Assessment of Serum Total Cholesterol, Low Density Lipoprotein and High Density Lipoprotein and Aspartate Transaminase Activity Among Healthy Sudanese Heavy Smokers, Wad Medani, Gezira State, Sudan (2017)

Mohamed Almasalami Musa Mustafa
B.Sc. Omdurman Ahlia University (2013)

A Dissertation

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Clinical Chemistry

Department of Clinical Chemistry
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Assessment of Serum Total Cholesterol, Low Density Lipoprotein and High Density Lipoprotein and Aspartate Transaminase Activity Among Healthy Sudanese Heavy Smokers, Wad Medani, Gezira State, Sudan (2017)

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Declaration

I authorized that my dissertation Assessment of Serum Total Cholesterol and LDL and HDL Ratio and Aspartate Transaminase Activity Among Healthy Sudanese Heavy Smokers, Wad Medani, Gezira State, Sudan (2018)

"", submitted by me, under the supervision D: Shams Eldein Mohammed Ahmed for the partial fulfillment for the award of Master degree in Medical Laboratory Sciences in Clinical Chemistry. University of Gezira Faculty of Medical Laboratory Sciences Department of Clinical Chemistry; Wad- Madani, Sudan and this is original and it was not submitted in part or in full, in any printed or electronic means, and is not being considered elsewhere for publication.

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Name: Mohamed Al masalami Musa Mustafa

Signature ........................................

Place: OmTalha

Date: // 2018
Dedication

For soul of my dear father & mother.
Candle of my life.
My brothers & sisters.
The source of my strength.
My friends.
Who support me.
Thank you for your presence in my Life
ACKNOWLEDGEMENT

Praise to god how gave me the health strength and patience to conduct this study.
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Assessment of Serum Total Cholesterol, LDL and HDL Ratio and Aspartate Transaminase Activity Among Healthy Sudanese Heavy Smokers, Wad Medani, Gezira State, Sudan (2018)

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ABSTRACT

Smoking is considered as major cardiovascular risk factor and is one of the main avoidable causes death in the world. It is also considered as an important factor in the stimulation of the development of atherosclerosis. This study aimed to measure the lipid profile concentration and AST enzymes activity. A total of 90 volunteers were participated in this study. Venous blood sample were taken from each and analyzed immediately by automation Mindary (BS200). Data was analyzed by Statistical Package of Social Science (SPSS) computer program Version 19. The results showed that the total cholesterol level were increased with increase in number of cigarette smoking Mean±SD (251±34) P-value, 0.000), and HDL level decreased with increase in number of cigarette smoking M±SD (23±3) P.value( 0.000) While LDL increased with increased in number of cigarette smoking Mean±SD (201±21) P-value (0.000), and aspartate transaminase (AST) enzyme increased with increase in number of cigarette smoking the longer the duration of smoking the highest aspartate transaminase level Mean±SD (71.6±13.9), (P.value 0.000). This result showed that total cholesterol, low density lipoprotein LDL and aspartate amino transaminase AST activity elevated, while high density lipoprotein HDL lower
تقييم مستوى الكولسترول الكلي والكولسترول العالي الكثافة والكولسترول المنخفض الكثافة ونشاط إنزيم الأسبارتيت ترانزامينيز في وسط المدخنين السودانيين الصحيين في مدينة ودمدني، ولاية الجزيرة السودان (2018)

محمد المسلمي موسى مصطفى

ملخص الدراسة

يعتبر التدخين عامل خطر رئيسي لأمراض القلب والأوعية الدموية ويعتبر أحد الأسباب الرئيسية التي يمكن أن يجلبها الموت في العالم. ويعتبر أيضاً عاملًا مهمًا في تطور القلب والشرايين. تهدف هذه الدراسة لقياس تركيز مصل الدهون ونشاط إنزيم الأسبارتيت ترانزامينيز ببعض الدراسات عن عوامل الخطر الإنجابية الرئوية. خلصت هذه الدراسة في ولاية الجزيرة ودمدني السودان.شارك في هذه الدراسة 90 متطوعًا. تم أخذ 3 مل من الدم الوريدي من كل متطوع. أجريت التحاليل باستخدام جهاز الطيف المرئي الضوئي (BS200) Mindary. كما تم تحليل البيانات ببرنامج الحزم التقنية الاجتماعية (SPSS) النسخة 19. أظهرت النتائج أن:

- اجمالي مستوى الكولسترول الكلي زاد مع زيادة عدد تدخين السجائر وكان المتوسط (34±15.2) ميلجرام/دليتر. معنوية كانت (0.000). وانخفاض في مستوي الكولسترول العالي الكثافة مع زيادة عدد تدخين السجائر وكان المتوسط (3±23) ميلجرام/دليتر. معنوية كانت (0.000). وارتفاع الكولسترول المنخفض الكثافة مع زيادة عدد تدخين السجائر وكان متوسط النتيجة (12±210) ميلجرام/دليتر. وقيمة المعنوية (0.000). وكذلك ازدياد إنزيم الأسبارتيت ترانزامينيز النشط مع زيادة عدد تدخين السجائر ومدة التدخين وكان متوسط النتيجة (9.1±71.6) ميلجرام/دليتر. وكانت المعنوية (0.000). خلصت هذه النتيجة أن مستوي الكولسترول الكلي والكولسترول المنخفض الكثافة ونشاط إنزيم الأسبارتيت ترانزامينيز مرتفع في حين أن البروتين الدهني العالي الكثافة منخفض.
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### ABBREVIATIONS

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<td>AMI</td>
<td>Acute Myocardial Infarction</td>
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<td>AspAT</td>
<td>Aspartate Aminotransferase</td>
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<td>AST</td>
<td>Aspartate Transaminase</td>
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<td>Cast</td>
<td>Cytosolic Aspartate</td>
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<td>CHD</td>
<td>Coronary Heart Disease</td>
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<td>CoA</td>
<td>Coenzyme A</td>
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<td>EC</td>
<td>Enzyme Commission</td>
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<td>FFA</td>
<td>Free Fatty Acid</td>
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<td>GLU</td>
<td>Glutamate</td>
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<td>HDL</td>
<td>High Density Lipoprotein</td>
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<td>HMG</td>
<td>Hydroxyl 3Methl Glatryl</td>
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<td>IDL</td>
<td>Intermediate Density Lipoprotein</td>
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<td>IHD</td>
<td>Ischemic Heart Disease</td>
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<td>LDL</td>
<td>Low Density Lipoprotein</td>
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<td>Mg/dl</td>
<td>Millie Gram Per Diluter</td>
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<td>NAD</td>
<td>Nicotinamide AdenineDinucleotide</td>
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<td>NADH</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate</td>
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<td>NEFA</td>
<td>Non Esterifies Fatty Acid</td>
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<td>PMP</td>
<td>Pyridoxamine Phosphate</td>
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<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
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<td>RCT</td>
<td>Reverse Cholesterol Transport</td>
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Chapter One
Introduction

1.1 General Introduction

Smoking is one of the major risk factors in the genesis of coronary atherosclerosis and development of coronary heart disease. The hazards of cigarette smoking are well recognized worldwide (Fagerstrm 2002) and (Ezzati and Lopez 2004), yet significant numbers of people continue to smoke in the developing countries (Reda and Moges 2012) and (Idris and Ibrahim 1998). In Sudan, prevalence of cigarette smoking in the adult population reached 12% (Idris and Ibrahim 1998). Alternatively, in some developed countries, although prevalence of cigarette smoking is almost double that of Sudan, it started to decrease over the last year (Gallus and Lugo 2012), and which is recognized as a major risk factor for the development of ischemic heart disease which may lead to alter the normal plasma lipoprotein pattern and incidence of developing cardiac heart diseases (CHD) which is directly related to the number of cigarette smoked, sudden death is 2-4 times more in heavy smokers than in non-smokers (Zamir2000). Lipid profile is a battery of laboratory studies to help determine the risk factors in coronary artery disease and a lipid profile usually includes total cholesterol, LDL-cholesterol (low density lipoprotein) which also is called ("bad" cholesterol) and HDL cholesterol (high-density lipoprotein) which also is called ("good" cholesterol) because it serves a protective function, it sucks up cholesterol found in the bloodstream and returns it to the liver for disposal while LDL-cholesterol carries cholesterol from the liver to the cells that need it so high levels of HDL are desired so that any free cholesterol will be quickly removed from the blood and Proper physical exercise is one way to increase the concentration of HDL-cholesterol (Vasudevan 20011 and Talaska 2009). Lipoproteins are macromolecules which composed of hydrophobic lipids bound to protein that transport lipids through aqueous plasma (i.e.) Lipoproteins are spherical or discoid aggregates of lipids and Apo proteins, which are classified into five groups in order of decreasing size and increasing density, these are: chylomicrons, VLDLs (very-low-density lipoproteins), LDLs (low density lipoproteins) and HDLs (high-density lipoproteins). Blood lipid tests should not be performed during stress or acute illness (e.g.) recent myocardial infarction [MI], stroke, pregnancy, trauma and use of certain drugs Koolman, and
Klaus2005) and (Wallach 2006). Lipids play an important role virtually in all aspects of biological life. Some of these roles include serving as hormones or hormone precursors, helping in digestion, providing energy, storage function and metabolic fuels; acting as functional and structural compounds in bio membranes and forming insulation to allow nerve conduction or to prevent heat loss (Odedeji Etukudo 2006). There is a dose response relationship between the number of cigarettes smoked per day and cardiovascular morbidity and mortality (Neki 2002). Smoking is practice where a substance is burned and the smoke tasted or inhaled. This is primarily done as from of recreational drug use (Surgeon 2004) as of 2000 1.22 billion people worldwide practice smoking assuming no change in prevalence it is predicted that 1.45 billion people will smoke in 2010 and 1.5 to 1.9 billion in 2025 (Guenon 2003) in 2004 the WHO projected 58.8 billion death to occur globally from which 5.4 million are tobacco attributed and 4.9 million as of 2007 (WHO 2004). Smoking act synergist calls with the risk factor elevated blood fat levels high blood pressure to greatly increase the risk of CVD (health care 2005). Elevated total cholesterol protect effectively against coronary artery disease (Kerry and Robert 2007). Smoking tend to increase blood total cholesterol levels and Aspartate transaminase (AST) activity in smokers compared to nonsmokers (Narkiewiz 2005) the biological mechanism by which smoking or component of cigarette smoke influence bone loss are not well understood they may include local and systemic toxic effects on bone collagen synthesis alteration in metabolism of adrenal cortical and gonado hormone or other undermined mechanism (Krall and Dawson 1991). The mechanism by which smoking increases the cardiovascular diseases are unclear. Recently it has been suggested that smoking adversely affects the concentration of plasma lipids and lipoprotein levels.
1.2. Justification
Cardiovascular disease complication associated with chronic hypercholesterolemia is found to be major health problem that is currently in increased. Accelerated cardiovascular disease (coronary heart disease, myocardial infarction) atherosclerosis including hypercholesterolemia was report to be associated to cigarette smoker. The enzyme Aspartate Transaminase (AST) activity it is found to biological marker which can be adversely at the smokers and this may be leading to increase the association of heart disease in cigarette smokers. Therefore study was conducted to high light the risk of alteration of lipid (total cholesterol level and Aspartate transaminase activity) in Sudanese cigarette smokers.

1.3: Objectives:
1.3.1 General Objective:
To assess the lipid level among cigarette smoking Sudanese population.

1.3.2. Specific Objective:
1- To measure serum cholesterol level for all the participant.
2- To measure of low density lipoprotein and high density lipoprotein.
3- To correlate TC, LDL, HDL, AST activity with number and duration of cigarette smokers.
2. Literature Review

2.1 Structure of cigarettes

Cigarettes are a small roll of porous paper containing a rod of chopped up Tobacco leaf. Cigarettes are designed so that the tobacco can be smoked, by lighting the cigarette and breathing in the smoke. At the mouth end of the cigarette there is a second layer of porous paper (called tipping paper) and a filter. The tipping paper is designed to allow fresh air to infiltrate when the smoker inhales. This fresh air reduces the harshness of the smoke. The filter cools the smoke and reduces the flow of smoke out of the cigarette. Cigarettes also contain additives such as sugars and flavorings’ which are used to increase shelf life, control the rate at which the cigarette burns and control the delivery of the chemicals. Cigarettes vary in strength, taste and intensity depending upon: The type of tobacco leaf that is used. Where on the tobacco plant it is taken from; The way the leaf is cured (Manecklee 1994)

![Structure of cigarette diagram](image-url)
2.2 Smoking tobacco:
Tobacco, one of the most widely used addictive substance in the world, is a plant native to the Americas and historically one of the half-dozen most important crops grown by Americas farmers. From 1617 to 1793 tobacco was the most valuable staple export from the English American mainland colliers and the united states. Until the 1960s, the united states not only grew but also manufactured and exported more tobacco than any other country. since 1964 conclusive epidemiological evidence of the deadly effects of tobacco consumption has led to a sharp decline in official support for producers and manufacturers of tobacco, in spite of its indisputably large contribution to the agricultural, fiscal, manufacturing, and exporting sectors of the economy. Tobacco smoking is the act of burning the dried or cured leaves of the tobacco plant and inhaling the smoke for social (such as peer pressure or ritualistic) purposes, self-medicating, or to satisfy addiction. the practice was common among native americans throughout North and South Americas, and was later introduced to the rest of the world, via trade, following European exploration of the Americas (Woo kc., et al. 1997). Tobacco smoke contains nicotine an addictive stimulant. The effect of nicotine in first time or irregular users is an increase in alertness and memory, and mild euphoria in chronic user's nicotine simply relieves the symptoms of nicotine withdrawal confusion restlessness, anxiety and insomnia withdrawal symptoms in chronic users begin to appear approximately 30 minutes after every dose also disturbs metabolism and suppresses appetite (manechlee 1994)

2.3 Chemicals components of cigarette
Chemical compounds found in all phases of cigarette smoke have been associated with independent negative effects on the smoker, which means they produce their own separate damage. These are just some of these chemicals found in cigarette smoke;

i. Nicotine:
Nicotine is harmful component of tobacco smoke, which cause an addiction to smoking its stimulate and increases activity in brain just like heroin, caffeine and cocaine the effect of nicotine in the first time acts as an agonist that attaches to nicotine acetylcholine receptor sites in the brain and body. Some of these neurons influence. Containing is a byproduct of the metabolism of nicotine which remains in the blood for up to 48 hours. Nicotine increase dopamine levels in the reward circuits...
of the brain (Parking et al., 1998) When A cigarette is smoked. Nicotine rich blood passes from the lungs to the brain within seven seconds and immediately stimulates the release of many chemical messengers (Robicsek 1979). Chemical properties;

i. Nicotine easily penetrates the skin.

ii. Nicotine will burn at a temperature below its boiling point.

iii. Nicotine is a hygroscopic oily liquid that is miscible with water in its Base from as a nitrogenous base

Nicotine from salts with acids that are usually solid and water.

(Diereville etal.,1993)

**ii. Polycyclicaromatic hydrocarbons:**

These chemical names may not mean very much to most people until they realize where else these chemicals are found and then it becomes evident just how harmful they are. The following is a list of some chemicals found in cigarette smoke . Is one component but is effect on a there thrombotic disease have been equivocal ,an earlier study suggested that co could be responsible for smoking relate cardiovascular alterations however more result data suggest that Carbon monoxide from cigarette smoke was an unlike cause of atherosclerosis or thrombus( (Diereville et al., 1993).

**iii. Tar**

Tar is a mixture of the compounds in cigarette smoke which condensate (turn a gas to a solid) once in the lungs to form a sticky brown substance, this is the cigarette smoke condensate. Tar is the part of cigarette smoke which causes the yellow-brown stains on teeth and fingers. Tar is made of nitrogen, oxygen, carbon hydrogen, carbon dioxide monoxide, and a wide range other chemicals. Chemicals have short and long term effects on health. The short term effects include coughing and shortness of breath ( Lakatos et al., 2007).

**2.4 Disease caused by smoking:**

Smoking harms nearly every organ of the body, causing many diseases, and reduces quality of life and life expectancy .it has been estimated that in England, 364,000 patients are admitted to hospitals each year due to diseases caused by smoking .This translates in to 7,000 hospital admission per week,or1,000 day .for every death caused by smoking approximately 20 smokers are suffering from a smoking related disease (Benouwitz 1998). Half of all teenagers who are currently smoking will die from diseases caused by tobacco if they continue to smoke .one quarter will die after 79
years of age and one quarter before, with those dying before 70 losing on average 21 years of life. It is estimated that between 1950 and 2000 six million 0.60 million people worldwide died from tobacco–related diseases (Benouwitz 1998). Smokers face higher risk than non–smokers for a wide variety of illnesses, many of which may be fatal however. Many, medical conditions associated with smoking while they may not be fatal, may cause years of debilitating illness or other problems (Mortality statistics 2005).

2.5 Cardiovascular diseases:

Heart disease or cardiovascular disease is the class of diseases, that involve the heart or blood vessels, while the term technically refers to any disease that affects Cardiovascular system (Maton et al., 1993). Cardiovascular diseases (CVD) is major cause of morbidity and mortality in the industrialized world (CVD) morbidity and mortality have increase dramatically over the last 30 years. An estimated 60 million Americans have some form of CVD, and approximately 1.5milion myocardial infarctions and 600,000 strokes occur every year CVD could be characterized as a disturbance in hydraulic (homodynamic) and /or electrical (electro physiologic) function. Coronary atherosclerosis (i.e vascular obstruction) has been designated as the ‘prime mover’ of cardiovascular disorders, such that a series of atherogenic risk factors were sought, and many were identified (e.g. hypercholestoleamia, hypertension diabetes, obesity). Both our understanding of the etiology of CVD and our ability to manage the epidemic are still limit. For example the Framingham heart study used epidemiologic techniques to identify important risk factors (smoking, diabetes, hypertension, and cholesterol) however, these traditional risk factors explained only part of risk for CHD in practical terms, this means that these standard risk factors fail to predict many of the new CHD cases. one of these factors essential hypertension is of practically unknown etiology (De Faire 1997)

2.6 Risk Factors of cardiovascular diseases:

The risk of developing CVD depends to a large extent on the presence of several risk factors. the major risk factors for CVD include tobacco use, high blood pressure, high blood glucose, lipid abnormalities. Obesity, and physical inactivity. the global variation in these known risk factors, such as age ethnicity, and dander. obviously cannot be modified, most of the risk is attributable to lifestyle and behavioral patterns, which can be changed (Wolf 1991).
2.6.1. Respiratory problems:
Respiratory problems can include increased coughing, phlegm, wheezing, chest colds and shortness of breath, even in smoke as little as one cigarette a week (Berliner et al., 1991).

2.6.2. Gastrointestinal Effects:
Peptic ulcer disease is more likely to occur in smokers than in non-smokers, when ulcers are present they heal less rapidly in smokers and are more likely to recur. Evidence is accumulating that smoking is a risk factor for the occurrence of chronic bowel disease (Crohns disease, as well, smoking may contribute to the recurrence of this disease (Hippe et al., 1997).

2.6.3 Effects on teeth and gums:
Tobacco use is an important factor in oral health, apart from its role in causing oral cancer, smoking has also been linked to periodontal disease in younger people (Hippe et al., 1997).

2.2 Plasma lipid
The major lipid present in plasma is fatty acid Triglyceride & cholesterol and phospholipids other lipid (Marshall and Benger 1994).

2.2.1. Fatty Acids
Are straight chain carbon compounds the major lipid present in plasma are fatty acid of varying lengths they may be saturated containing no double bonds Monounsaturated with one or polyunsaturated with more than one (Philip 1994). Fatty acids are variable in length and can be classified as short –chain (4-6 carbon atoms) medium-chain (8-12 carbon atom) or long–chain (>12 carbon atoms) most fatty acid in our diet are of the long-chain variety and contain an even number of carbon atoms (Bishop and Schoeff 2000). May be esterifies with glycerol to form Triglycerides, or be non esterifies or free (NEFA or FFA). Plasma FFA liberated from adipose tissue are transported, mainly bound to albumin to the liver and muscle where they are metabolized. They provide a significant proportion of the energy requirements of the body (Philip 1994).
2.2.2 Triglyceride:
Are fatty acid esters of glycerol each containing three different fatty acids (Philip 1994). Molecules attach to one molecule of glycerol by ester bonds. because of the large number of possible forms fatty acids each fatty acid in the triglyceride molecule can potentially be different in structure producing many possible structural forms of Triglyceride (Bishop and Schoeff 2000). They are transported from The intestine and the liver to various tissues, such as adipose tissue, as lipoproteins, following hydrolysis, fatty acids are taken up, reesterifies and stored as Triglyceride. plasma Triglyceride concentrations rise after a fatty meal and remain increased for several hours. (Bishop and Schoeff 2000).

2.2.3 Cholesterol:
Steroid of unsaturated alcohol with high molecular consists of no polar rings, so is completely insoluble in water. is an unsaturated steroid alcohol containing 4 rings (A-B-C and D) and single C-H chain tail (Bishop and Schoeff 2000) steroid, is a precursor to many physiologically important steroids, such as bile acids and steroid hormones (Philip 1994) Cholesterol is required to build and maintain membranes it modulates membranes fluidity over the range of physiological temperatures (Koran et al., 1991).

2.2.4 Phospholipids:
Phospholipids is similar in structure to triglyceride but containing phosphate and nitrogenous base in place of the fatty acid they are important of cell membranes and lipoproteins maintaining the solubility of non-polar lipids and cholesterol the most common phospholipids found on lipoproteins and in cell membranes the two fatty acids in phospholipids are normally 14-24 carbon atoms long, with one fatty acid commonly saturated and the other un saturated. because phospholipids contain both hydrophobic fatty acid C-H chains and a hydrophilic head group found Elevated plasma concentration of lipid Bishop and Schoeff 2000) particularly cholesterol are causally related to pathogenesis of atherosclerosis the process responsible for the majority of cardiovascular disease cerebrovascular and peripheral vascular disease is the common cause of death (Marshall 2004).

2.3 Function of lipid:
1-Important of lipid in biological process:
2-Energy source (carbohydrate-lipids)
3- energy storage (most stored in body in the form lipid)
4- cell membrane structural component (phosphoglycerate-sphingolipid)
5- Hormone steroid hormone are chemical messenger that allow tissue of the body
6- vitamin a absorption dietary fat serves as carrier lipid soluble vitamin

(Katherine 2001).

2.3.1 Lipoproteins:
2.3.1.1 Structure of Lipoproteins:
Lipoprotein particles range in size from 10 to 1000 nanometers. The largest lipoproteins are about one tenth the size of a red blood cell. The density of lipoproteins increases in proportion to their ratio of proteins to lipids. In general, as the density of a lipoprotein increases, the size of the particles decreases. The outer layer of a lipoprotein consists of a water-soluble (hydrophilic) layer of apolipoproteins, phospholipids and cholesterol. The center of a lipoprotein is composed of cholesterol esters, triglycerides, fatty acids and fat-soluble vitamins like Vitamin E.

Structure of Lipoproteins (Allain et al., 1974).

2.3.1.2 Classification of lipoproteins:
Triglycerides rich particles include:
A. Chylomicrons; which transport exogenous lipid from the intestine to all cell.
B. Very low density lipoproteins (VLDL); which transport endogenous lipid from the liver to cell.
C. Intermediate density lipoproteins (IDL); which are usually undetectable in normal plasma, it is normally a transient intermediate lipoprotein formed during the conversion of VLDL to LDL.

Because of their large size, these particles reflect light and plasma containing high concentration appears turbid or milkly (lippedema), if turbid plasma sample is left
standing for 18 hours at 4 °C the larger Chylomicrons. Because of their low density, rise to form a creamy layer on the surface. The smaller, denser VLDL and IDL particles do not rise and the sample remains diffusely turbid (Whitby et al., 1987).

**Cholesterol rich particles:**

**A. Low density lipoproteins (LDL);**

Formed from VLDL, transport cholesterol to cells.

**B. High density lipoproteins (HDL)**

Are involved in the transport of cholesterol from the peripheral Lp(a) is similar in composition to LDL but has a higher protein content, it contains a protein which is structurally similar to the clotting factors. It is synthesized in the liver and normally present in low plasma concentrations (Whitby et al., 1987).

**Classification of lipoproteins** (Whitby et al., 1987).

2.4. Cholesterol:

**2.4.1 Structure of cholesterol:**

White crystalline substance, C27H45OH, found in various foods, that is normally synthesized by the liver. Cholesterol is only slightly soluble in water; it can dissolve and travel in the water-based bloodstream at exceedingly small concentration.
2.4.2 Function of cholesterol:
Cholesterol is essential constituent of cell membrane. Cholesterol is required to build and maintain membranes. It is also a precursor of bile acid including vitamin D. A cell can either synthesize cholesterol or acquire it from its environment. A cell can also acquire cholesterol from outside the cell through plasma membranes. Low density lipoprotein (LDL) receptor which mediates lipoprotein internalization into the cell. Satisfied it need for cholesterol by balance between synthesis and import (Dominiczak 1999). Cholesterol is the precursor molecule in several biochemical pathways.

In the liver, cholesterol is converted to bile, which is then stored in the gallbladder. Cholesterol is required to build and maintain intracellular transport. Cholesterol is essential for the structure. Cholesterol is an important precursor molecule for the steroid hormones, including the adrenal gland hormones (cortical and aldosterone) as well as the sex hormones (progesterone, estrogens, and testosterone), and their derivatives (Koren et al., 1991).

2.4.3 Source of cholesterol:
Either endogenous (body) or exogenous (diet). Diet contributes about 100-700 mg/day of cholesterol, about 500-1000 mg/day synthesized in the liver and other tissue, about 600-1000 mg/day excreted from biliary tract about 50% of which can be reabsorbed in to the blood through liver. 74% located into skin, adipose tissue, intestine and muscle cell (stationary pool). The remain portion (mobile pool) bound to protein mainly albumin in from of lipoprotein and transported through blood esterifies with fatty acid to form ester cholesterol. 1/3 present as free cholesterol (Bishop et al., 2006).

2.4.4 Synthesis of cholesterol:
The liver is the major site of cholesterol synthesis, although cholesterol is also produced in many other organ and tissues (Bishop et al., 2006).

Focusing on the enzyme the regulated sterol intermediates and the location of enzymes in the cell sterol are synthesized from the two carbon building block acetyl CoA. The soluble enzyme ace to acetyl CoA thiolase interconvert acetyl CoA and ace to acetyl CoA which are then condensed by 3-hydroxy-3methyl glutaryl (HMG) –CoA synthases to from HMG –CoA. There are two form of HMG-CoA synthases, mitochondrial form involved in cyto genesis predominate in the liver (Vance 2002).
HMG reductases catalyzes the reduction of HMG CoA to mevalonate. HMG CoA reductases mevalonic acid is converted into squalene after series of converted into squalene modified to yield cholesterol. The Synthesis of cholesterol in the liver regulated by the intracellular cholesterol concentration and activity of HMGCO reductases. Rate determined by enzyme of cholesterol biosynthetic pathway (Vance 2002). Hormonal effects cholesterol biosynthesis: Insulin stimulates HMG.COA reductases activity. Glucagon antagonizes the effect of insulin. Thyroid hormone HMG.COA reductases activity. The drug levitation which is used to treat hypercholesterolemia blocks endogenous cholesterol synthesis by inhibiting HMG.COA reductase (Davidson and Sittman1999).

2.4.5. Cholesterol absorption:
The average diet estimated to contain approximately 300 to 450 mg of cholesterol per day which mostly comes from the consumption of human A similar amount of cholesterol enters the gut from billiard secretion and the turnover and release intestine (Ash wood 2001). In order to be absorbed cholesterol is solubilised by formation of mixed micelles containing unsterilized cholesterol fatty acid monoglyceried phospholipids and conjugate bile acid these micelles also facilitate cholesterol transport across the luminal cell traffic in absence bile acids (Tietz 1976). Digestion and absorption of both cholesterol and triglycerides are severely impaired on the average 30- 60% dietary and intestinal cholesterol is absorbed daily to maximum of 1g/day when the oral intake reaches 3g/day (Tietz 1976).

2.4.6. Transportation of cholesterol:
Cholesterol consists of no polar rings, so is completely insoluble in water. Therefore, it has to be transported in lipoproteins as either bile salts or cholesterol esters. There are different densities of lipoproteins high density (HDL) and low density (LDL) is sometimes known as "bad" cholesterol and is responsible for transporting cholesterol form the liver. HDL is known as "good" cholesterol is transported in the watery fluids of the body in lipoproteins. These carriers are water soluble on the outside and fat soluble on the inside. The Water soluble coating allows cholesterol and other fats to move throughout the body without clogging blood vessels and other tissues (Vila et al., 2004). The food fed on the cholesterol and triglycerides, after absorption from the intestine of the Chylomikronen and then transported into the tissue. Different density lipoproteins (VLDL, IDL and LDL) carry themselves recorded and produced
cholesterol from the liver to the tissues. HDL cholesterol takes from the tissues and brings it back to the liver. The cholesterol in lipoproteins is predominantly esterifies with fatty acids. The range of these fatty acids is strongly influenced by the food (Korean et al., 1991).

2.4.7 Normal Range of cholesterol:
Cholesterol concentration at birth below 2.5mmol/l (100mg/dl) increase slowly but not exceed than 4.0 mmol/l (160 mg/dl) in children in adult 5.2 mmol/l (200 mg/dl) more in men than women during reproductive years (affected by age and sex) (Philly 1994.).

Cholesterol as predictor of cardiovascular diseases
Cholesterol is novel and traditional biochemical risk markers, of ischemic heart disease morbidity, and mortality in both diabetics and non-diabetics. Besides total cholesterol, elevated triglycerides high LDL Cholesterol have also been shown to independently increase cardiovascular mortality in diabetic’s patients. Recently prospective studies illustrated that many cases of cardiovascular diseases like stroke, ischemic heart disease and myocardial infarction, occur with normal level of cholesterol, which create the need of anew sensitive predicator for cardiovascular diseases (William, et al 1998)

2.5 Plasma lipids and cardiovascular disease:
Plasma cholesterol concentration is correlated with the incidence of ischemic heart disease. But not necessarily as cause and effect. There is nuclear cut – off between normal values and increased risk, however it appears to be a particularly high risk, with a rapidly rising incidence. of ischemic heart disease if plasma total cholesterol exceeds 6.0 mmol/L(235mg/dl).

is a positive correlation between the risk of developing ischemic heart disease and raised plasma total and LDL –cholesterol concentration and a negative one with plasma raised HDL-cholesterol concentration. Lowering high plasma LDL-cholesterol concentration reduce the risk of cardiovascular disease. Hypercholesterolemia, is just one of the major risk factor of cardiovascular disease. other include smoking and hypertension (Stryer 1998).
2.5.1 Hypercholesterolemia:

2.5.1.1 Primary Hypercholesterolemia:

The familial incidence of Hypercholesterolemia, often associated with an increased risk of ischemic heart disease. Men adult level of total cholesterol (TC) vary in different communities throughout the world from about 3.9 mmol/L (150 mg/dl) to over 7 mmol/L (275 mg/dl). When community levels are compared with IHD mortality rates, a striking corris observed. The international atherosclerosis project, which investigated autopsy material from over 20,000 individuals in various cities throughout the world, demonstrated also a strong association between the mean total cholesterol levels for various communities and the prevailing severity of Atheroma (Murray et al., 1999). Primary Hypercholesterolemia is transmitted by an autosomal dominant inheritance. Homozygote commonly develop flat coetaneous and tendon exanthemas, and usually die from ischemic heart disease in early adult life. In heterozygote the number of LDL receptors is reduced by about 50 percent and the plasma concentrations are about twice these in normal subject. They have a 10 to 20-fold higher risk of developing ischemic heart disease than those with normal plasma concentration (Murray et al., 1999).

2.5.1.2 Secondary Hypercholesterolemia:

The commonest disorders that may produce a secondary increase in plasma total cholesterol concentration are:

i. Primary Hypothyroidism
ii. Nephritic syndrome
iii. Chronic renal failure
iv. Cholestasis
v. Diabetes mellitus
vi. Some drugs (Whitby 1987).

These disorders must be excluded in any patient presenting with hypercholesterolemia.

2.5.3. Treatment of Hypercholesterolemia:

A. Nicotinic acid:

Nicotinic acid reduces the plasma levels of both VLDLs and LDLs by inhibiting hepatic VLDL secretion, as well as suppressing the flux of FFA release from adipose
tissue by inhibiting lipolysis. In addition, nicotinic administration strongly increases the circulating levels of HDLs.

**B. Cholestyramine or cholesterol (resins):**

These compounds are non-absorbable resins that bind bile acids which are then not reabsorbed by the liver but excreted. The drop in hepatic reabsorption of bile acids releases a feedback inhibitory mechanism that had been inhibiting bile acid synthesis.

**C. Ezetimibe**

functions to reduce intestinal absorption of cholesterol, thus effecting a reduction in circulating cholesterol. The drug functions by inhibiting the intestinal brush border transporter involved in absorption of cholesterol (Bucoio and David 1973).

**2.6. Atheroma (Atherosclerosis):**

In most developed countries this is responsible for more deaths than any other disease. It causes narrowing of the lumen of arteries, and it is the major causes of disability and death from heart disease, cerebral infraction and ischemia of the lower limbs, due mainly to accumulation of lipids, proliferation of smooth muscle cells and formation of fibrous tissue (Anderson 1988). The considering further the importance of plasma lipid, it may be recalled that about 70% of cholesterol is carried low density lipoproteins (LDL), about 20% in the high density lipoproteins and only a small percentage in very low density lipoproteins (VLDL). The level of LDL relates closely to total cholesterol (TC) levels, and prospective studies which have included lipoprotein assays have demonstrated that LDL levels are predictive of the risk of IHD. Plasma levels of HDL are related inversely to the risk of IHD (Murray et al., 1999).

**2.7 Relationship between smoking and lipid:**

Smoking is a major cause of atherosclerosis — a buildup of fatty substances in the arteries. Atherosclerosis occurs when the normal lining of the arteries deteriorates, the walls of the arteries thicken and deposits of fat and plaque block the flow of blood through the arteries. In coronary artery disease, the arteries that supply blood to the heart become severely narrowed, decreasing the supply of oxygen-rich blood to the heart, especially during times of increased activity. Extra strain on the heart may result in chest pain (angina pectoris) and other symptoms. When one or more of the coronary arteries are completely blocked, a heart attack (injury to the heart muscle) may occur. In peripheral artery disease, atherosclerosis affects the arteries that carry blood to the arms and legs. As a result, the patient may experience painful cramping.
of the leg muscles when walking (a condition called intermittent claudicating). Peripheral artery disease also increases the risk of stroke (Bottcher and Falk 1999).

2.8 Lipoproteins:
Lipoproteins are macromolecular complexes that carry hydrophobic plasma lipids, particularly cholesterol and triglycerides, in the plasma. They transport essentially all of the cholesterol and esterified lipids in blood.

Lipoprotein structure:
Lipoproteins are spherical particles made up of hundreds of lipid and protein when larger triglycerides rich lipoproteins are present in high concentration, plasma can appear turbid or milky to naked eyes. The major lipids of lipoproteins are cholesterol, triglycerides and phospholipid. Triglycerides and the esterified form of cholesterol (cholesterol esters) are non-polar lipids that are insoluble in aqueous environments (hydrophobic) and compromise the core of the lipoproteins. Phospholipids and a small quantity of free (unesterified) cholesterol, which are in both lipid and aqueous environments (amphipathic), cover the surface of the particles. A family of protein called apolipoproteins also occupy the surface of the lipoprotein and play a crucial role in the regulation of lipid transport and lipoprotein metabolism (Harrison’s 1998).

2.8.1 Types of plasma lipoproteins:
Four major classes and two minor classes of lipoproteins identified, based on particles size, chemical composition, physiochemical characteristics, flotation characteristics and electrophoretic mobility.

Four major lipoproteins are:
1. Chylomicrons
2. Very low density lipoprotein (VLDL)
3. Low density lipoprotein (LDL)
4. High density lipoprotein (HDL)

Two minor lipoproteins are:
1. Intermediate density lipoproteins (LDL)
2. Lipoprotein (a)

The protein moiety of lipoprotein is composed of several specified proteins called as apolipoproteins.

VLDL accounts for most of the triglycerides in the plasma, LDL carry most of the cholesterol normal plasma. The LDL is about 50% by weight cholesterol and 20% by
weight protein. HDL is about 50% by weight protein and 50% by weight lipid (Hevel, Holdsten and Brown 2012).

2.8.1.1 Chylomicrons:
Chylomicrons are large particles produced by intestine that are rich in triglycerides (85-95%) of exogenous (dietary) origin and relatively poor in free cholesterol and phospholipids.
- Contains 1-2% of protein by weight.
- Because of high lipid/protein ratio, Chylomicrons are considerably less dense than water and float without centrifugation.
- High Chylomicrons content results in milky plasma in which Chylomicrons accumulate as a floating creamy layer when left undisturbed for several hours.
- Apo lipoproteins in Chylomicrons include:
  Apo B - 48
  Apo A – I present in newly secreted particle
  Apo A - IV
  Apo C – I
  Apo C – II acquired from other lipoproteins in the circulation
  Apo C - III
  Apo E
Interaction of Chylomicrons and lipoprotein lipase results in a smaller particles depleted in triglycerides and some surface elements which is referred to as Chylomicrons remnant. Peripheral metabolism of Chylomicrons mainly yields triglycerides.

2.8.1.2 Very low density lipoprotein (VLDL):
- VLDL is a major transport vehicle for endogenously synthesized triglycerides and is predominantly synthesized in liver.
- VLDL particles are smaller than Chylomicrons and are rich in triglycerides.
- They have a lower lipids/protein ratio and thus float at a somewhat higher density.
- When excessive amount of VLDL are present the plasma appears turbid.
- VLDL triglycerides which are of endogenous origin, mainly hepatic constitute about half the particles mass.
- Cholesterol and phospholipids make up about 40% of the particles and about 10% of mass is made of protein.
• Apo protein present here are:
Apo B - 100
Apo C
Apo E

VLDL particle size varies widely with concomitant variation of chemical composition. Large particles are rich in triglycerides and apo C and smaller particles are poorer in these two components. Smaller particles depleted of triglycerides and surface material result from hydrolysis of VLDL by lipoprotein lipase. These particles are referred to as VLDL remnants and intermediate density lipoprotein (IDL) (Tietz fundamentals1999).

2.8.1.3 Low density lipoprotein (LDL):
• LDL is the principal vehicle for the transport of cholesterol from liver to the body cells. LDL is formed in the circulation by progressive removal of triglycerides from VLDL.
• LDL consists of about 50% of total lipoprotein mass in human plasma.
• LDL particles are much smaller than the triglycerides rich lipoproteins.
• Cholesterol most of which is esterifies accounts for about half of the LDL mass.
• About 25% of LDL mass is protein moiety (apo-100, with trace of apc).
• Discrete sub fractions of LDL have been identified that differ somewhat in their sizes and chemical composition. The smaller species of LDL contain lower amounts of cholesterol ester resulting in lower ratio of cholesterol to apo B in these particles than in larger species of LDL.
• Increased amount of smaller particles have been found in patients with several common form of dyslipoproteinemia that are associated with coronary artery disease (CAD) (Tietz fundamentals1999).

2.8.1.4 High density lipoproteins:
• HDL are small particles consisting of 50% protein (mostly apo A- I and apo - II but some apo C and E), 30% phospholipids and only trace of triglycerides.
• HDL can be separated into 2 major subclasses hdl2 and hdl3 which differ in their density, particle size and composition.
• These are synthesized in the liver and in the intestine and appear to play a significant role in the transport of cholesterol from peripheral cells to liver, where cholesterol is removed from the body via bile.
• Small increments in HDL-C, impact significantly in CHD rates with 2% to 4% reduction in CHD risk for each 1gm/dl increase in HDL-C levels.
• According to the Helsinki heart study, an elevated risk of CHD depended more on HDL-C than LDL-C.
• The antiatherogenic properties of HDL-C have been ascribed to its multiple functions, including reverse cholesterol transport, maintenance of endothelial function and protection against thrombosis (Tietz fundamentals1999).

### 2.8.2 Transport of Dietary Lipids (Exogenous Pathway):

The exogenous pathway of lipoprotein metabolism permits efficient transport of dietary lipids. Dietary triglycerides are hydrolyzed by lipases within the intestinal lumen and emulsified with bile acids to form micelles. Dietary cholesterol, fatty acids, and fat-soluble vitamins are absorbed in the proximal small intestine. Cholesterol and retinol are esterifies (by the addition of a fatty acid) in the enterocyte to form cholesterol esters and retinyl esters, respectively. Longer-chain fatty acids (>12 carbons) are incorporated into triglycerides and packaged with apoB-48, cholesterol esters, retinyl esters, phospholipids, and cholesterol to form Chylomicrons. Nascent Chylomicrons are secreted into the intestinal lymph and delivered via the thoracic duct directly to the systemic circulation, where they are extensively processed by peripheral tissues before reaching the liver. The particles encounter lipoprotein lipase (LPL), which is anchored to a glycosylphosphatidylinositol-anchored protein, GPIHBP1, that is attached to the endothelial surfaces of capillaries in adipose tissue, heart, and skeletal muscle. The triglycerides of Chylomicrons are hydrolyzed by LPL, and free fatty acids are released. Apo-II, which is transferred to circulating Chylomicrons from HDL, acts as a required cofactor for LPL in this reaction. The released free fatty acids are taken up by adjacent myocytes or adiposities and either oxidized to generate energy or esterifies and stored as triglyceride. Some of the released free fatty acids bind albumin before entering cells and are transported to other tissues, especially the liver. The Chylomicrons particle progressively shrinks in size as the hydrophobic core is hydrolyzed and the hydrophilic lipids (cholesterol and phospholipids) and apolipoproteins on the particle surface are transferred to HDL, creating Chylomicrons remnants. Chylomicrons remnants are rapidly removed from the circulation by the liver through a process that requires apoE as a legend for receptors in the liver. Consequently, few, if any, Chylomicrons or Chylomicrons
remnants are present in the blood after a 12-hour fast, except in patients with disorders of Chylomicrons metabolism (Tietz fundamentals 1999).

2.8.3 Transport of Hepatic Lipids (Endogenous Pathway):

The endogenous pathway of lipoprotein metabolism refers to the secretion of apoB-containing lipoproteins from the liver and the metabolism of these triglyceride rich particles in peripheral tissues. VLDL particles resemble Chylomicrons in protein composition but contain apoB-100 rather than apoB-48 and have a higher ratio of cholesterol to triglyceride (1 mg of cholesterol for every 5 mg of triglyceride). The triglycerides of VLDL are derived predominantly from the esterification of long-chain fatty acids in the liver. The packaging of hepatic triglycerides with the other major components of the nascent VLDL particle (apoB-100, cholesterol esters, phospholipids, and vitamin E) requires the action of the enzyme microsomal triglyceride transfer protein (MTP). After secretion into the plasma, VLDL acquires multiple copies of apoE and Apolipoproteins of the C series by transfer from HDL. As with Chylomicrons, the triglycerides of VLDL are hydrolyzed by LPL, especially in muscle, heart, and adipose tissue. After the VLDL remnants dissociate from LPL, they are referred to as IDLs, which contain roughly similar amounts of cholesterol and triglyceride. The liver removes approximately 40–60% of IDL by LDL receptor–mediated endocytosis via binding to apoE. The remainder of IDL is remodeled by hepatic lipase (HL) to form LDL. During this process, most of the triglyceride in the particle hydrolyzed, and all Apolipoproteins except apoB-100 are transferred to other lipoproteins. The cholesterol in LDL accounts for more than one-half of the plasma cholesterol in most individuals. Approximately 70% of circulating LDL is cleared by LDL receptor–mediated endocytosis in the liver. Lipoprotein(a) [Lp(a)] is a lipoprotein similar to LDL in lipid and protein composition, but it contains an additional protein called Apolipoproteins(a) [apo(a)]. Apo(a) is synthesized in the liver and attached to apoB-100 by a disulfide linkage. The major site of clearance of Lp (a) is the liver, but the uptake pathway is not known (Tietz fundamentals 1999).

2.9 Aspartate transaminase (AST):

Aspartate transaminase (AST), also called aspartate aminotransferase (AspAT/ASAT/AAT) or serum glutamic oxaloacetic transaminase (SGOT), is a pyridoxal phosphate (PLP)-dependent transaminase enzyme (EC 2.6.1.1). AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate
and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells (Kirsch et al., 1984).

2.9.1 Function:
Aspartate transaminase catalyzes the interconversion of aspartate and \( \alpha \)-ketoglutarate to oxaloacetate and glutamate. Aspartate (Asp) + \( \alpha \)-ketoglutarate \( \leftrightarrow \) oxaloacetate + glutamate (Glu) Reaction catalyzed by aspartate aminotransferase As a prototypical transaminase, AST relies on PLP as a cofactor to transfer the amino group from aspartate or glutamate to the corresponding ketoacid. In the process, the cofactor shuttles between PLP and the pyridoxamine phosphate (PMP) form (Kirsch et al., 1984) The amino group transfer catalyzed by this enzyme is crucial in both amino acid degradation and biosynthesis. In amino acid degradation, following the conversion of \( \alpha \)-ketoglutarate to glutamate, glutamate subsequently undergoes oxidative deamination to form ammonium ions, which are excreted as urea. In the reverse reaction, aspartate may be synthesized from oxaloacetate, which is a key intermediate in the citric acid cycle (Berg 2006).

2.9.2 Isoenzyme:
Two Iso enzymes are present in a wide variety of eukaryotes. In humans:
1. GOT1/cAST, the cytosolic Isoenzyme derives mainly from red blood cells and heart.
2. GOT2/mAST, the mitochondrial iso enzyme is present predominantly in liver. These iso enzymes are thought to have evolved from a common ancestral AST via gene duplication, and they share a sequence homology of approximately 45%. (Hayashi et al 1990).

2.9.3 Clinical significance:
AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells (Goldberg and Kirsch 1996).
AST may be elevated also in diseases affecting other organs, such as
1-myocardial infarction.
2- acute pancreatitis.
3-acute hemolytic anemia.
4-severe burns.
5-acute renal disease.
6-musculoskeletal diseases.
7-trauma (Gaze DC 2007).
AST was defined as a biochemical marker for the diagnosis of acute myocardial infarction in 1954. However, the use of AST for such a diagnosis is now redundant and has been superseded by the cardiac troponins. AST (SGOT) is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health. (Gaze DC 2007).

**Previous Study:**

Alteration of lipid profile can adversely causing dyslipidemia in smokers and the changes become more mark with the increase number of cigarette smoked and with the increase of the duration of smoking in years, smoking cause an increase in oxidize LDL cholesterol level which plays the key role for a atherosclerotic process A high levels of LDL-C, are strongly associated with the development of coronary artery disease while a low level of HDL-C remains significant independent predictor of coronary artery disease. Stampfer MJ, Sacks FM, Simonetta S A (1991) prospective study of cholesterol, apolipoporroteins and the risk of myocardial infarction N Engl J v (325); N;373-80.
Chapter Three
3. Materials and Methods

3.1.1 Study design:
Descriptive cross sectional laboratory based study.

3.1.2 Study approach:
A quantitative method was used to measure total cholesterol level and LDL-C and HDL-C and Aspartate transaminase (AST) activity in Sudanese cigarette smokers.

3.1.3 Study area and duration:
This study was carried out in Wad Medani, Gezira State, Sudan in period from (August to November 2017).

3.1.4 Inclusion criteria:
Any volunteer adult healthy heavy smoker agrees to participate in the study.

3.1.5 Exclusion criteria:
- Any patient with renal and heart disease.
- Diabetes mellitus.
- Hypertensive.

3.1.6 Sample size:
The study included 90 male healthy heavy smokers.

3.1.7 Ethical Considerations:
Ethical approval obtained from University of Gezira research committee. Informed consent was provided to participant and all participants gave an oral consent.

3.1.8 Data Collection tools:
Questionnaire was used for information.

3.1.9 Study variables:
- Ages and sex.
- Duration and number of cigarette smoked

3.2 Material:
- For collection and examination: questionnaire, permit, lithium heparin container, syringe, gloves, Sterile alcohol preps, Tourniquet, Sterile gauze pads, paper adhesive tape, centrifuge.
The chemical analyzer (MindaryBS200) was used for lipid profile (total cholesterol, LDL, HDL) and aspartate transaminase (AST) activity.

3.3 Method:

3.3.1 Sample collection and preparation:
Three ml of venous blood specimen were collected by sterile, disposable syringe in lithium heparin containers, plasma was separated after centrifuged for 3000 Rm for 15 minutes and kept at-20C.

3.3.2 Method of Estimation:

1. Estimation of (Cholesterol):
Cholesterol esterase enzymatic assay (appendix-1).

2. Estimation of (HDL-C):
Homogeneous enzymatic assay (appendix-2).

3. Estimation of (LDL-C):
Homogeneous enzymatic assay (appendix-3)

4. Estimation of Aspartate transaminase (AST):
Homogeneous enzymatic assay (appendix-4)

5. Data analysis:
Data was analyzed and tabulated using the (Statistical Package for Social Sciences) (SPSS), program version 19. P-value < 0.05 considered significant.
Chapter Four

4. Results and Discussion

4.1 Results

Ninety smokers participated for this study divided into three groups, group (1) smoke up to (10) cigarette/day, group (2) smoke between (10) to (20) cigarette/day, group (3) smoke above (20) cigarette/day. Positive correlation between lipid profile and duration of cigarette smoking was also reported (Muscat and Haris1991) claimed that the level of total cholesterol level in the blood of the smokers increase with the increase of the number of smoked cigarette, recently Altoum (2007) observed strong correlation between the level of total cholesterol in the blood of the smoke and number of cigarette smoked.

Table (1) Comparison the mean of total cholesterol level between the three cigarette smoker groups.

<table>
<thead>
<tr>
<th>Groups of Cigarette / Day</th>
<th>No</th>
<th>Mean</th>
<th>Std Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1—10 Cigarettes</td>
<td>30</td>
<td>133</td>
<td>17</td>
<td>0.000</td>
</tr>
<tr>
<td>Group 11—20 Cigarettes</td>
<td>30</td>
<td>196</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>&gt; 20 Cigarettes</td>
<td>30</td>
<td>251</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>
Table (2) Comparison the mean of LDL-C level between the three cigarette smoker groups.

<table>
<thead>
<tr>
<th>Group of Cigarette / Day</th>
<th>No</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1—10 Cigarettes</td>
<td>30</td>
<td>110</td>
<td>20</td>
<td>0.000</td>
</tr>
<tr>
<td>Group 11--20 Cigarettes</td>
<td>30</td>
<td>144</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>&gt; 20 Cigarettes</td>
<td>30</td>
<td>201</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Table (3) Comparison the mean of HDL-C level between the three cigarette smoker groups.

<table>
<thead>
<tr>
<th>Groups of Cigarette / Day</th>
<th>No</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1—10 Cigarettes</td>
<td>30</td>
<td>48</td>
<td>6.7</td>
<td>0.000</td>
</tr>
<tr>
<td>Group 11--20 Cigarettes</td>
<td>30</td>
<td>38.8</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>&gt; 20 Cigarettes</td>
<td>30</td>
<td>23</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Table (4) Comparison the mean of aspartate transaminase activity between the three cigarette smoker groups.

<table>
<thead>
<tr>
<th>Groups Of Cigarette / Day</th>
<th>No</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1—10 Cigarettes</td>
<td>30</td>
<td>31.7</td>
<td>8.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Group 11—20 Cigarettes</td>
<td>30</td>
<td>32.7</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>&gt; 20 Cigarettes</td>
<td>30</td>
<td>71.6</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Discussion

Smoking is one of the major risk factors in the genesis of coronary atherosclerosis and development of coronary heart disease. The hazards of cigarette smoking are well recognized worldwide (Fagerstrom2002 and (Ezzati and, Lopez 2004), yet significant numbers of people continue to smoke in the developing countries (Reda and Moges 2012). In Sudan, prevalence of cigarette smoking in the adult population reached 12% (Idris and Ibrahim 1998). This study confirms that the serum cholesterol level increase in relation to the number of cigarette smoked per/day (The higher the smoked cigarette the highest the cholesterol level). This study showed significantly increased in total cholesterol (133±17mg/dl), (196±26mg/dl), (251±34 mg/dl) P-value (0.000) in three groups respectively. This result was agree with study done by Rustogi., (Japan) et al and NS Neki., et al in Indian) and (Anila et al (Indian 2007). The current study proved that LDL level increased with increased in the number of cigarette smoked per/day (110±20mg/dl), (144±22mg/dl), (201±21mg/dl) P-value 0.000) in three groups respectively. free radicals combine with LDL-C particles causing them to more easily burrow into the artery cell walls clogging the arteries & hastening the atherosclerotic process, This result was agree with study done by D.A. Odedeji., et al Pakistan (2006). The present study confirm that smoking has strong effects on HDL concentration (the highest cigarette smoked per/day the lower HDL concentration (133±17 mg/dl), (196±26 mg/dl), (251±34 mg/dl) P-value 0.000) in three groups respectively. This result was agree with study done by Rosenson., et al and Maeda., et al 2003(Arch Intern Med), Brischetto., et al (American J Cardio). The risk for CHD events in smokers increased significantly increase in total cholesterol and decrease in HDL cholesterol which may lead to alter the incidence of developing cardiac heart diseases, this varied considerably by gender and region (Nakamura (2009). This study proved that (AST) level within the normal range in individuals who smoke less than 20 cigarette per/day for duration of less than 10 years but it is high in those whom smoke more than 20 cigarette per/day above 20 years, (31.7±8.2 mg/dl), (32.7±8.8 mg/dl), (71.6±13.9 mg/dl) P-value 0.000) in three groups respectively. This result was agree with study done by Anyanwu and Etukudo, (2002). This study conclude that the more number of cigarette smoked per/day and the longer the duration of smoking, the highest level of total cholesterol, LDL, AST, and the lower HDL level.
Chapter Five

Conclusions and Recommendations

5.1 Conclusions

. The study concludes that serum total cholesterol level, LDL and aspartate transaminase (AST) activity was increased in healthy heavy smokers.
. The serum HDL level was decreased in healthy heavy smokers.
. Total cholesterol and LDL, Aspartate transaminase(AST) activity significant
. Total cholesterol level was found affected .elevated in the blood of Sudanese smokers by the duration of smoking and number of cigarette smoked. The age of smokers has no significance the level of total cholesterol. The level of Aspartate transaminase (AST) activity has being found not to be affected by the duration of smoking nor in the number cigarette per day or age of smokers.

5.2 Recommendations

. Lipid profile should be regularly monitored for smoker to avoid the complication of cardiovascular disease and atherosclerosis.
. Health education for the community about the hazard and complication of cigarette smoking should be implemented.
. The government must play an obvious role is the war against cigarette smokers and effective international plans to reduce smoking among population.
REFERENCES


Johnkennedy , N.(2010). Effect of smoking on lipid profile among adult smoking in owerrri, NIGERIA. *Journal of Medical Laboratory Science*; 1(2): 2048 -497X.

Kerry, Robert I; Hamby (2007) increase good cholesterol with heart disease health ivillage come heart health subject patient 102-106.


Nakamura, K.(2009). Smoking worsens effects of adverse lipid profile on CHD risk Heart; Advance online publication.


Appendices
University of Gezira
Faculty of Medical Laboratory Sciences
Questionnaire
Evaluation of Serum Total Cholesterol, LDL&HDL Ratio and Aspartate Transaminase Activity Among Healthy Sudanese Heavy Smokers. Sudanese Wad Madani, Gezira State, Sudan (2017)

Name…………………………………………………………………………………………
Serial number………………………………………………………………………………
Age…………………………………………………………………………………………
Sex…………………………………………………………………………………………
Locality……………………………………………………………………………………
Number of cigarette per/day……………………………………………………………..
Duration of smoking……………………………………………………………………..

Other diseases: Yes (  )     No (  )
If the answer is yes, mention the disease / diseases
………………………………………………………………………………………………
………………………………………………………………………………………………

2. Budgets:

<table>
<thead>
<tr>
<th>Materials</th>
<th>Number</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton</td>
<td>1 weal</td>
<td>10</td>
</tr>
<tr>
<td>Syringes</td>
<td>1 box</td>
<td>80</td>
</tr>
<tr>
<td>Tourniquet</td>
<td>1 piece</td>
<td>30</td>
</tr>
<tr>
<td>Plain tube</td>
<td>2 box</td>
<td>60</td>
</tr>
<tr>
<td>Gloves</td>
<td>1 box</td>
<td>70</td>
</tr>
</tbody>
</table>
Appendix-1-

TC (Total Cholesterol):

**Clinical significance:** Cholesterol is a main component of cell membranes and lipoprotein and it is the precursor for steroid hormones and bile acids synthesizing. Cholesterol is transported in plasma by low-density lipoprotein. The level of the individual’s total cholesterol is used in screening early atherosclerosis and monitoring the clinical effect of drugs or low-fat diet.

**Method**

Cholesterol oxidase- Peroxidase (CHOD-POD) method

**Reaction Principle**

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightleftharpoons{\text{CHE}} \text{Cholesterol} + \text{Fatty acid}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{CHO}} \text{4-Cholestenone} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4\text{H}_2\text{O}
\]

By the catalysis of CHE and CHO, Cholesterol ester is catalyzed to yield H2O2, which oxidates 4- Aminoantipyrine with phenol to form a colored dye of quinoneimine. The absorbency increase is directly proportional to the concentration of cholesterol.

**Reference Intervals:**

For adult : up to 200 mg/dl

S.I. Units Serum / Plasma ≤ 5.2 mmol/l
Appendix-2-

HDL-C (High Density Lipoprotein - Cholesterol)

Clinical significance: HDL cholesterol is inversely related to the risk of developing coronary artery disease. A low HDL/LDL cholesterol ratio is directly related to the risk of developing coronary artery disease. A high HDL cholesterol is associated with the "longevity" syndrome.

Method

Direct method

Reaction Principle

(1) LDL, VLDL, Chylomicrons $\rightarrow$ Cholestenone + H2O2

$$2\text{H}_2\text{O}_2 \xleftarrow{\text{Catalase}} \text{2H}_2\text{O} + \text{O}_2$$

(2) HDL $\rightarrow$ Cholestenone + H2O2

$$\text{H}_2\text{O}_2 + \text{HDAOS} + 4\text{-aminoantipyrin} \xrightarrow{\text{POD}} \text{Quinoneimine}$$

The System monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the HDL-cholesterol concentration.

Reference Intervals:

For adult: more than 40 mg/dl

Conventional Units Serum more than 1-1.15 mmol/L
Appendix-3-

LDL-C (Low Density Lipoprotein - Cholesterol)

Clinical significance

LDL-Cholesterol is directly related to the risk of developing coronary heart disease. A low HDL/LDL-Cholesterol ratio is directly related to the risk of developing coronary artery disease. Elevated LDL-Cholesterol is the primary target of cholesterol-lowering therapy.

Method

Direct method

Reaction Principle

(1) HDL, VLDL, Chylomicrons $\leftrightarrow$ Cholestenone + H2O2

$2\text{H}_2\text{O}_2$ $\text{Catalase}$ $2\text{H}_2\text{O} + \text{O}_2$

(2) LDL $\text{CHE} + \text{CHO}$ $\leftrightarrow$ Cholestenone + H2O2

$\text{H}_2\text{O}_2 + \text{TOOS} + 4$-aminoantipyrin $\leftrightarrow$ POD Quinoneimine

The System monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the LDL-cholesterol concentration.

Reference Intervals:

For adult: 90----160 mg/dl

Conventional Units Serum 0-4.11 mmol/L
Appendix-4-

AST (Aspartate Aminotransferase)

**Clinical significant:** Aspartate aminotransferase (EC 2.6.1.1, AST), formerly called Glutamic Oxalacetic Transaminase (GOT), is present in both cytoplasm and mitochondria of cells, belonging to the transaminase family, which catalyze the conversion of amino acids and α-oxoglutarate by transfer of amino groups. AST is commonly found in various human tissues. The heart muscle is found to have the most activity of the enzyme, secondly in the brain, liver, gastric mucosa, skeletal muscle and kidneys. The serum AST present low activity in the healthy human body, but when these tissues injury or damage, AST is released into blood and results in high blood AST activity. Measurement of AST in serum and plasma is mainly used for the diagnosis of heart muscle damages, liver damages and skeletal muscle diseases as well as for monitoring the treatment. The AST/ALT ratio is often used for differential diagnosis in liver diseases. While the ratio < 1, it indicates mild liver damage, otherwise it is associated with severe, often chronic liver diseases.

**Method:** UV-assay according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) without pyridoxal phosphate activation.

**Reaction Principle**

\[
\text{L-aspartate} + \alpha\text{-oxoglutarate} \rightarrow \text{oxaloacetate} + \text{L-glutamate}
\]

\[
\text{L-malate} + \text{NAD}^+ (\text{MDH} - \text{Malate} \rightarrow \text{oxaloacetate} + \text{NADH} + \text{H}^+
\]

Dehydrogenase, EC1.1.1.37) In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and α-oxoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with NADH being oxidized to NAD+. The rate of the photometrically determined NADH decrease is directly proportional to the rate of formation of oxaloacetate and thus the AST activity.

**Reference Intervals:**

- **Male:** ≤35 U/L ≤0.58 μkat/L
- **Female:** ≤31 U/L ≤0.52 μkat/L