profile.

## **2. Statement of the problem**

The prevalence of non-communicable disease is rising rapidly; by 2020 in Africa it is expected to cause almost three-quarters deaths as communicable disease. It is anticipated to surpass maternal, Perinatal, and nutritional diseases as the most common causes of death by 2030. A study conducted in Nigeria indicates Dyslipidaemia was highly prevalent in all of its geopolitical zones with the consistent pattern being low HDL-Cholesterol and high LDL-C. Overall, the prevalence of dyslipidaemia ranged from 60% among apparently healthy Nigerians to 89% among diabetic Nigerians [24]. Another Study conducted in Ethiopian Diabetic patient shows the most common dyslipidemia was high level of serum LDL followed by hypercholesterolemia and hypertriglyceridemia [25]. In developing countries like Ethiopia which is been engaged in combating many communicable diseases, dyslipidaemia and other non-communicable disease also require equal notice and baseline research must be conducted to plan better strategies that potentially reduce the future burden.

Recent studies have shown that Apo B provides better information regarding risk of coronary artery disease. Apo B identifies high-risk dyslipidaemia phenotypes that are not detected by standard lipid profile in type 2 diabetic patients. Its addition to standard lipid profile could aid in timely introduction of lipid lowering therapy in unidentified high risk patients and thus reduce mortality and morbidity due to future cardiovascular complications [26]. The Apolipoprotein concentrations are minimally influence from biological variables when

compared with lipid measurements. The ApoB100/Apo A-I ratio showed a significance correlation with glycated hemoglobin indicating the adverse effect of prolonged hyperglycaemia on the Apolipoproteins [27].

In the present Study, In addition to classical lipid profile and Apolipoprotein different lipid ratios were calculated that can determine CVD risk, TC/HDL, TG/HDL, LDL/HDL and Apo B/Apo A. Hence this study will try to provide baseline data on the correlation between Apolipoproteins A, B, lipid ratios and common lipid panels to be used as a tool for assessing dyslipidaemia in Type 2 diabetic patients.

### **3. Significance of the study**

Since Diabetic dyslipidaemia is characterised by retention of atherogenic particles, which are depleted of cholesterol, measuring LDL or VLDL cholesterol may not reflect the actual number of these atherogenic particles, while the plasma concentration of Apo B indicates their cumulative number[28]. The significance of this study is to determine the correlation of lipid profile and Apolipoprotein in diabetic patients in our country concrete circumstances that can serve as useful information to understand magnitude of dyslipidaemia in type 2 Diabetics and The use of Apolipoprotein markers that could be the next step in assessing the patient risk, and represent an additional to conventional lipid markers.

## **4. Literature review**

Cardiovascular diseases are the main cause of mortality around the globe and the main risk factors are diabetes mellitus, hypertension, cigarette smoking, dyslipidaemia, obesity and physical inactivity [29]. In terms of attributable deaths, the leading CVD risk factor is raised blood pressure, which cause 13 % of global death, followed by tobacco use (9%), raised blood glucose (7%), physical inactivity and obesity (6 %) [30].

Diabetic Dyslipidaemia developed from Dietary intake is known risk factor for atherosclerosis. A comparison study was conducted in 1994 in relationship between dietary intake, lipoprotein, Apolipoproteins in 423 Taipei, China and 420 Framingham, USA study subjects. According to the study, shift from stir frying lard (fat derived from pig abdomen) to the use of soybean oil in China explain for favourable lipid profile due to the fact that stir frying needs fat or oil to absorb heat and gives better flavour. similarly In addition to (body mass index) BMI and smoking habit; carbohydrate intake, polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) consumption shows relatively better lipid profile association than other dietary intakes [31]. Another study conducted in USA in 2011 shows low carbohydrate and low saturated fat intake decrease the risk of dyslipidaemia by lowering LDL, non HDL cholesterol and Apo b level compared with low carbohydrate and high saturated fat intake [32].

Another risk factor indicator is Waist circumference, which is a better pointer than BMI and waist to hip ratio because it is a measure of total abdominal fat accumulation that can't be measured by the above measurements. And it shows central adiposity through thrombotic mechanism of Apo B. A study on the association of waist circumference with Apo A and Apo B ratio in black and white Americans In 1999, indicate a positive correlation independent of age, increased waist circumference was associated with increased Apo B, TG, LDL, blood glucose, TC, insulin and decreased Apo A and HDL [33].

The effect of physical exercise on atherosclerosis has been a controversial issue for many years one of the reasons for this is the interference of different diet. A study conducted on the effect of physical exercise on lipid, lipoprotein and Apolipoproteins in New Zealand 2006, compare subjects with sedentary life style and those who perform 12 week of aerobic exercise, support the other studies finding and shows, increase in the Apo-A1: Apo-B ratio seen only among the exercising group which is associated with a decrease in Apo B level, suggesting that this measure is sensitive to light exercise and responds in a Relatively short period. The Study adds that this method is appropriate for monitoring CHD risk factor change during short-term light-exercise intervention [34]. Another study in Brazil conducted in footballers in 2011 shows the association between physical exercise and LDL and TG is insignificant because they had gone under stressful training and must maintain their fitness by high energy diet like high carbohydrate rich diet that increase plasma triglyceride which is independent risk factor for CHD [35].

Many studies in the past times reveal the fact that tobacco smoking is risk factor for Mets because of its effect on waist circumference, blood lipids, blood pressure and direct negative effect on insulin resistance. A population based cohort study conducted in association between smoking, component of metabolic syndrome and lipoprotein particle size in 24,389 men and 35,078 women aged between 18 and 80 years from 2006-2012 on Netherland, shows independent of sex and BMI, dose dependent daily tobacco smoking contribute to the occurrence of Mets and with increasing BMI current smoker have lower level of HDL and

Apo A1 and HDL to Apo A1 ratio and higher level of TG, Apo B and waist circumference. In contrary the finding indicate no consistent association between smoking and blood pressure or blood glucose[36].

Alcohol consumption has cardio protective effect which depends on dose, amount and type, A cross sectional study on The Correlation between Alcohol Consumption, Lipids, Apolipoproteins and Coronary Heart Disease in 105 subjects aged from 35-75 years in France 2008; tried to assess the above fact with evidence and the finding shows Alcohol intake was positively associated with male gender, cigarette smoking and inversely associated with prevalence of diabetes and hypertension, with moderate alcohol intake.

Similarly in other studies light to moderate alcohol consumption (up to 1 drink daily for women and 1-2 drinks daily for men) is greatly linked with decreasing CVD risk however excessive consumption cause damaging of HDL- C[11]. Article review on Wine, Beer, Alcohol and Polyphenols on Cardiovascular Disease in Spain 2012, explains the type of beverages which is more cardio protective and emphasize moderate and regular red wine and beer consumption have better beneficial role with respect to reduced risk of CVD. But A person blood type, genetics, age, gender, drug supplement and healthy diet (including fruit vegetable and whole grain) are the important factor that maintain the beneficial effect [37]. Since cigarette and Alcohol used together their additive effect is dependent on their dose there is no evidence that shows the effect of consumption of both can worse the risk in comparison with using cigarette and Alcohol alone [38].

Now a day's Khat plant (*Catha edulis* forsk) is widely used for its pleasant stimulant effect in many countries. A Yemen article review in 2007 tried to explain some of the effect of chat on circadian rhythm of myocardial infarction that shifts from early morning in non-Chewers to afternoon in khat chewers [39]. Though some researchers argue on the harmful effect of the plant by stating its hypoglycaemic effect on diabetic patients, others oppose the idea because a chance for diabetic khat chewer person not to follow the appropriate dietary regulation is very high which is accompanied by consuming sweet drinks that account for increased harmful effect of khat[40]. Similar study in Ethiopia in 2010 on the effect of khat on CVD risk like blood pressure indicate khat chewers have high diastolic blood pressure that is associated with the presence of Cathionine in chat leaf[41].

Many studies indicate that the Apolipoprotein B (ApoB) level, represents the total number of atherogenic lipoprotein particles (each particle contains a single ApoB molecule), better associated with CHD than the LDL cholesterol level in untreated as well as statin-treated individuals [28]. Patients were randomized to treatment with rosuvastatin, atorvastatin, or simvastatin to compare the efficacy and safety of the most widely prescribed statins. In untreated patients, an LDL-C level of 100 mg/dl was approximately equivalent to an Apo B level of 90 mg/dl. But in patients treated with a statin to an LDL-C goal of less than 100 mg/dl, only 48% reached their Apo B goal of less than 90 mg/dl [42].

In cross-sectional population based survey conducted in Taiwan 2013, indicate in comparison with Apo B/LDL-C, LDL-C/HDL-C, Apo B /ApoA1, LDL-C, HDL-C, and TG, ApoA1/HDL-C was highly associated with diabetes[43].After the recent discovery of Apolipoproteins, Apo B/Apo A which shows the balance between anti atherogenic and atherogenic, has been better predictor of CVD risk than traditional lipid profiles [14].

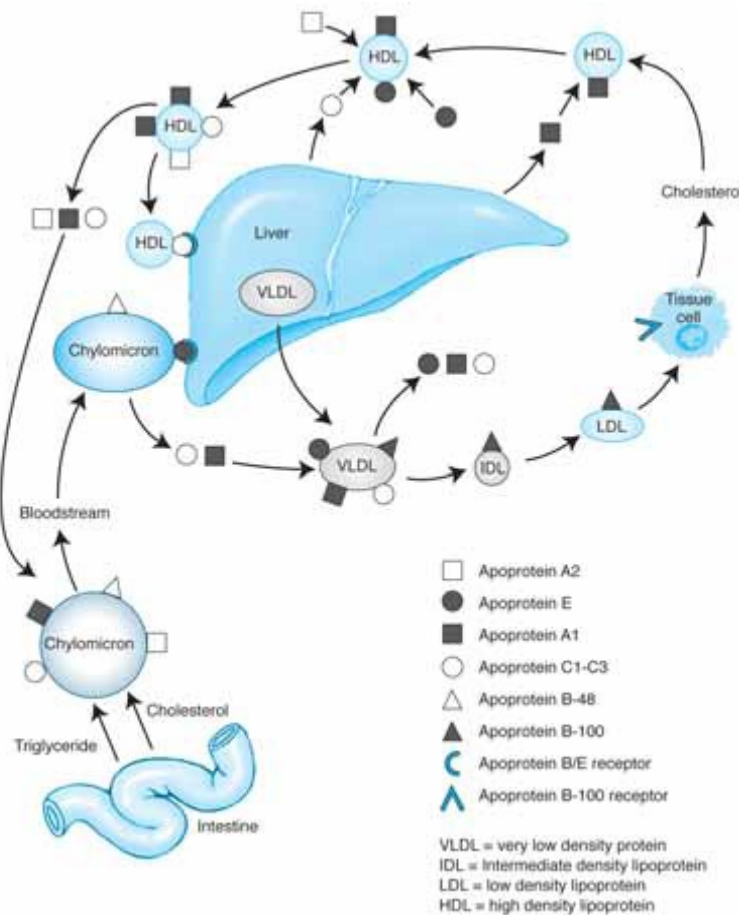


Figure 1.1 Liver synthesis and metabolism of lipids from Apolipoprotein perspective from Arneson clinical chemistry a laboratory perspective (2016)

In a Study conducted to evaluate the Lipid profile including Non-HDL Cholesterol levels and LDLC/HDL-C ratio in type II Diabetic Patients as markers of diabetic dyslipidaemia, Sex and age matched group of diabetic and normal subjects were included and a significant increase in non HDL cholesterol and LDL/HDL ratio was revealed in diabetic subjects.

That strength the notion of using Non-HDL Cholesterol and LDL/HDL ratio as more representative of all atherogenic lipoproteins, and to use them as markers of Diabetic dyslipidaemia than using LDL Cholesterol alone [18]. Another Study conducted to assess Apo B/Apo A ratio as a predictor of CVD in India shows the ratio reflects both atherogenic

and antiatherogenic lipid related risk, can be used as a better predictor better than traditional lipid markers like LDL and HDL to analyse Atherogenicity[44].

A study of lipid profile levels in diabetics and non-diabetics taking TC/HDL ratio and LDL/HDL ratio into consideration in India in 2014 shows The TC/HDL ratio is a specific and sensitive index of cardiovascular risk and predictor of CHD especially with values above 6.0 [45]. Another Study, Ratio of Triglycerides to HDL Cholesterol is an Indicator of LDL Particle Size in Patients With Type 2 Diabetes and Normal HDL Cholesterol Levels. TG-to-HDL cholesterol molar ratio  $> 1.33$  distinguishes the small and large LDL size pattern. This ratio may be used to identify diabetic patients with atherogenic lipid profile and may be relevant for assessing CAD risk. Especially in newly diagnosed patients, it may be useful for the selection of patients who need aggressive treatment of lipid abnormalities early in the course of diabetes or before the onset of clinical cardiovascular disease [46].

An Observational Study conducted in Cameroon in 2012 on micro and macro vascular complication of diabetics mellitus; risk factors and effect of diabetic check-up reveal the prevalence of Micro vascular complication, retinopathy was 23.6% in diabetic patients. They also reported major Macro vascular complication, CAD was 23.6%. It was concluded that the results of vascular complications exhibited in subjects were due to less glycaemic control practice [47].

Different study on dyslipidaemia were conducted in Ethiopia, A Cross sectional Study on Dyslipidaemia among diabetic patients in Southern Ethiopia shows Significantly higher mean serum levels of TC and LDL in type 2 DM than type 1 DM[25].

Other studies that were conducted in glycaemic control of diabetic patients are as follows. A study conducted in assessment of magnitude of glycaemic control and its associated factors in type 2 diabetic patients in Ethiopia, 2015 shows 80 % of diabetic patients shows poor

glycaemic control, which means none of the patients involved in the study has HbA1c determination due to expensiveness and unavailability of the test in the country. It was also found longer duration of diabetics and being on insulin therapy associated with poor glycaemia control [48]. A Crosssectional study accompanied on insulin resistance and dyslipidaemia in type 2 diabetic patients in 2015, shows Poor glycaemic control seems to be related to diabetic dyslipidaemia, and many of the patients in this study had uncontrolled dyslipidaemia[49].

Likewise A study conducted in Dyslipidaemia Associated with Poor Glycaemic Control in Type 2 Diabetes Mellitus and the Protective Effect of Metformin Supplementation reveal type 2 DM patients in 2012 indicate Improved glycaemic control and dyslipidaemia were observed in patients on combination therapy of metformin and Glibenclamide that shows good glycaemic control could result in improvement of lipid profile and patient could minimize their cardiovascular risk and underline combination therapy[50].

As the above studies indicate there are limited studies in assessment of dyslipidemia using Apolipoprotein in Ethiopia and this study will try to provide baseline data on the correlation between Apolipoproteins A and B and common lipid panels to be used as a tool for assessing dyslipidaemia in diabetic patients.

## **5. Objective**

### **5.1. General objective**

- To assess dyslipidaemia using lipid profile and Apolipoprotein in Type 2 Diabetic patients

## **5.2. Specific objective**

- To evaluate the association of lipid profile and Apolipoprotein in Type 2 diabetic patients
- To evaluate the relationship between anthropometric variables, BMI, BP, FBS and lipid profile including Apolipoproteins

## **Hypothesis**

There is no association between classical lipid profile and Apolipoproteins in Type 2 diabetic patients.

## **6. Methodology**

### **6.1. Study design**

Cross sectional study was conducted at Tikur Anbessa specialized hospital

### **6.2. Study area and period**

The study was conducted in Tikur Anbesa specialized hospital (TASH). It is found in Lideta Sub City, Addis Ababa Ethiopia. It is the largest referral hospital in the country with 700 beds and is the main teaching hospital for both clinical and preclinical training of most disciplines. The hospital has separate endocrinology centre which provide specialized service for patients with different endocrinological problem. Every year about 10,000 clinic visits of the patients with type 2 diabetes provided service at this centre. The endocrinology unit provide two clinics visit every week for diabetic patients. The study was conducted from 15/10/2014 to 24/10/2016. Blood analysis was done at international clinical laboratories, which is internationally accredited.

### **6.3. Source population**

All type 2 diabetes patients who have follow up at diabetic clinic of TASH.

### **6.4. Study population**

Study population was Type 2 diabetes outpatients attending diabetic clinic of TASH during data collection period.

## **6.5. Inclusion and Exclusion criteria**

### **6.5.1. Inclusion criteria**

Patients who were diagnosed to have type 2 diabetes.

Volunteers who participate in the study

### **6.5.2. Exclusion criteria**

Pregnant or lactating women

Patients taking lipid lowering drug.

Subjects who had other debilitating chronic disease like liver disease

### **6.6. Sample size**

Convenient sampling was used to select study subjects to assess the association between Apolipoproteins and classical lipid profile in type 2 diabetic patients. According to International Diabetic Federation's (IDF) 3.5% prevalence of type 2 diabetes in Ethiopia. Where N- number of patients, Z- confidence interval (95%), P – Proportions 3.5%, d- the level of confidence is 5%.

$$N = (Z_{\alpha/2})^2 P (1-P)$$

$$N = (1.96/0.035)^2 0.035(1-0.035) = 106$$

But Due to reagent shortage minimum of 50-70 subjects was expected to participate and 70 study subjects were involved.

### **6.7. Sampling procedure**

Anthropometric measurements and Fasting Blood sample were collected by trained nurses from volunteer participants after they sign the consent form prepared both in Amharic and English language. Assigned nurses were trained for accurate height, weight and Blood pressure measurement and blood collection.

## 6.8. Data collection method

The data collection had three approaches

- **Anthropometric measurement** BP, Height and weight were measured with the subject in light clothes without shoes, and Body Mass Index (Kg/m<sup>2</sup>) was calculated.
- **Blood collection** Blood sample was collected after overnight fasting. 5mL of blood was collected from study subjects by employing standard infection prevention procedures. The collected aliquot of blood serum was used to determine FBS, TG, TC, HDL-C, LDL-C, Apolipoprotein A1 and B100 at ICL Addis Ababa, Ethiopia.
- **Biochemical measurement** TC was determined by enzymatic method using cholesterol esterase. TG concentration was determined by standardized enzymatic procedures using glycerol phosphate oxidase assay. HDL-C was measured using Ultra-HDL assay which is a homogeneous method for directly measuring HDL-C concentrations in serum. LDL-C was determined by combination of detergents and phosphorous compounds. Participants' FBS was determined using standardized glucose oxidase method. Apolipoproteins were measured by immune turbidimetric assay. Lipid profile, Apolipoprotein and FBS concentration was reported as mg/dl.

## 6.9. Study variables

### 6.9.1. Dependent variables

- Apolipoproteins A1 and B100, TC, TG, HDL, LDL

### 6.9.2. Independent variables

- Fasting blood sugar
- Age
- Sex
- Blood Pressure
- Body Mass Index

### **6.10. Data management and analysis**

Data entry was done using SPSS version 20 software. Data was checked for accuracy and consistency and error in the data was corrected. Data was checked for normality and logarithmical transformation was done for non-normally distributed data then correlation was done for categorical and linear data. P values less than 0.05 was considered significant.

### **6.11. Quality assurance**

The quality of data was maintained through training given for data collectors who were experienced nurses on the objective of the study, Anthropometric measurement, blood collection and how to retain confidentiality and privacy of the study subjects. The collected data was checked for completeness and accuracy and corrected on daily basis before leaving the facilities. Data was labelled before entry and edited appropriately by the principal investigator (PI) and entered into statistical computer package using SPSS version 20 and checked for missing data by PI. During the blood analysis, internal quality control (IQC) materials were performed before study participants' samples. The IQC samples results were equivalent to expected known values of the analyte under analysis. They were assigned in three levels mainly used to ensure good precision of analysis. In addition the laboratory actively participate in External Quality assurance (EQC) programs that it is accredited by USA based Joint Commission International for four times in a row and also received first level National Quality Excellence Award from the Ethiopian Quality Award (EQA) organization.

### **6.12. Ethical consideration**

Ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of College of Health Sciences, Department of Laboratory Sciences of Addis Ababa University. Clearly written information sheet was given to all subjects who agreed to participate. Informed consent was obtained from all subjects on a prescribed form. The confidentiality of the study data was maintained by coding of samples and data.

### **6.13. Dissemination of result**

The study on completion could serve as a reference material and a baseline to researchers and experts for further study. To reach these bodies the finalized paper will be submitted to College of Health Sciences, Department of Laboratory Sciences. So it can serve as a reference in the library. In addition, a copy of this material will be given to different laboratories to consider the final result on their standard operating procedure. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant workshops and seminars.

## **7. Result**

### **7.1. Study Subjects**

A total of seventy diabetic patients 32 (45%) male and 38(55%) female were participated in this study. The age ranged from 31 to 63 years median of 47 (**Table1.1**). There is no missing anthropometric and lipid profile data of all the study participants. From the total of 70 study

participants' normal lipid profile were 34(49%) TC, 4(5.7%) HDL, 36(51%) LDL, 40(57%) TG, 43(61%) Apo A1 and 31(44%) Apo B100.

**Table 1.1 Age and sex description of type 2 Diabetes Patients (N=70)**

Sex	Age group			
	31-41	42-52	53-63	Total
Male (Column %)	10(55.6)	11(42.3)	11(44)	32(45.7)
(Row %)	31.2	34.4	34	100
Female (Column %)	8(44.4)	15(57.5)	15(56)	38(54.3)
(Row %)	21.1	39.5	38.6	100
Total (column %)	18(100)	26(100)	26(100)	70(100)
(Row %)	25.7	37.1	37.1	100

**Table 1.2.Lipid profile, Apolipoprotein and Lipid ratio in Age matched type 2 Diabetic subjects (N=70)**

Age group	Mean ±SD										
	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	Apo A1 mg/dl	ApoB 100 mg/dl	Non HDL mg/dl	TC/HDL	TG/HDL	LDL/HDL	Apo B100/ApoA1
31-41	195±58	130±36	37±15	135±66	99±16	127±36	160±69	6.3±3.7	3.9±1.8	4.5±3.3	1.3±0.4
42-52	206±57	140±38	36±12	137±60	92±19	126±46	169±62	6.4±3	4.3±1.9	4.4±2.6	1.4±0.6
53-63	198±58	147±43	38±11	133±51	102±23	152±54	160±56	8±13	4.2±1.5	3.7±2	1.6±0.8
X <sup>2</sup> (p value)	7.5(0.28)	3.38(0.76)	4(0.67)	9(0.47)	1(0.84)	1(0.7)	1(0.78)	1.3(0.72)	1.3(0.72)	2.8(0.43)	20(0.38)

TC, LDL and TG value directly related with age of study subjects. While HDL and Apo A value remains in the same pattern. Apo B value has direct relationship with Age. Non HDL value is normal only among 42 -52 years old. But like other profiles it also increases with increasing age. The highest TC/HDL was observed in 42-52 years old. TG/HDL shows the same amount of increase in all age group. The highest LDL/ HDL ratio exhibited in 42-52 years old. Likewise Apo B/Apo A ratio increase with increasing Age.

**Table 1.3. Lipid profile, Apolipoprotein and Lipid ratio in sex matched type 2 Diabetic subjects (N=70)**

	Mean ±SD										
<b>Gender</b>	<b>TC</b> mg/dl	<b>TG</b> mg/dl	<b>HD</b> <b>L</b> mg/dl	<b>LDL</b> mg/dl	<b>Apo</b> <b>A1</b> mg /dl	<b>Apo</b> <b>B100</b> mg/dl	<b>Non</b> <b>HDL</b> mg/dl	<b>TC/H</b> <b>DL</b>	<b>TG/H</b> <b>DL</b>	<b>LDL/H</b> <b>DL</b>	<b>Apo</b> <b>B100/A</b> <b>po A1</b>
<b>Male</b>	196±5 5	138± 41	34± 11	131± 57	90±1 2	130± 44	163± 60	6.3±2.6	4.4±1.7	4.3±2.4	1.5±0.6
<b>Female</b>	205±5 6	141± 37	39± 12	139± 58	102± 23	137± 49	166± 62	7.3±9.7	4±1.8	4.2±2.7	1.4±0.7
<b>X<sup>2</sup>(p)</b>	1.2(0. 61)	2.48( 0.29)	6(0. 04)	1.56( 0.67)	7(0.0 4)	1(0.6 9)	1.1(0. 06)	0.9(0.4 1)	2(0.11)	0.5(0.9 6)	0.5(0.96 )

In terms of Gender Lipid profile of study subjects in both sexes shows an increase beyond normal range except Triglyceride. Female exhibit increased TC, LDL, Apo B100, non HDL than males but lipid ratios in both sexes had the same pattern.

**Table 1.4.Lipid profile, Apolipoprotein and Lipid ratio in BMI matched type 2 Diabetic subjects (N=70)**

BMI	Mean±SD										
	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	Apo A1 mg/dl	Apo B100 mg/dl	Non HDL mg/dl	TC/H DL	TG/H DL	LDL/H DL	Apo B100/A poA1
<b>18-24 kg/mm<sup>2</sup></b>	190 ±60	129 ±32	38±1 4	128± 63	106 ±22	140± 52	153± 66	6±3.2	3.9±1.7	4±3	1.4±0.7
<b>25-29 kg/mm<sup>2</sup></b>	202 ±55	146 ±42	36±1 0	140± 53	93± 18	124± 44	168± 59	8±10	4.4±1.8	4.3±2.5	1.4±0.6
<b>&gt;30 kg/mm<sup>2</sup></b>	219 ±50	151 ±40	34±9	141± 56	87± 10	148± 38	185± 50	7±2	4.6±1.6	4.4±2	1.7±0.5
<b>X<sup>2</sup>(p)</b>	2.5( 0.28 )	6.4( 0.01 )	1.8(0. 39)	1(0.9 7)	1.1( 0.76 )	1(0.9 2)	1.7(0. 2)	1(0.3 88)	1.3(0.5 2)	1.1(0.78 )	1.2(0.81 )

*<18.5 kg/mm<sup>2</sup> Underweight 18.5-25 kg/mm<sup>2</sup> Normal 25-29.9 kg/mm<sup>2</sup> Over weight*

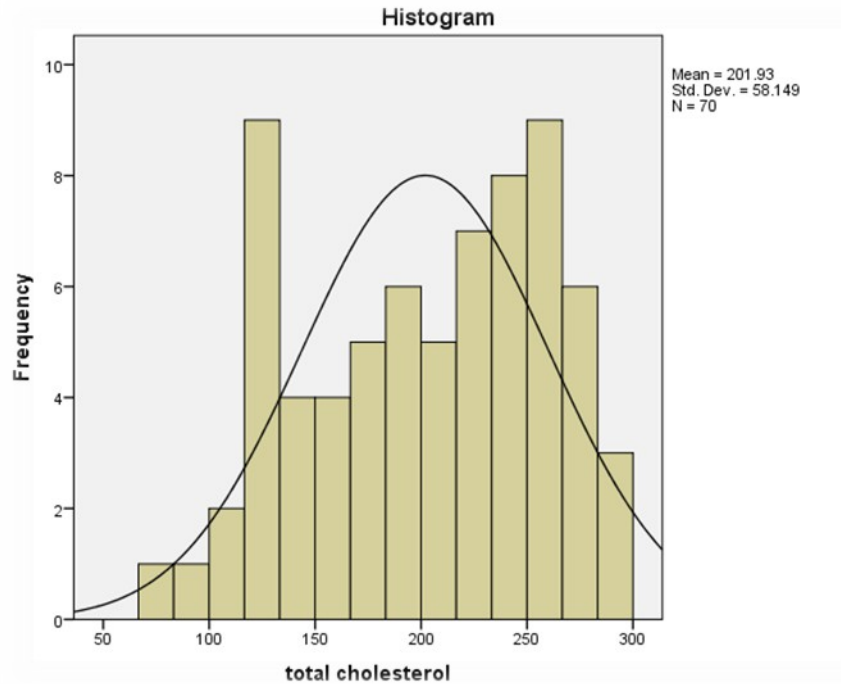
*>30 kg/mm<sup>2</sup> Obesity*

As table 1.4 shows over weight (BMI 25-29) and obese (BMI>30) patients exhibits higher atherogenic value, with increased TC, LDL, Apo B100, Non HDL and lipid ratios. In contrary their protective cholesterol, HDL and Apo A1 tend to decrease

## **7.2. Laboratory variables**

### **7.2.1. Total Cholesterol (TC)**

The minimum and maximum was 76 and 297 mg/dl respectively, with mean of 201 and standard error 6.9. The median was 206 mg/dl (with 95% CI of 185,237). The overall distribution has Skewness -0.25 and Kurtosis -1.13. The 2.5th and 97.5th percentile value of TC was 90.7mg/dl and 295.5 mg/dl respectively. The overall distribution of data (Using Shapiro-Wilk normality test) has not shown statistically significant difference from Gaussian distribution ( $p = 0.06$ ).



**Figure 1.2.** Distribution of Total Cholesterol in Type 2 diabetic patients (N=70)

The level of TC was tested for association with different host factors. It was found not to be associated with them.

**Table 1.5.** Association of host factors with Total cholesterol

	Sex <sup>a</sup>	Age <sup>b</sup>	BMI <sup>b</sup>	FBS <sup>b</sup>	BP <sup>c</sup>
P value	0.76	0.12	0.07	0.21	0.09
r	-	-	-	-	-
X <sup>2</sup> (d.f)					7.23(2)

*a- Independent sample t test*

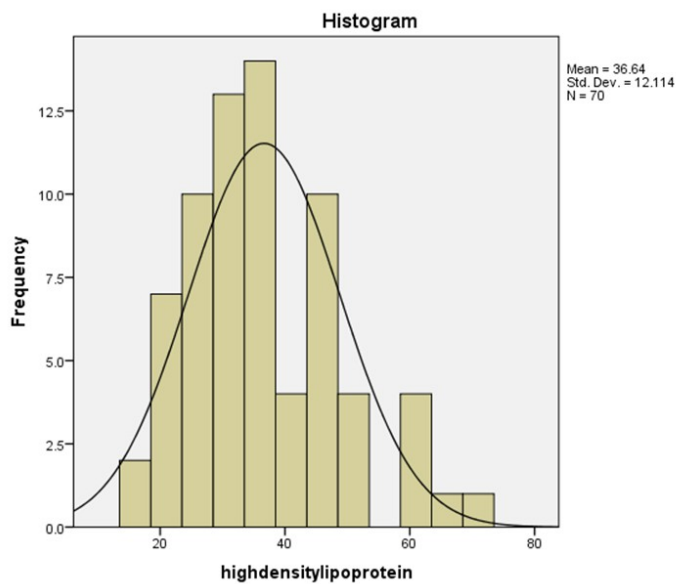
*b- spearman's rank correlation*

*c- Kruskal-Wallis rank test*

Even if there is a slight increase in Total cholesterol in increasing age group they don't have statistically significant association.

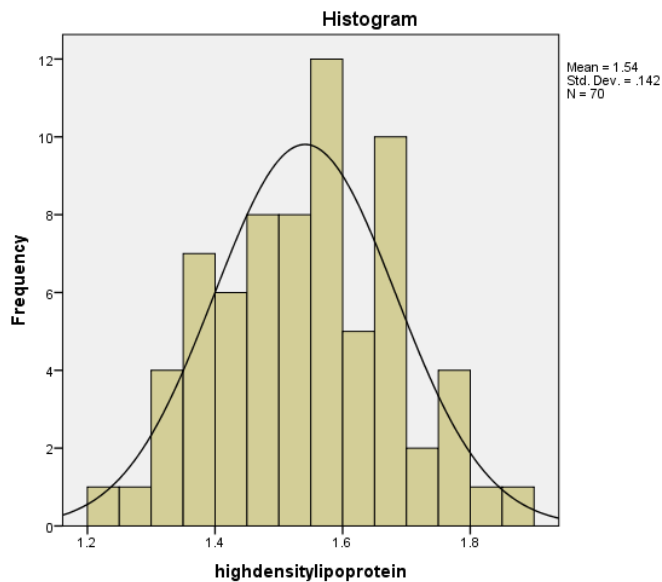
### 7.2.2. High Density Lipoprotein (HDL)

The minimum and maximum value was 16 and 72 mg/dl respectively, with mean of 36.6 and standard error 1.45. The median was 35 mg/dl (with 95% CI of 30, 38). The overall distribution (figure 9.2) has Skewness 0.7 and Kurtosis 0.18. The 2.5th and 97.5th percentile value of HDL was 17.5mg/dl and 65.8 mg/dl respectively. The overall distribution of data (Using Shapiro-Wilk normality test) has statistically significant difference from Gaussian distribution ( $p = 0.04$ ).



**Figure 1.3.** Distribution of HDL in Type 2 diabetic patients (N=70)

After logarithmically normalized and 2.5<sup>th</sup> and 97.5<sup>th</sup> percentile values were computed, the antilog of these values was found to be 17.5-65.8 mg/dl (**figure1.4**). Using Shapiro-Wilk normality test, the distribution has not stastically significant difference from Gaussian distribution (p = 0.06).



**Figure 1.4.**The level of HDL distribution after data was logarithmically normalized.

The level of HDL was tested for association with different host factors. HDL value was found to be associated with sex

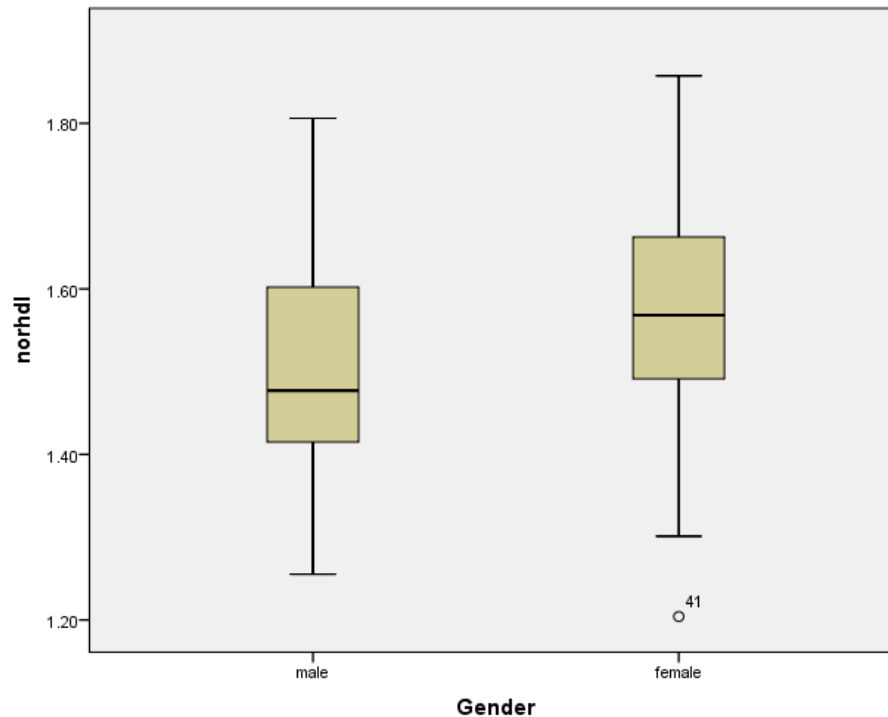
**Table 1.6.**Association of host factors with HDL

	Sex <sup>a</sup>	Age <sup>b</sup>	BMI <sup>b</sup>	FBS <sup>b</sup>	BP <sup>c</sup>
P value	0.04	0.38	0.4	0.12	0.35
r	0.24	-	-	-	-
X <sup>2</sup> (d.f)	-	-	-	-	43.3(2)

a- Mann-Whitney two sample rank sum test

b- spearman's rank correlation

c- Kruskal-Wallis rank test



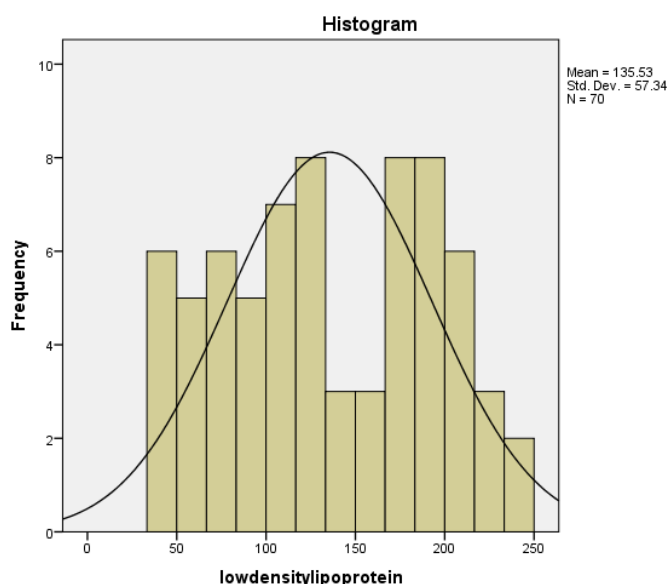
P.value 0.04

**Figure 1.5.** Box-whisker of Gender specific HDL Value of Type 2 Diabetic patients (N=70)

### 7.2.3. Low Density Lipoprotein

#### (LDL)

The minimum and maximum value was 36 and 241 mg/dl respectively, with mean of 135 and standard error 6.8. The median was 128 mg/dl (with 95% CI of 108,168). The overall distribution (Figure 3.1.) has Skewness 0.02 and -1.2 Kurtosis. The 2.5th and 97.5th percentile value was 41.4 mg/dl and 235.6 mg/dl respectively. The overall distribution of data (Using Shapiro-Wilk normality test) has not statistically significant difference from Gaussian distribution.  $p=0.08$



**Figure 1.6.** Distribution of Low density lipoprotein among Type 2 Diabetic patients (N=70)

The level of LDL was tested for association with different host factors. And shows no association

**Table 1.7.** Association of LDL with host factors

	Sex <sup>a</sup>	Age <sup>b</sup>	FBS <sup>b</sup>	BMI <sup>b</sup>	BP <sup>c</sup>
P value	0.85	0.68	0.79	0.29	0.13
r	-	-	-	-	-
X <sup>2</sup> (d.f)					8.12(3)

*a- Independent sample t test*

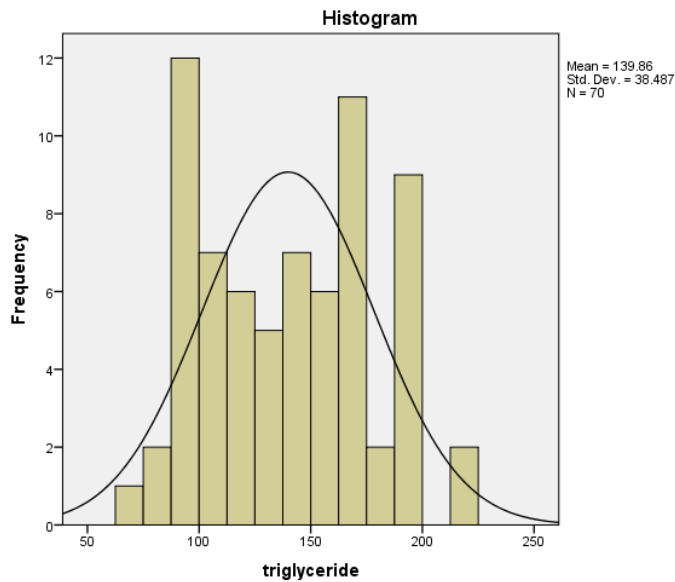
*b- spearman's rank correlation*

*c- Kruskal-Wallis rank test*

#### 7.2.4. Triglyceride (TG)

The minimum and maximum value was 70 and 222 mg/dl respectively, with mean of 139 and standard error 4.6. The median was 143 mg/dl (95% CI of 115,155). The overall distribution is shown in Figure 3.1.), Skewness 0.17 and -1.0 Kurtosis. The 2.5th and 97.5th percentile value

is 80 mg/dl and 220.5 mg/dl respectively. The overall distribution of data (Using Shapiro-Wilk normality test) has not statistically significant difference from Gaussian distribution ( $p = 0.08$ ).



**Figure 1.7.** Distribution of Triglyceride among type 2 Diabetic patients (N=70)

**Table 9.1.11.** Association of TG with host factors

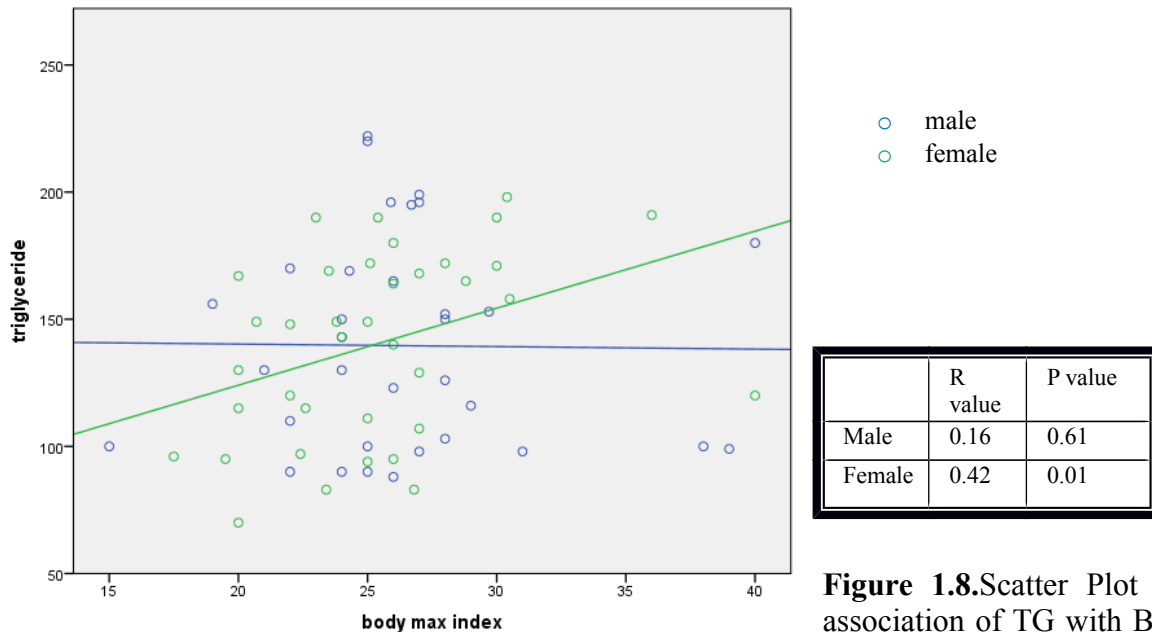
	Sex <sup>a</sup>	Age <sup>b</sup>	BMI <sup>b</sup>	FBS <sup>b</sup>	BP <sup>c</sup>
P .value	0.45	0.05	0.04	0.59	0.23
r			0.25		
X <sup>2</sup> (d.f)					10(4)

*a. Independent sample t test*

*b. spearman's rank correlation*

*c. Kruskal-Wallis rank test*

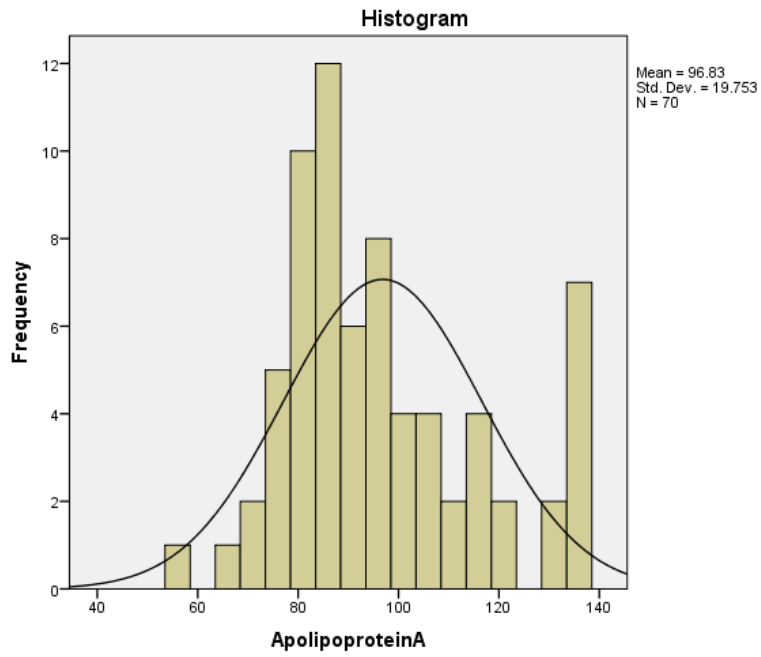
The level of TG was tested for association with different host factors and it was found to be associated with BMI.



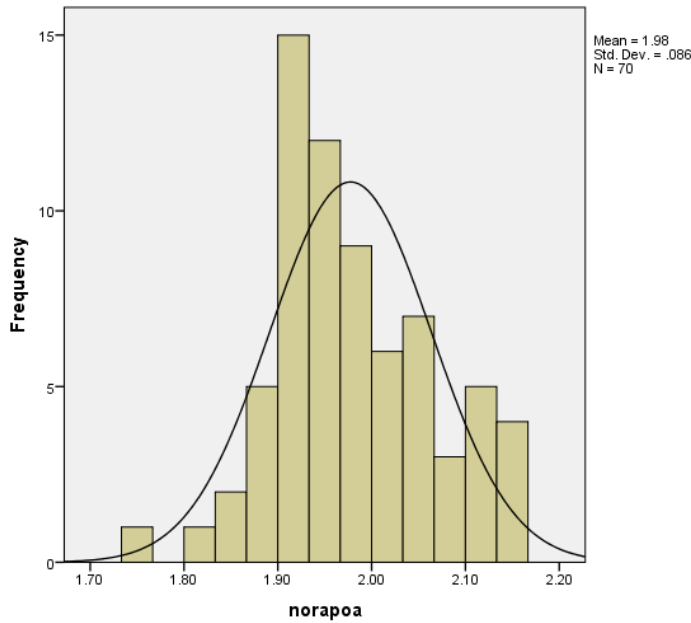
**Figure 1.8.**Scatter Plot for association of TG with BMI in gender specific Type 2 Diabetic patients (N=70)

### 7.2.5. Apolipoprotein A1 (Apo A1)

The minimum and maximum value is 56 and 194 mg/dl respectively, with mean of 100.9 and standard error 2.3. The median is 91mg/dl (95% CI of 86, 96). The overall distribution (**figure 1.9**) has Skewness 1.7 and 2.5 Kurtosis. The 2.5th and 97.5th percentile value is 64.5mg/dl and 189.4mg/dl respectively. The overall distribution of data (Using Shapiro-Wilk normality test) has statistically significant difference from Gaussian distribution ( $p = 0.00$ ).



**Figure 1.9.** Distribution of Apolipoprotein A among Type 2 Diabetic patients (N=70)



**Figure 1.10** Apo A value distribution after logarithmically normalized

The Value of Apo A tested for association with different host factors and found to be positively associated with sex and negatively associated with FBS, BMI and BP.

**Table 1.9** Association of Apo A with host factors

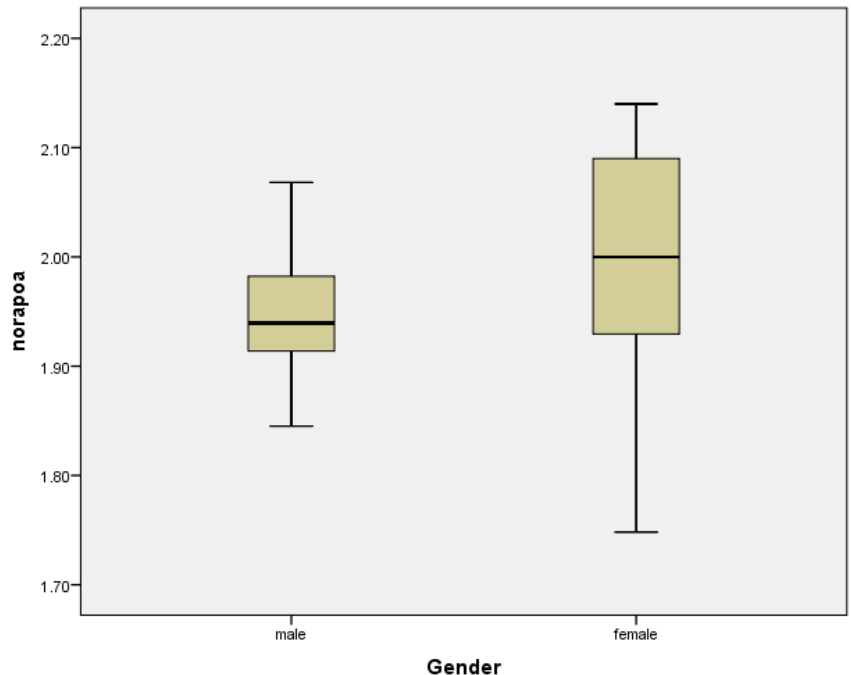
	Sex <sup>a</sup>	Age <sup>b</sup>	FBS <sup>b</sup>	BMI <sup>b</sup>	BP <sup>c</sup>
P value	0.01	0.98	0.00	0.00	0.01
r	0.31	-	-0.40	-0.35	
X <sup>2</sup> (d.f)					8(3)

*a- Mann-Whitney two sample rank sum test*

*b- spearman's rank correlation*

*c. Kruskal-Wallis rank test*

The median Apo A value for male and females was 87 (95% CI 83-92) and 98 (95% CI 90-111), respectively. The difference was statistically significant,  $p=0.01$  (**figure 1.10**). The central 95% distribution of Apo A for Male and Female subjects was 86-94 and 95-110 mg/dl, respectively.

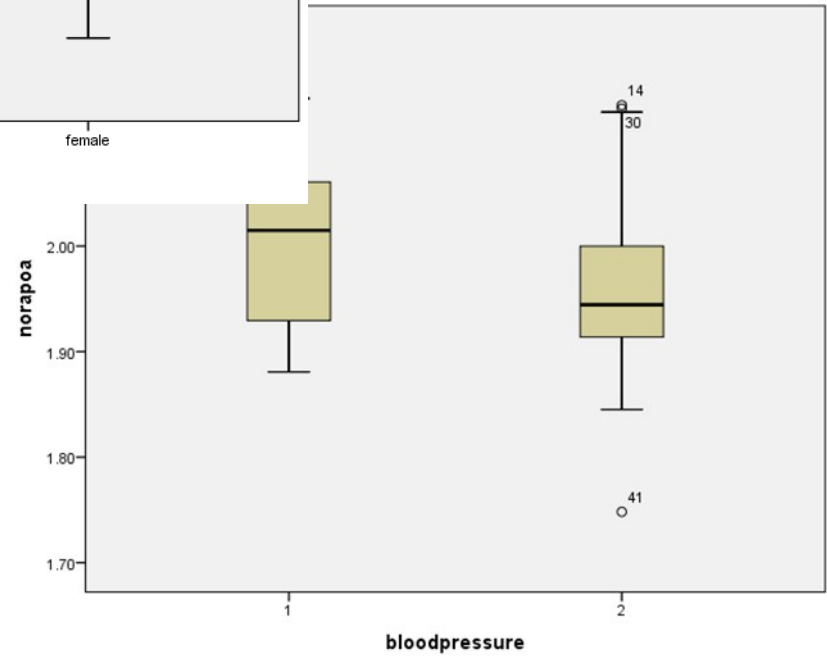


P value 0.01

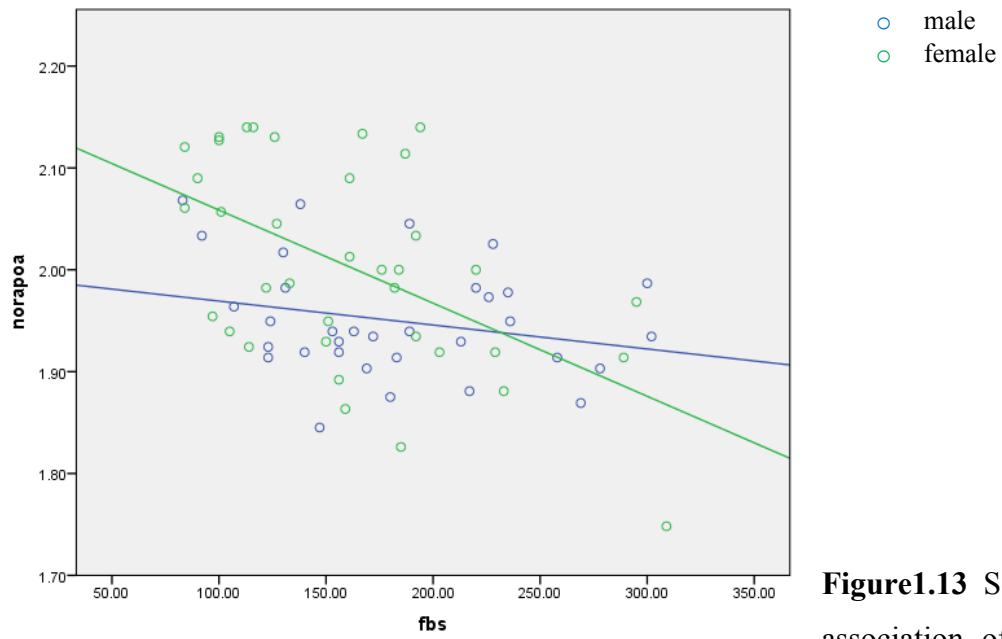
**Figure 1.11.**Box-whisker of Gender specific Apo A Value in Type 2 Diabetic patients (N=70).

1.<120/80mm/hg  
2.>120/80 mm/hg

P.value 0.01



**Figure1.12.**Box whisker of different BP specific Apo A Value in Type 2 Diabetic patients (N=70)

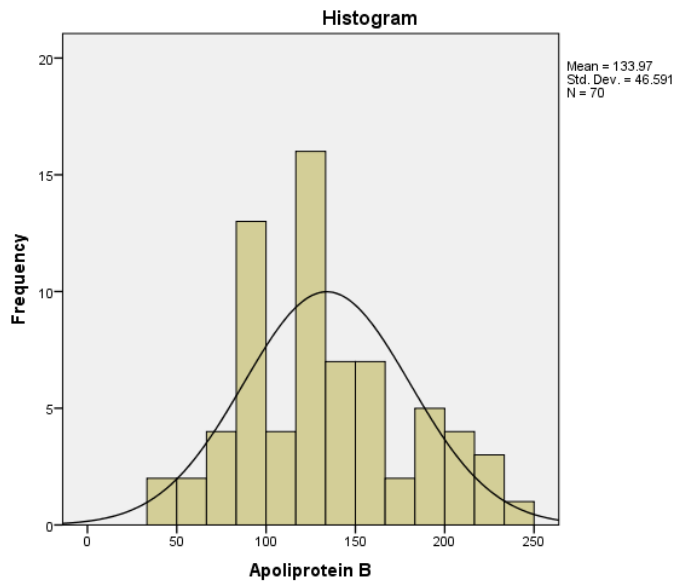


**Figure1.13** Scatter Plot for association of Apo A with FBS in gender specific Type

2 Diabetic patients (N=70)

### 7.2.6. Apolipoprotein B100(Apo B100)

The minimum and maximum value was 38 and 245mg/dl respectively, with mean of 133.9 and standard error 5.5. The median was 126 mg/dl (95% CI of 120,136). Skewness 0.39 and -0.38 Kurtosis. The 2.5th and 97.5th percentile value was 42.6mg/dl and 226.4mg/dl respectively. The overall distribution (Using Shapiro-Wilk normality test) has not stastically significant difference from Gaussian distribution, ( $p = 0.08$ ).



**Figure 1.14.** Distribution of Apo B100 among type 2 Diabetic Patients (N=70)

The Value of Apo b was not associated with different host factors

**Table 1.10.** Association of Apo B100 with host factors

	Sex <sup>a</sup>	Age <sup>b</sup>	FBS <sup>b</sup>	BMI <sup>b</sup>	BP <sup>c</sup>
P.value	0.06	0.16	0.36	0.96	0.86
r	-	-	-	-	-
X <sup>2</sup> (d.f)					11(4)

*a- Independent sample t test*

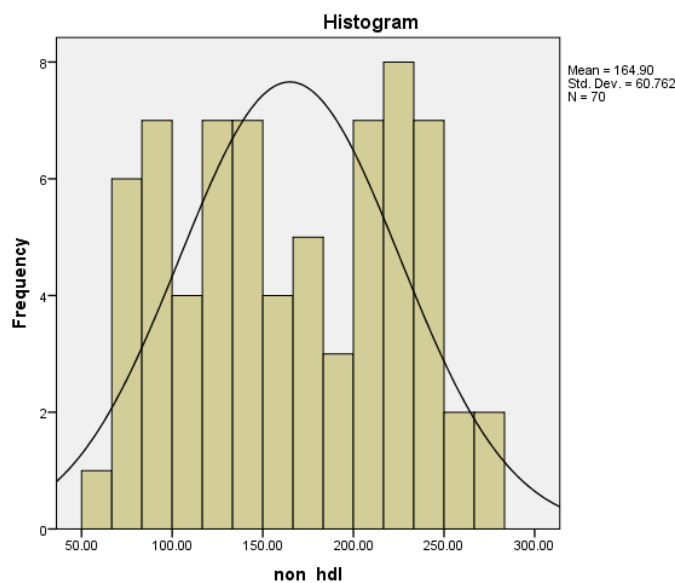
b- spearman's rank correlation

c- Kruskal-Wallis rank test

The median Apo b value for male and females was 89 (95% CI 80-110) and 91 (95% CI 83-123), respectively. The difference was not statistically significant,  $p=0.58$ . The central 95% distribution of Apo b for Male and Female subjects was 92-116 and 98-126 mg/dl, respectively.

### 7.2.7. Non HDL

The minimum and maximum value was 60 and 275mg/dl respectively, with mean of 164.9 and standard error 7.3. The median was 162 mg/dl (95% CI of 138,204). Skewness -0.01 and -1.3 Kurtosis. The 2.5th and 97.5th percentile value of non HDL was 66 mg/dl and 271 mg/dl respectively. The overall distribution (Using Shapiro-Wilk normality test) has not shown statistically significant difference from Gaussian distribution ( $p = 0.07$ ).



**Figure 1.15** Distribution of nonHDL among Type 2 Diabetic patients (N=70)

The Value of non HDL was not associated with different host factors

**Table 1.11.** Association of non HDL with host factor

	Sex <sup>a</sup>	Age <sup>b</sup>	BMI <sup>b</sup>	FBS <sup>b</sup>	BP <sup>c</sup>
P value	0.71	0.54	0.14	0.06	0.88
r					
X <sup>2</sup> (d.f)					12(3)

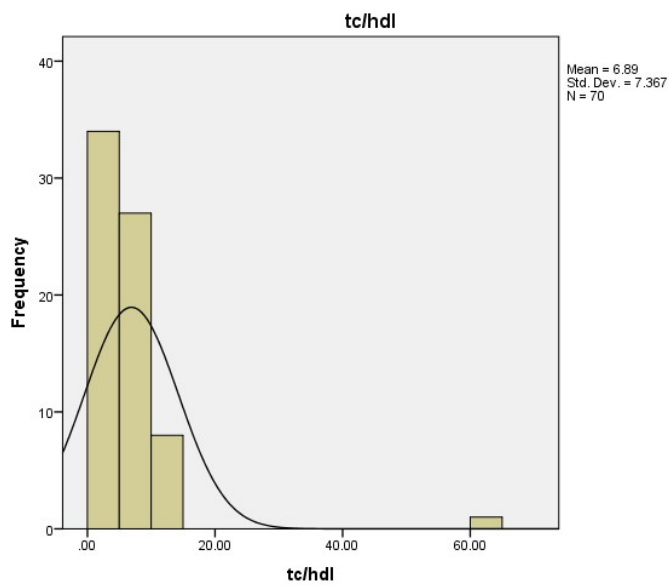
a- Independent sample t test

b- spearman's rank correlation

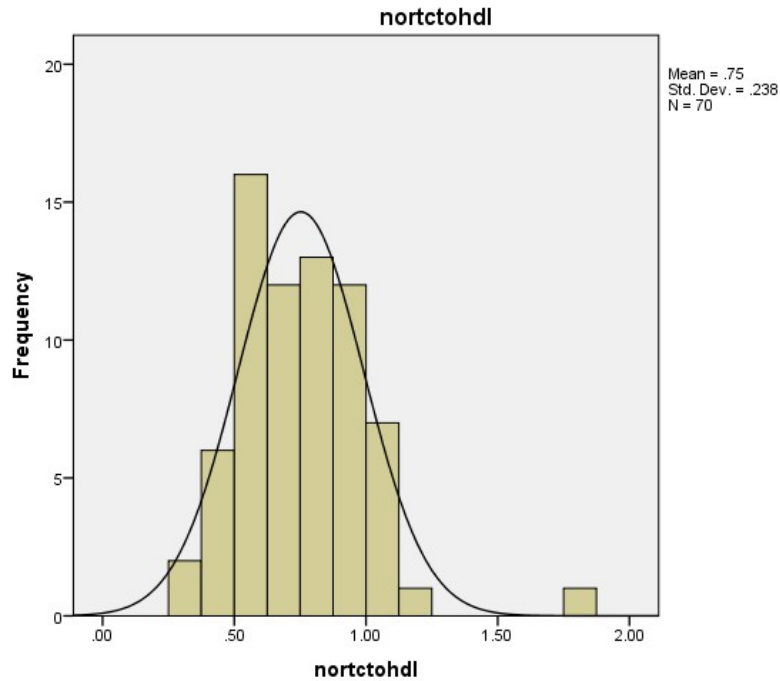
c- Kruskal-wallis rank test

### 7.2.8. TC to HDL ratio

The minimum and maximum value was 2.3 and 6.3 mg/dl respectively, with mean of 6.9 and standard error 0.8. The median was 5 mg/dl (95% CI of 4.2, 7.2). The overall distribution has Skewness 6.6 and 50 Kurtosis. The 2.5th and 97.5th percentile distribution of TC to HDL ratio was 2.3 mg/dl and 24.6mg/dl respectively. The overall distribution of data (Using Shapiro-Wilk normality test) has statistically significant difference from Gaussian distribution (p = 0.00).



**Figure 1.16** Distrbution of TC/HDL among Type 2 Diabetic Patients (N=70)



**Figure 1.17.** Distribution of TC to HDL value after logarithmically normalized (P= 0.08)

The value TC to HDL was tested for correlation and found to be associated with BP

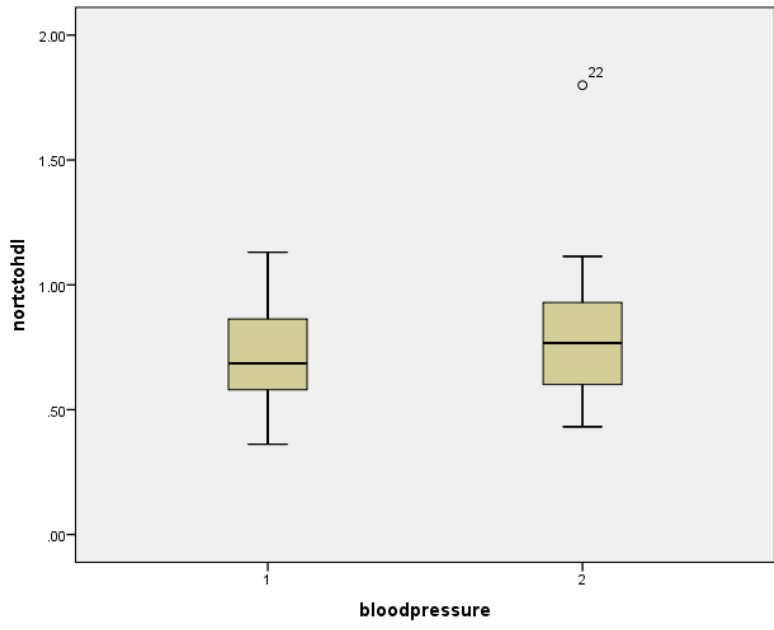
**Table 1.12** Association of TC to HDL ratio with host factors

	Sex <sup>a</sup>	Age <sup>b</sup>	FBS <sup>b</sup>	BMI <sup>b</sup>	BP <sup>c</sup>
P.value	0.49	0.72	0.23	0.16	0.01
r	-	-	-	-	-
X <sup>2</sup> (d.f)					11(3)

*a- Mann-Whitney two sample rank sum test*

*b- spearman's rank correlation*

*c- Kruskal-Wallis rank test*



1.<120/80mm/hg  
2.>120/80mm/hg

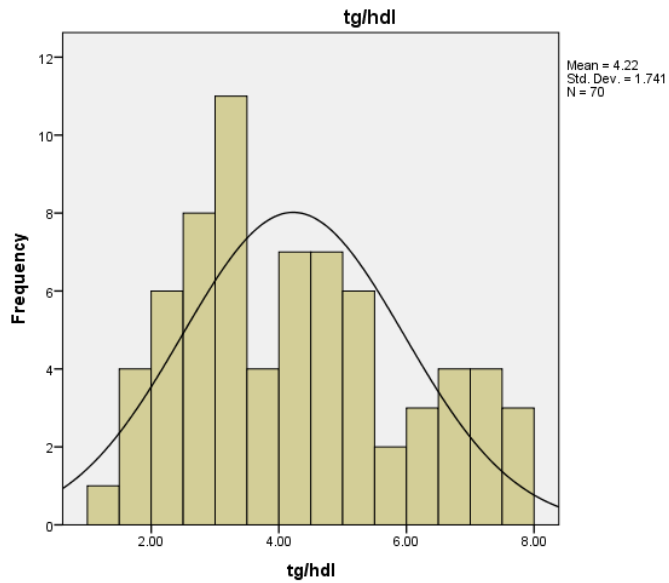
P.value 0.01

**Figure1.18.**Box whisker of BP specific TC to HDL ratio Value among Type 2 Diabetic

Patients (N=70)

**7.2.9. TG to HDL ratio**

The minimum and maximum value was 1.3 and 7.8 mg/dl respectively, with mean of 4.2 and standard error 0.21.The median was 4.2(95% CI of 3.4, 4.6). Skewness 0.39 and -0.83 Kurtosis. The 2.5th and 97.5th percentile value of TG to HDL ratio was 1.5mg/dl and 7.7mg/dl respectively. The overall distribution of data (Using Shapiro-Wilk normality test) has not shown statistically significant difference from Gaussian distribution(p = 0.07).



**Figure 1.19** Distribution of TG/HDL among Type 2 Diabetic patients (N=70)

TG/HDL was tested for association with different host factors and found to be positively associated with BP

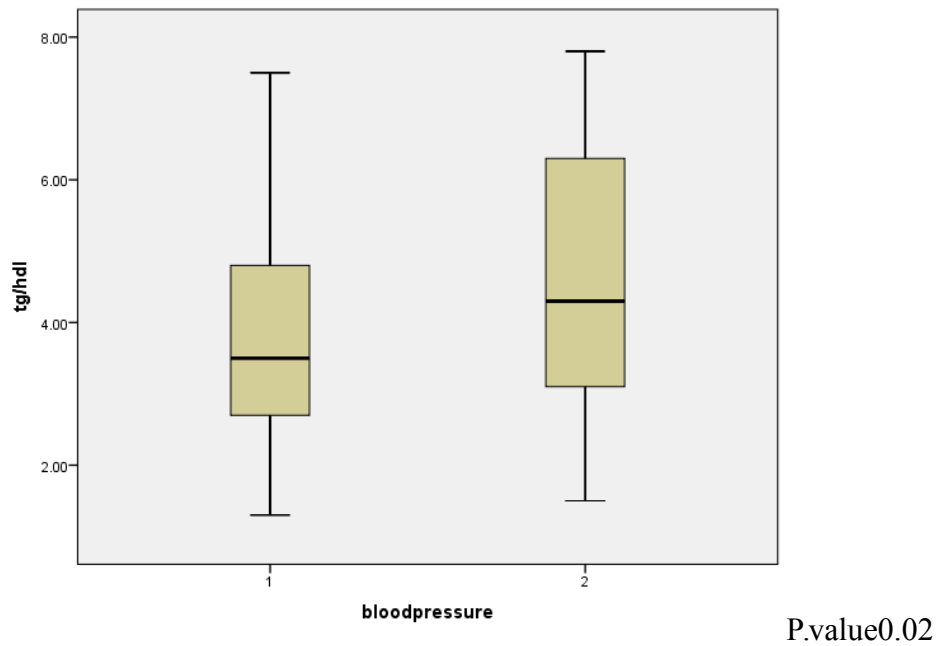
**Table 1.13** Association of TG/HDL with host factors

	Sex <sup>a</sup>	Age <sup>b</sup>	FBS <sup>b</sup>	BMI <sup>b</sup>	BP <sup>c</sup>
P .value	0.67	0.78	0.86	0.28	0.02
r	-	-	-	-	
X <sup>2</sup> (d.f)					7(3)

*a- Independent sample t test*

*b- spearman's rank correlation*

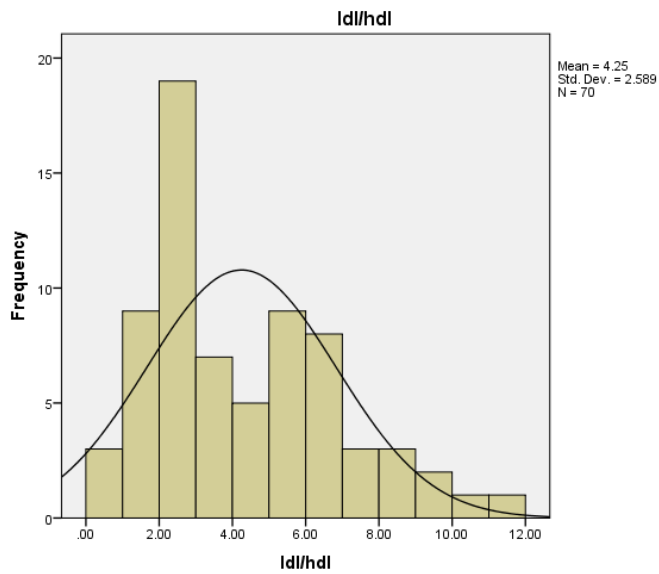
*c- Kruskal-wallis rank test*



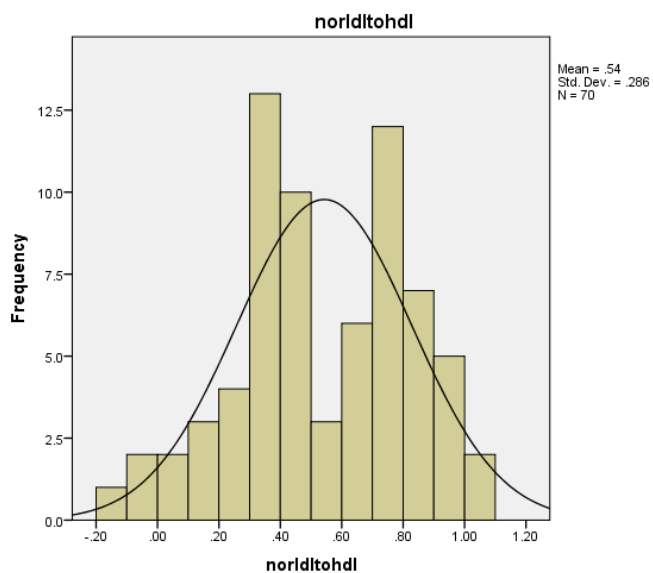
**Figure 1.20** Box whisker of BP specific TG to HDL ratio Value in Type 2 Diabetic patients (N=70)

#### 7.2.10. LDL to HDL ratio

The minimum and maximum value was 0.77 and 11.2 mg/dl respectively, with mean of 4.2 and standard error 0.31. The median was 3.2 mg/dl (95% CI of 2.6, 5.2). The overall distribution has Skewness 0.83 and -0.01 Kurtosis. The 2.5th and 97.5th percentile value of LDL to HDL ratio was 0.87mg/dl and 10.9mg/dl respectively. The overall distribution of the data (Using Shapiro-Wilk normality test) has shown stastically significant difference from Gaussian distribution ( $p = 0.00$ ).



**Figure 1.21** Distribution of LDL/HDL among Type 2 Diabetic patients (N=70)



**Figure1.22** Distribution of LDL/HDL value among all participants after logarithmically normalized

LDL/HDL ratio was tested for Association with host factors found to be associated with BP

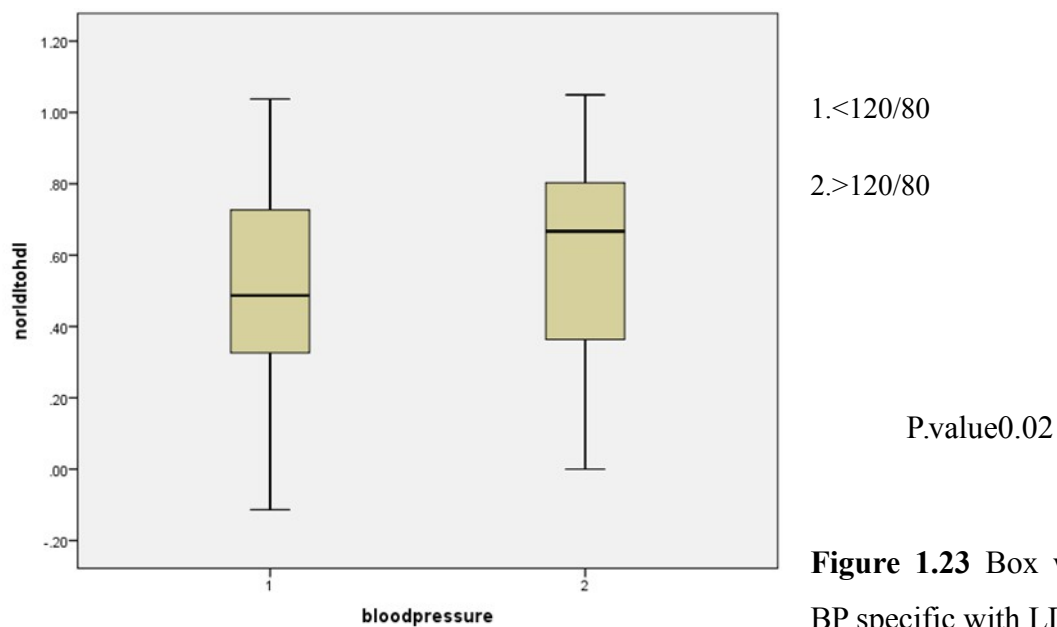
**Table 1.14** Association of LDL/HDL ratio with host factors

	Sex <sup>a</sup>	Age <sup>b</sup>	BMI <sup>b</sup>	FBS <sup>b</sup>	BP <sup>c</sup>
P value	0.6	0.98	0.27	0.21	0.02
r					-
X <sup>2</sup> (d.f)					13(3)

a- Mann-Whitney two sample rank sum test

b- spearman's rank correlation

c- Kruskal-wallis rank test

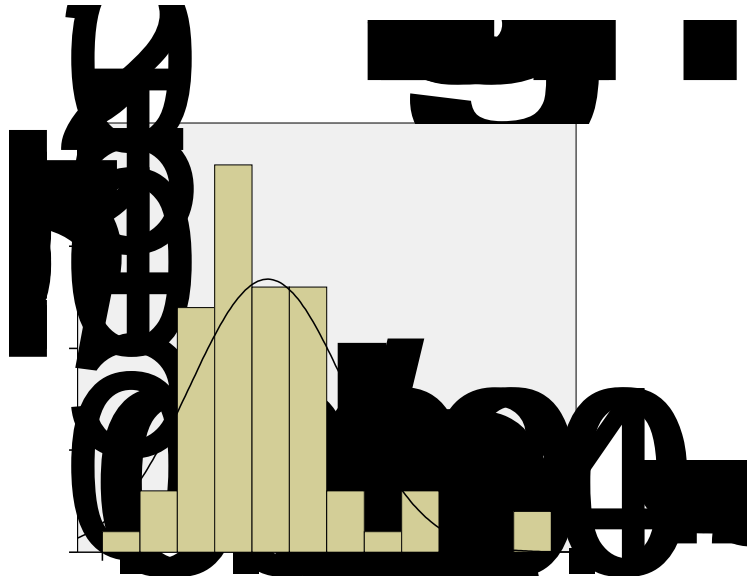


**Figure 1.23** Box whisker of BP specific with LDL to HDL ratio Value in Type 2 Diabetic patients (N=70)

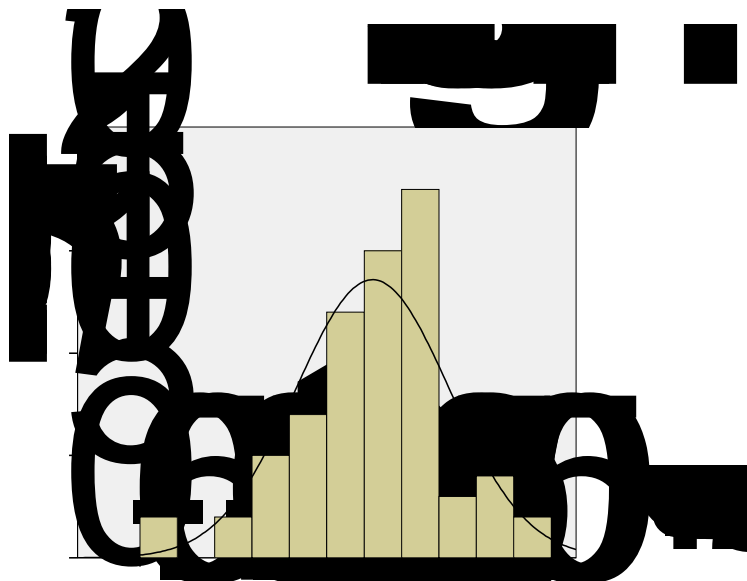
### 7.2.11. Apo B100 to Apo A1 ratio

The minimum and maximum value was 0.32 and 3.95 mg/dl respectively, with mean of 1.5 and standard error 0.08. The median was 1.3 mg/dl (95% CI of 0, 2). Skewness 1.4 and 3.2

Kurtosis. The 2.5th and 97.5th percentile value of Apo b to Apo a ratio was 0.34 mg/dl and 0.38 mg/dl respectively. The overall distribution (Using Shapiro-Wilk normality test) has a statistically significant difference from Gaussian distribution, ( $p = 0.00$ ).



**Figure 1.24** Distribution of Apo B100 /Apo A1 among Type 2 Diabetic Patients (N=70)



**Figure 1.25** Distribution of Apo B100 /Apo A1 ratio value among all participants after logarithmically normalized

Apo B 100/Apo A1 ratio was tested for association with host factor and found to be associated with FBS

**Table 1.15** Association of Apo B100/Apo A1 with host factors

	Sex <sup>a</sup>	Age <sup>b</sup>	BMI <sup>b</sup>	FBS <sup>b</sup>	BP <sup>c</sup>
P value	0.68	0.38	0.17	0.18	0.49
r	-	-	-	-	-
X <sup>2</sup> (d.f)					14(4)

*a- Mann-Whitney two sample rank sum test*

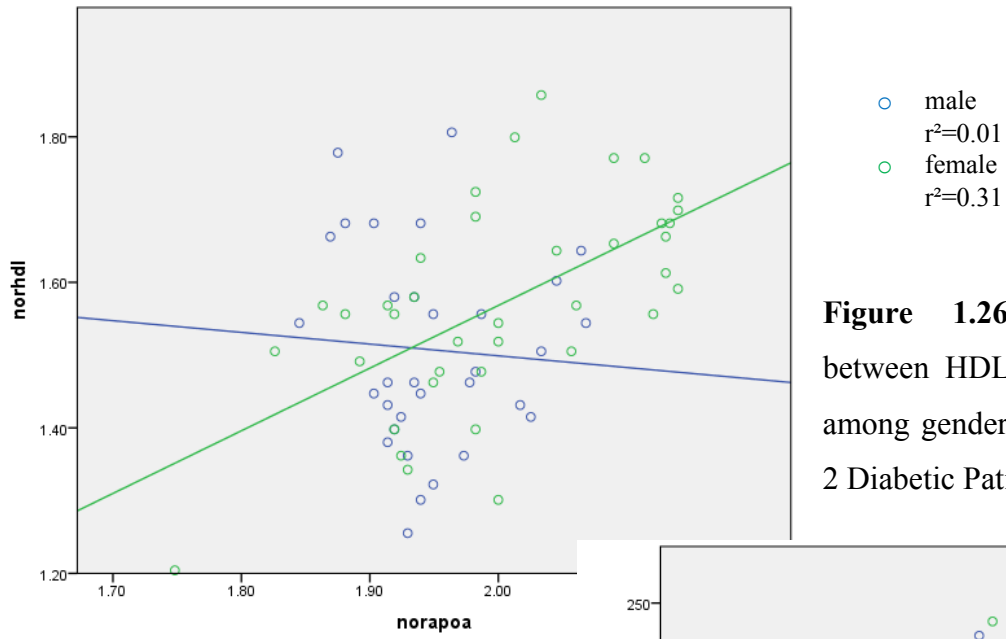
*b- spearman's rank correlation*

*c- Kruskal –wallis rank test*

**Table 1.16 Correlation between lipid profile, Apolipoprotein A1, B100 and lipid ratio**

Pearson correlation P.value (level of significance)	TC	HDL	TG	LDL	APO A1	APO B100	NonHDL	TC/HDL	TG/HDL	LDL/HDL	Apo B100/Apo A1
TC	-	-0.15 0.23	<b>0.71</b> <b>0.00</b>	<b>0.91</b> <b>0.00</b>	-0.91 0.12	0.09 0.43	<b>0.97</b> <b>0.00</b>	<b>0.29</b> <b>0.01</b>	<b>0.55</b> <b>0.00</b>	<b>0.72</b> <b>0.00</b>	<b>0.85</b> <b>0.00</b>
HDL	-	-	-0.1 0.52	<b>-0.3</b> <b>0.00</b>	<b>0.4</b> <b>0.00</b>	-1.4 0.26	<b>-0.34</b> <b>0.01</b>	<b>-0.28</b> <b>0.02</b>	<b>-0.73</b> <b>0.00</b>	<b>-0.66</b> <b>0.00</b>	<b>-0.4</b> <b>0.00</b>
TG	-	-0.1 0.52	-	<b>0.59</b> <b>0.00</b>	-0.2 0.06	0.15 0.22	<b>0.69</b> <b>0.00</b>	<b>0.33</b> <b>0.00</b>	<b>0.66</b> <b>0.00</b>	<b>0.44</b> <b>0.00</b>	<b>-0.6</b> <b>0.00</b>
LDL	-	-	-	-	- <b>0.19</b> <b>0.04</b>	<b>0.54</b> <b>0.00</b>	<b>0.94</b> <b>0.00</b>	<b>0.32</b> <b>0.01</b>	<b>0.64</b> <b>0.00</b>	<b>0.88</b> <b>0.00</b>	<b>0.7</b> <b>0.00</b>
APO A1	-	-	-	-	-	-0.2 0.12	<b>-0.3</b> <b>0.01</b>	<b>-0.38</b> <b>0.00</b>	<b>-0.38</b> <b>0.00</b>	<b>-0.37</b> <b>0.00</b>	<b>-0.6</b> <b>0.00</b>
APO B100	-	-	-	-	-	-	0.16 0.18	0.18 0.13	0.14 0.25	0.21 0.08	<b>0.9</b> <b>0.00</b>
Non HDL	-	-	-	-	-	-	-	<b>0.84</b> <b>0.00</b>	<b>0.67</b> <b>0.00</b>	<b>0.84</b> <b>0.00</b>	<b>0.7</b> <b>0.00</b>
TC/HDL	-	-	-	-	-	-	-	-	<b>0.88</b> <b>0.00</b>	<b>0.95</b> <b>0.00</b>	<b>0.7</b> <b>0.00</b>
TG/HDL	-	-	-	-	-	-	-	-	-	<b>0.82</b> <b>0.00</b>	<b>0.6</b> <b>0.00</b>

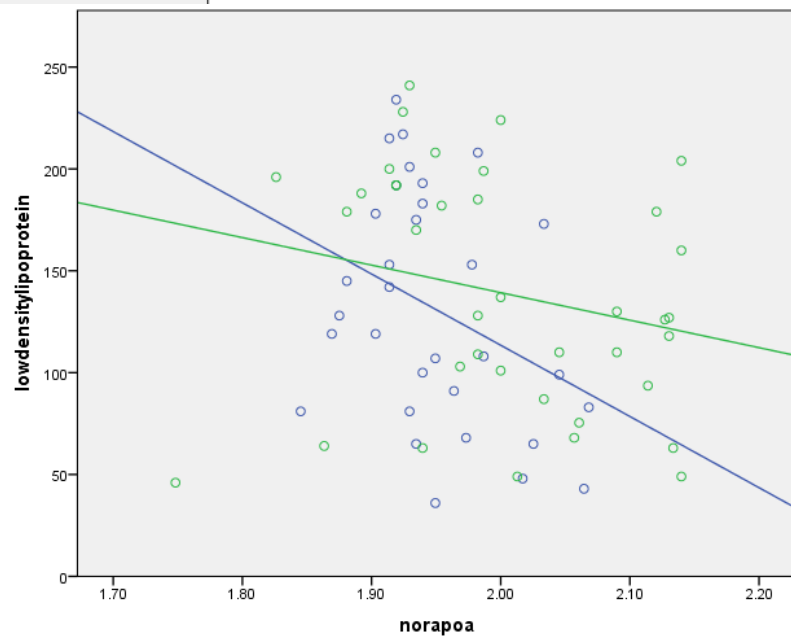
Scatter plot for lipid parameter correlation based on gender

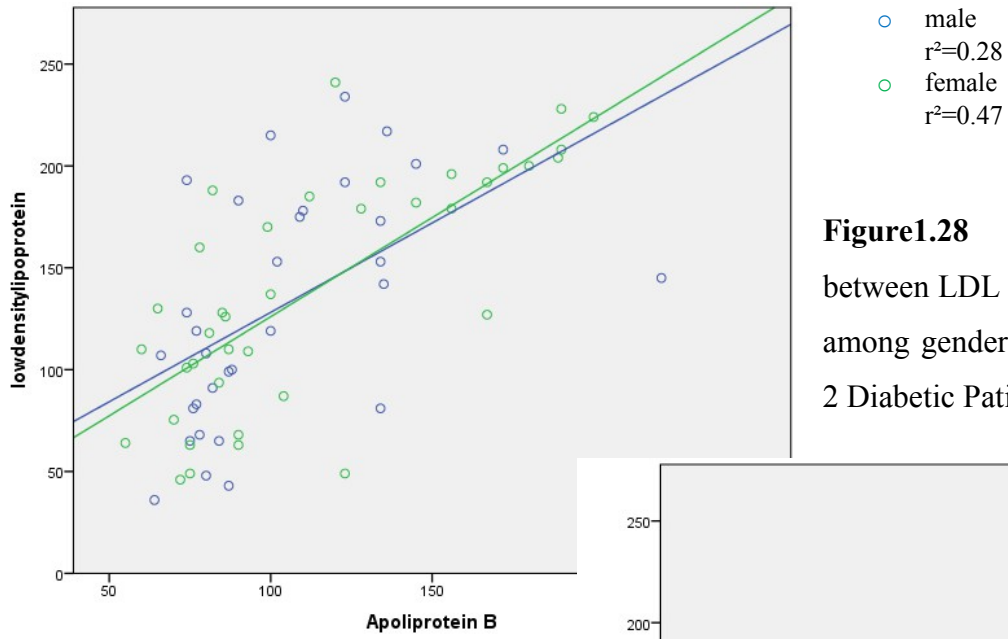


**Figure 1.26** Correlation between HDL and Apo A1 among gender specific Type 2 Diabetic Patients (N=70)

○ male  
 $r^2=0.12$   
 ○ female  
 $r^2=0.05$

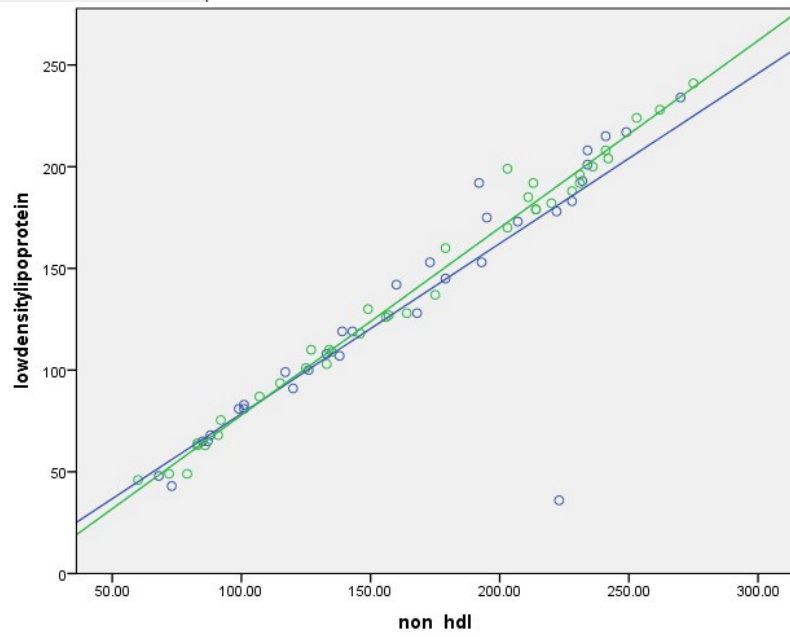
**Figure 1.27** Correlation between LDL and Apo A1 among gender specific Type 2 Diabetic Patients (N=70)



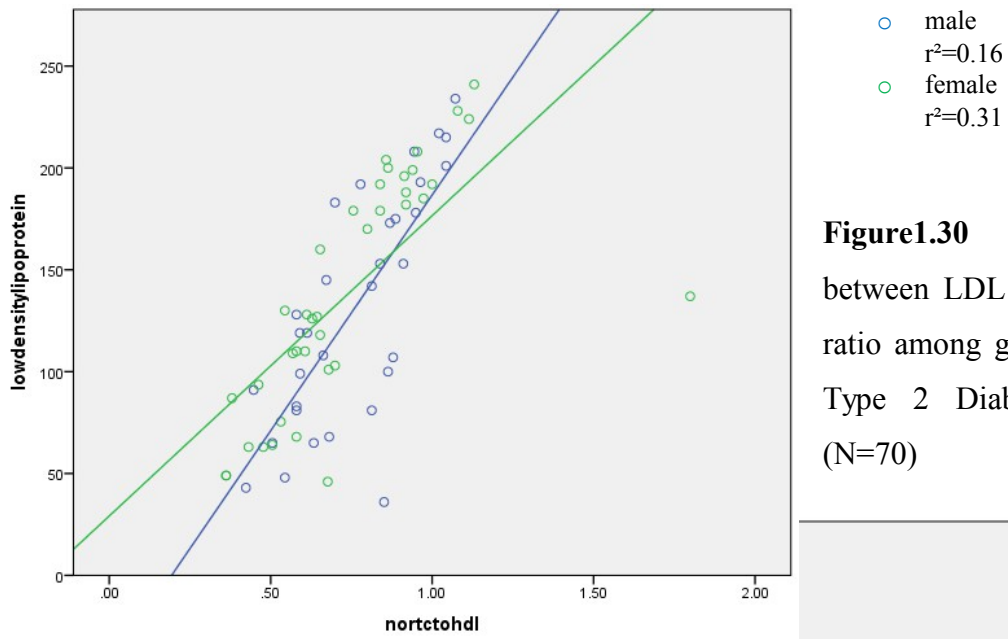


**Figure 1.28** Correlation between LDL and Apo B100 among gender specific Type 2 Diabetic Patients (N=70)

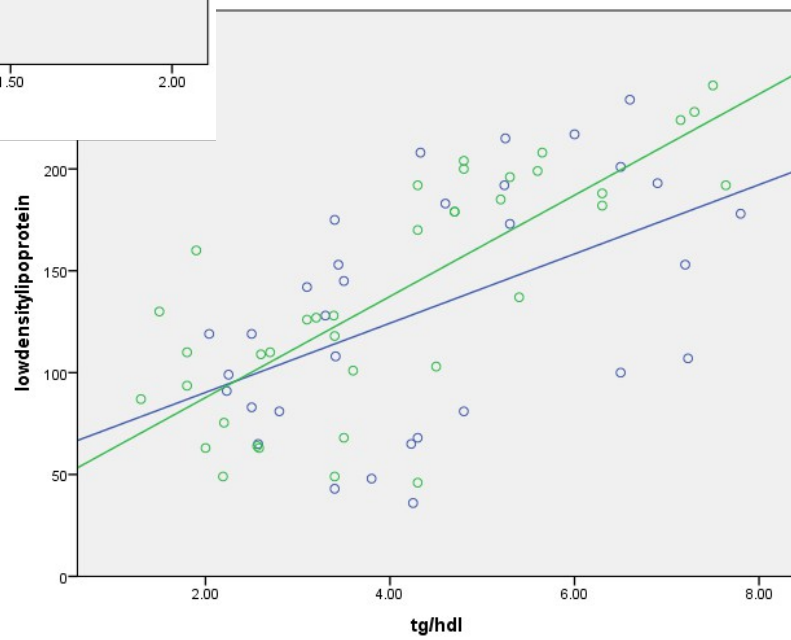
○ male  
 $r^2=0.75$   
 ○ female  
 $r^2=0.98$



**Figure 1.29** Correlation between LDL and non HDL among gender specific Type 2 Diabetes patients (N=70)



○ male  $r^2=0.25$   
 ○ female  $r^2=0.59$



**Figure 1.31** Correlation between LDL and TG/HDL ratio among gender specific Type 2 Diabetic patients (N=70)

## 8. Discussion

In the present study Dyslipidemia observed in study participants were, high TC 36(51%), low HDL 48(68%),high LDL 34(48.6%), high TG 30(42.9%),low Apo A1 27(39%),high Apo B 100 39(56%),high non HDL 47(67%),high TC/HDL 40(57%),high TG/HDL 58(83%) and high LDL/HDL 44(63%),high Apo B100/Apo A1 54(77%). The finding Supported by Henok etal study in which dyslipidemia observed in diabetic southern Ethiopian patients was high level of serum LDL, hypercholesterolemia and hypertriglyceridemia [25].Likewise Mohamed etal in Libya, indicate the commonest lipid abnormality was low HDL and hypertriglyceridemia [51].Similarly, Many Western epidemiological studies have shown diabetic dyslipidemia characterized by hypertriglyceridemia and low levels of HDL [52].

In the study, Female Gender was positively associated with Apo A1 and HDL. Similarly Iranian study shows Women had higher plasma levels of HDL-C compared to men[X].In a related study conducted by Yuthika etal Serum HDL-C were significantly higher in diabetic female in comparison to males, which is not so in controls, indicating gender influence on lipid in diabetics [53].In contrary Reidenger study present Apo A is non significantly associated with gender [54].

Another finding is positive association of TG with BMI. Similarly The serum triglyceride in diabetics having BMI >30 (obese) was increased as compared to patients having BMI <30 (non-obese) [55]. A Study in China also reveals the correlation between TG and BMI was the largest [56]. It is also reported by Albanian study that the levels of TG was higher in the obese group of the patients than the other group, and the differences were statistically significant (p value 0.01) [57].Hypertriglyceridemia predisposes the patients to life threatening complications like diabetic ketoacidosis, coronary artery disease [58].

Another important finding is the correlation of BP with TC/HDL, TG/HDL, LDL/HDL in other hand Goal etal present there is no association between hypertensive patients and LDL/HDL and TC/HDL [59]. Though Nigerian Study report The atherogenic index of plasma (AIP), defined as logarithm of (TG/HDL-C), has recently been proposed as a predictive marker for plasma atherogenicity and is positively correlated with cardiovascular disease risk like raised BP which consistent with present study[60].

Since each lipid particle contains one molecule of the atherogenic Apo B, it is direct measure of the number of potentially atherogenic particles in the different conventional lipid

components [22]. In present study Apo B has positive association with LDL which is supported by Iranian Study which shows they had positive correlation with each other ( $P < 0.05$ ) [61]. Likewise a study in Manipal shows the Correlation between Apo B100 and LDL ( $r = 0.712, p < 0.001$ ) in uncontrolled Diabetes Mellitus. Diabetes mellitus affects the LDL metabolism by two opposing phenomenon. It decreases the LDL clearance to increase LDL levels and also directly removes the VLDL Apo B to lower the LDL levels. The resultant concentration thus depends upon the relative magnitude of these two processes [27].

In the current Study HDL was positively correlated with Apo A 1 supported by Korean Study which emphasize Simultaneous measurement of ApoA-I provides a more accurate risk assessment for future development of T2DM and report correlation between two parameters [62].

LDL/HDL ratio is significantly associated with TC, LDL negatively associated with HDL. LDL-C/HDL-C ratio that reflects the two-way traffic of cholesterol can be used as markers of dyslipidemia in Type II Diabetic patients entering and leaving the arterial intima in a condition where LDL-C and HDL-C value increase alone [45].

TG/HDL ratio is negatively associated with HDL and positively associated with LDL. It distinguishes the small and large LDL size pattern. This ratio may be used to identify diabetic patients with an atherogenic lipid profile and may be relevant for assessing CAD risk. Especially in newly diagnosed patients, it may be useful for the selection of patients who need aggressive treatment of lipid abnormalities early in the course of diabetes or before the onset of CVD [23].

Apo B100 to Apo A1 ratio positively associated with LDL. Likewise It was indicated by Milanetalgreater the Apo B/Apo A ratio, the larger will be the amount of cholesterol from atherogenic lipoproteins circulating through the plasma compartment and likely to induce endothelial dysfunction and trigger the Atherogenicity process [21]. Therefore the Study present the Strong positive association of TG/HDL, LDL/HDL, Non HDL and Apo B with atherogenic marker LDL that help in diagnosis of diabetic dyslipidemia.

## **9. Conclusion and Recommendation**

Diabetic patients have a dyslipidemia characterized by low serum levels of high density lipoprotein, high serum levels of TC, LDL and TG which play a substantial role in acceleration of CVD complication. Apolipoprotein A1 and B 100 and lipid ratios TC/HDL, LDL/HDL, TG/HDL and Apo B100/Apo A1 are associated with classical lipid profiles in TASH, that help in diagnosis of dyslipidaemia in type 2 Diabetic patients. Since Apolipoprotein measurement is less affected by Biological variability like fasting and lipid ratios are easy to calculate physicians may consider their implication on further complication that is initiated through dyslipidemia in type 2 diabetes patients. Future studies should be done for additional analysis of the relevance of Apolipoprotein for type 2 diabetic patients taking lipid lowering drug in larger sample size. More over Positive association of TG with BMI in TASH indicates the importance of maintaining healthy weight. Finally the positive association of blood pressure with lipid ratios like TC/HDL, TG/HDL and LDL/HDL highlight the significance of controlling blood pressure of diabetic patients to minimize future risk.

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## **Annex I**

### **Information sheet for study participants**

The informed consent will be obtained after reading/let them read the information sheet to the study participants. This information sheet will be read by personal investigator at study sites.

You are invited to participate in this study. The aim of this study is to assess dyslipidemia using Apolipoprotein, lipid profile in diabetic patients. This study involves collecting 5 ml of blood from study participant and it will serve as a baseline data for researchers and physicians with regard to apolipoprotein association with classical lipid profile and risk factors.

- a. **Purpose:** the purpose of this study is to assess dyslipidemia using apolipoprotein and lipid profile in Diabetic patients in Black lion specialized hospital.
- b. **Duration:** the duration of this study depend on the availability of study subjects and it can take about 3-4 months. However, specimen from you is collected only once.
- c. **Procedure to be carried out:** the procedure is easy and simple; first you will be asked to fill questions in a questioner and then you will be asked to provide 10ml of blood from vein. The sample will be transported to Laboratory for analysis.
- d. **Risk and discomfort:** There will be minor discomfort during collection of samples. Your hand will feel pain during collection of blood. During collection of samples from your hand appropriate precaution will be taken and all samples will be collected by trained health professionals. Appropriate medical care will be provided to you if needed.
- e. **Expected benefits:** The information gained from yours and others study participant will be used as a baseline of apolipoprotein reference range for physicians
- f. **Confidentiality:** We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. If a research article or publication comes from this study, you will not be identified by name. The information we collect from you as part of the study will be protected by a password on the computer that can only be accessible to personnel involved in the study.
- g. **Voluntary Participation and Withdrawal from the Study:** The participation is completely voluntary and you have the right not to participate in this study. You can stop participating in the study at any time after giving your consent. This decision will not affect in any way yours current or future medical care in the health facility.

**Contact information:** If you have any questions about this study you can contact the following investigators and the ethical committee for further information.

Meron Amsalu: 0934756965

Samuel Kinde: 0911854686

School of medical laboratory sciences Department (office): 0112755170

## **Annex II**

**Amharic version of participant's information sheet**

በአዲስ አበባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል በማስተርስ ዲግሪ ተማሪ የስኬር ህመምን በደም ውስጥ የሚገኝ የቅባት ክምችት መጠን መጨመር ልኬት በሚል ርዕስ የተዘጋጀ የመመሪያ ጥናት ፅሁፍ ተሳታፊ እንዲሆኑ በትህትና ይጠየቃሉ። እርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፍቃደኝነት ላይ የተመሰረተ ሲሆን ፈቃደኛ ካልሆኑ ወይም ጥናቱን ለማቋረጥ የሚወስኑ ቢሆን እንኳን ማንኛውንም የጤና አገልግሎት ያገኛሉ። በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅፁ ላይ በፅሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅቧል።

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንደሚውል መስማማት ይጠበቅቧል። ከተወሰደው ናሙና ላይ የሚገኙ መረጃዎች ለስራው አግባብነት ላላቸው ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቧል። ይሁን እንጂ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጹ መረጃዎችን ማለትም ስምና አድራሻ የስልክ ቁጥርን ጨምሮ አይገልጽም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚውል የርስዎን ናሙና ለማወቅ የሚያስችል መለያቁጥር ጥቅም ላይ እንዲውል ይደረጋል። በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታና የኑሮ ዘይቤ የሚቀርብሎትን ጥያቄ መመለስ ይጠበቅቧል።

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

ሚሮን አምሳሉ

ጥባይል 0934756965

ኢ.ሜይል: mer552408@gmail.com

ለመሳተፍ ከተስማሙ ሙሉ ፈቃደኛ ለመሆንዎ ቀጥለው በስምምነት ቅጽ ላይ ባለው ባዶ ቦታ ፊርማዎን ያይዙ።

የሚስጥር ቁጥር \_\_\_\_\_ የጥናቱ ተሳታፊ ፊርማ \_\_\_\_\_

የጥናቱ ተሳታፊ ስም \_\_\_\_\_

**Annex III Test protocol**

**Enzymatic method for Glucose**

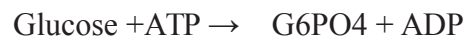
**Test principle**

Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G6P-DH) specifically oxidizes glucose-6-

phosphate to gluconate-6-phosphate with the concurrent reduction of NAD<sup>+</sup> to NADH. The increase in absorbance at 340nm is proportional to the glucose concentration in the sample.

### **Reaction: Enzymatic Method**

Enzymatic approaches to the measurement of glucose have been explored. Enzymatic methods are specific for glucose. The hexokinase method is the reference method for glucose. The method involves two coupled reactions:



G6PD 6-phosphogluconate



The increase in absorbance of NADPH at 340 nm is measured as directly proportional to glucose. The hexokinase reaction may also be coupled to an indicator reaction and measured through the development of a colored product.

### **Specimen**

Glucose in serum can be measured by this method. Glucose levels in serum or plasma are 10% to 15% higher than those in whole blood. Serum or plasma must be separated within 1 hour to prevent degradation by glycolysis. Glucose is stable for 24 hours in whole blood when preserved with sodium fluoride.

### **Reference Ranges**

Serum or plasma (fasting) 70–120 mg/dl

## **Enzymatic method for Total cholesterol**

### **Principle**

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reaction that hydrolyze cholesterol ester and oxidize 3-OH group of cholesterol on of the reaction product H<sub>2</sub>O<sub>2</sub> is measured quantitatively in a peroxidase catalyzed reaction that produce a color. Absorbance is measured at 500nm in which the color intensity is proportional to cholesterol concentration.

### *Cholesterol Esterase*

T. cholesterol esters → Cholesterol + free fatty acid

### *Cholesterol Oxidase*

Cholesterol + O<sub>2</sub> → Cholest-3-ene-4-one + H<sub>2</sub>O<sub>2</sub>

### *Peroxidase*

2 H<sub>2</sub>O<sub>2</sub> + 4-aminoantipyrine → 4 H<sub>2</sub>O + chromogen

Elevated level of cholesterol associated with increased risk of CHD. Serum cholesterol help to asses patients' risk status.

### **Interference**

Remove sample from red cells after blood clots or plasma has been centrifuged. The peroxidase

Assay can be susceptible to increases in uric acid, ascorbic acid, bilirubin, hemoglobin, or other reducing substances. Samples should have only the normal amount of these substances present.

### **Specimen**

Nonhemolyzed serum or plasma, free from clots. The patient need not be fasting if this is the only lipid test requested. However, if total cholesterol is requested as part of a lipid Panel, the patient must be fasting for 10 to 12 hours.

### **Reference Ranges**

Male (25–29 year old) 130–234 mg/dl

Female (25–29 years old) 130–231 mg/dl

Based on coronary heart disease risk:

Child <170 mg/dl

Adult <200 mg/dl

### **Enzymatic method for Triglyceride**

Triglycerides are composed of three fatty acids and a glycerol moiety. Analyzing a serum or plasma sample for triglycerides typically involves four reactions. Triglyceride measured enzymatically using a couple reaction in which triglyceride hydrolyzed to glycerol. And glycerol kinase catalyses the transfer phosphate from ATP to glycerol thus forming glycerol phosphate and pyruvate kinase transfer phosphate phosphoenol pyruvate to ADP to form ATP and final conversion of pyruvate to lactate.

### **The Reaction**

*Lipase (Bacterial)*

Triglycerides → 3 fatty acids + glycerol

*Glycerol Kinase*

Glycerol + ATP → glycerol-3-phosphate + ADP

*Pyruvate Kinase*

ADP + phosphoenol pyruvate → ATP + pyruvate

*Lactate Dehydrogenase*

NADH + H + pyruvate → NAD + lactate

### **The Specimen**

Serum, fasting 10 to 12 hours recommended

### **Reference Ranges (Age-Specific)**

Male (25–29 years old) 45–204 mg/dl

Females (25–29 years old) 42–159 mg/dl

National Cholesterol Education Program risk factors (adult male):

Optimal <150 mg/dl

High 150–199 mg/dl

Hypertriglyceridemic 200–499 mg/dl

Very high >499 mg/dl

### **Homogenous method for HDL**

#### **Principle**

Homogeneous HDL-C assays do not use precipitation, nor do they require a centrifugation/separation step. This improves the yield of HDL recovered from the specimen. One method uses an antibody to apolipoprotein B-100 to bind LDL and VLDL in the sample. This leaves the HDL-C to react with the second reagent, which contains enzymes and

substrate for cholesterol analysis. In a second method, a synthetic polyanion reagent binds the sites on VLDL and LDL particles, blocking their products from forming cholesterol colored products. The second reagent added has detergent, enzymes, and substrate that react with the HDL-C in the sample. Only the HDL particle cholesterol is allowed to form a colored product and can be measured.

### **Specimen**

Serum, plasma

### **Reference Ranges (Age-Specific)**

Men (25–29 years old) 31–63 mg/dl

Women (25–29 years old) 37–83 mg/dl

NCEP risk factors (adult male):

Low risk >59 mg/dl

High risk <40 mg/dl

### **LDL measurement**

### **Principle**

LDL-L reagent is produced by using a combination of detergents and phosphorous compounds which specifically bind HDL, VLDL and chylomicron (CM) but not LDL. The combination protects HDL, VLDL and CM from the reaction by cholesterol esterase and cholesterol oxidase. Consequently LDL-cholesterol is selectively exposed to react with both enzymes.

LDL (ester-cholesterol) + H<sub>2</sub>O CE Cholesterol + Fatty Acids

Detergent & Phosphorous Compounds

Free Cholesterol + O<sub>2</sub>

CO Delta<sup>4</sup> – Cholestenone + H<sub>2</sub>O<sub>2</sub>

2H<sub>2</sub>O<sub>2</sub> + HDAOS Peroxidase 4H<sub>2</sub>O + Quinone dye

(λ<sub>max</sub> 585 nm)

HDAOS = N-(2-hydroxy-3-sulfopropyl)-3, 5 dimethoxyaniline.

### **Reference Range**

<130 Desirable LDL-C Concentration

130-160 Borderline high risk LDL-C

>160 High risk LDL-C

### **Apolipoprotein A measurement**

#### **Test principle**

Apo AI assay quantifies Apolipoprotein AI based on immune turbidimetric assay. The reagent uses a goat polyclonal antibody specific for human Apolipoprotein AI. The antibody binds to the Apo AI in the serum forming light scattering immune complexes, which increase the turbidity of the sample. Since the increase in turbidity is proportional to the amount of Apo AI in the sample, the Apolipoprotein AI concentration can be determined by measuring this increase in turbidity. The increase in turbidity is measured at 800 nm. Apolipoprotein AI in the sample is quantitatively determined.

#### **Specimen**

Non fasting Serum or plasma

#### **Reference Range**

Apo AI 94–199 mg/dl

### **Apolipoprotein B measurement**

#### **Test principle**

Apo B assay quantifies Apo lipoprotein B based on immune turbidimetric assay. The antiserum used in the kit is a goat polyclonal antibody specific for human Apolipoprotein B. The Apo B antibody interacts with the Apo B in the serum forming immune complexes. The immune complexes cause an increase in light scattering, which can be measured at 600 nm. Since the increase in turbidity is proportional to the amount of Apo B in the sample, the Apolipoprotein B concentration can be determined by measuring this increase in turbidity. Apolipoprotein B in the sample is quantitatively determined.

#### **Specimen**

Non fasting Serum or plasma

#### **Reference Range**

ApoB-100 55–125 mg/dl