Sudan University of Science and Technology

College of Graduate Studies

Influence of Smoking on Serum Total Cholesterol, High Density Lipoprotein Cholesterol and Low Density Lipoprotein CholesterolLevels among Sudanese Male Smokers in Khartoum State

أثر التدخين فى مستوي الكوليسترول الكلي والكوليسترول الجيد والكوليسترول السيء لدى السودانين المدخنيين الذكور بولاية الخرطوم

A dissertation submitted for partial fulfillment for the requirements of M.Sc. degree in Clinical Chemistry

By

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2015

بِسْمِ اللّهِ الرّحْمَنِ الرّحِيمِ

قال الله تعالى :

وَوَصَّيْنَا الْإِنسَانَ بِوَالِدَيْهِ إِحْسَانًا ۖ حَمَلَتْهُ أُمُّهُ كُرْهًا وَوَضَعَتْهُ كُرْهًا ۖ وَحَمْلُهُ وَفِصَالُهُ ثَلَاثُونَ شَهْرًا ۚ حَتَّىٰ إِذَا بَلَغَ أَشُدَّهُ وَبَلَغَ أَرْبَعِينَ سَنَةً قَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَصْلِحْ لِي فِي ذُرِّيَّتِي ۖ إِنِّي تُبْتُ إِلَيْكَ وَإِنِّي مِنَ الْمُسْلِمِينَ

صدق الله العظيم
سورة الأحقاف الآية 15

Dedication

This work dedicated to

My husband and daughters

My mother and father

My brother and sisters

My friends

To all my prophesiers in the

Clinical chemistry department

Acknowledgements

Thanks first and last to ALLAH who enables me to Conduct this study by grace of him and donate strength and patience. I would like to express my sincere appreciation of thanks to my supervisor Dr. Mariam Abbas for continuous help and guidance during this work. Great thanks to my family, they are always supporting and encouraging me with their best wishes. Thanks also are extending to all my teachers in Sudan University of Science and Technology. My appreciation is also to all volunteers from who blood samples were collected. Finally, I wish to express my great thanks to any people who helped me in this work.

Abstract

Cigarette smoking is widely spread throughout the world and the effects of smoking on human health are serious.

This is a case control study was done in Khartoum state during February to March 2015, to evaluate serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol levels in male smokers.

Sixty smokers were enrolled as test group and forty non smokers as control group (age was matched) (16 – 65 years), blood specimens were collected from both groups and serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol concentrations were analyzed by using enzymatic methods.

Statistical analysis was done by using SPSS,the results showed significant increase in cholesterol concentration compared with control group (p=0.000) ,and significant decrease in HDLc level compared with control group (p=0.000), and significant increase in LDLc level compared with control group (p=0.052).

The results also showed insignificant weak positive correlation between cholesterol concentration and age, insignificantweak negativecorrelation between HDLc concentration and age,and insignificant weak positive correlation between LDLc concentration and age (p=0.179,r=0.176); (p=0.067,r=-0.238); (p =0.233 ,r=0.156) respectively.

Statistical analysis also showed insignificant weak positive correlation between cholesterolwith duration of smoking per year,insignificantvery weak positive correlation between LDLc concentration with duration of smoking per year, and insignificant very weak negative correlation

between HDLc concentration with duration of smoking per year (p=0.091, r=0.220); (p=0.463, r=0.096); (p=0.349, r=-0.123) respectively.

Statistical analysis also showed insignificant very weak positivecorrelation between cholesterol concentration and LDLc concentration with number of cigarette per day,but showed significant weak negativecorrelation between HDLc concentration and number of cigarette per day (p=0.774, r=0.038); (p=0.774, r=0.038); (p=0.052, r=-0.253) respectively.

The study results revaled that smoking habit leads to significant increase in serum cholesterol level,significant decrease in serum HDL cholesterol level, and significant elevation in serum LDL cholesterol level compare to non smokers.. Results also revealed insignificant weak positivecorrelation between cholesterol and LDLc concentration and age, insignificant weak negativecorrelation between HDLc concentration and age,and insignificantweak positive correlation between cholesterolwith duration of smoking, insignificantvery weak positive correlation between LDLc concentration with duration of smoking, and insignificant very weak negative correlation between HDLc concentration with duration of smoking.Finally, results showed insignificant very weak positivecorrelation between cholesterol concentration and LDLc concentration with number of cigarette per day,but showed significant weak negative correlation between HDLc concentration and number of cigarette per day .

مستخلص الدراسة

تدخين السجاير ينتشر على نطاق واسع في جميع انحاء العالم وله اثار خطيرة على صحة الانسان.

اجريت هذه الدراسة المقطعية في منطقة الخرطوم مابين فبراير الى مارس 2015 لتقويم مستوى الكولسترول بين المدخنين.

تم اختبار 60 شخص من المدخنين (كمجموعة اختبار) و 40 شخص غير مدخن (كمجموعة ضابطة) (اعمارهم بين 16-65) تم جمع العينات من كلا المجموعتين وتم قياس الكولسترول باستخدام طريقة الانزيمات.

اجري التحليل الاحصائي باستخدام برنامج التحليل الاحصائي اظهرت النتائج ان هناك زيادة ذات دلالة احصائية في متوسط تركيز الكولسترول ونقصان ذا دلالة احصائية في متوسط تركيز الكولسترول الجيد وزيادة ذات دلالة احصائية في متوسط تركيز الكولسترول السيء لدى المدخنين مقارنة مع المجموعة الضابطة القيمة المعنوية (0.00)(0.00)(0.052) على التوالي.

ايضا اظهرت النتائج وجود علاقة طردية ضعيفةجدا ليست ذات دلالة احصائية بين تركيز الكولسترول الكلي و عمر المدخن وعلاقة عكسية ضعيفة ليست ذات دلالة احصائية بين الكولسترول الجيد وعمر المدخن و علاقة طردية ضعيفةجدا ليست ذات دلالة احصائية بين تركيز الكولسترول السيء و عمر المدخن (معامل بيرسون للارتباط= 0.176)(مستوى المعنوية=0.179)،(معامل بيرسون للارتباط=0.238)(مستوى المعنوية=0.076),(معامل بيرسون للارتباط=0.156)(مستوى المعنوية= 0.233)على التوالي.

ايضا اظهرت النتائج وجود علاقة طردية ضعيفة ليست ذات دلالة احصائية بين مدة التدخين بالسنوات و تركيز الكولسترول الكلي وجود علاقة طردية ضعيفة جدا ليست ذات دلالة احصائية بين مدة التدخين بالسنوات و تركيز الكولسترول السيء ووجود علاقة عكسية ضعيفة جدا ليست ذات دلالة احصائية بين تركيز الكولسترول الجيد ومدة التدخين بالسنوات (معامل بيرسون للارتباط= 0.220)(مستوى المعنوية=0.091)،),(معامل بيرسون للارتباط=0.096)(مستوى المعنوية= 0.463) ,(معامل بيرسون للارتباط=0.123-)(مستوى المعنوية=0.349على التوالي.

ايضا اظهر التحليل الاحصائي وجود علاقة طردية ضعيفة جدا ليست ذات دلالة احصائية بين عدد السيجارات في اليوم وتركيز الكولسترول الكلي والكولسترول السيء (معامل بيرسون للارتباط= 0.038)(مستوى المعنوية=0.774)،(معامل بيرسون للارتباط=0.038)(مستوى المعنوية0.774),بينما توجد علاقة عكسية ضعيفة ذات دلالة احصائية بين عدد السيجارات في اليوم وتركيز الكولسترول الجيد (معامل بيرسون للارتباط= 0.253-)(مستوى المعنوية=0.052).

اظهرت نتائج الدراسة ان عادة التدخين تسبب ارتفاع في تركيز الكولسترول الكلي و الكولسترول السيء وانخفاض في تركيز الكولسترول الجيد، وايضا اظهرت علاقة طردية ضعيفةجدا ليست ذات دلالة احصائية بين تركيز الكولسترول الكلي و عمر المدخن وعلاقة عكسية ضعيفة ليست ذات دلالة احصائية بين الكولسترول الجيد وعمر المدخن و علاقة طردية ضعيفةجدا ليست ذات دلالة احصائية بين تركيز الكولسترول السيء و عمر المدخن. كما اظهرت الدراسة وجود علاقة طردية ضعيفة ليست ذات دلالة احصائية بين مدة التدخين بالسنوات و تركيز الكولسترول الكلي وجود علاقة طردية ضعيفة جدا ليست ذات دلالة احصائية بين مدة التدخين بالسنوات و تركيز الكولسترول السيء ووجود علاقة عكسية ضعيفة جدا ليست ذات دلالة احصائية بين تركيز الكولسترول الجيد ومدة التدخين بالسنوات واخيرا اظهرت النتائج وجود علاقة طردية ضعيفة جدا ليست ذات دلالة احصائية بين عدد السجائر في اليوم وتركيز الكولسترول الكلي والكولسترول السيء بينما توجد علاقة عكسية ضعيفة ذات دلالة احصائية بين عدد السيجارات في اليوم وتركيز الكولسترول الجيد.

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Abbreviations

Abbreviation	Full term
ABCA1	ATP-binding cassette transporter A1
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
Fh	familial hypercholesterolemia
HDLc	high-density lipoprotein Cholesterol
HMG-CoA	3-hydroxy-3-methylglutaryl CoA
IDL	intermediate-density lipoprotein
LCAT	lecithin-cholesterol acyltransferase
LDLc	low-density lipoprotein Cholesterol
LPL	Lipoprotein lipase
PLTP	phospholipid transport protein
RCT	Reverse cholesterol transport
S1P and S2P	site-1 and -2 protease
SCAP	SREBP cleavage activating protein
SREBP	sterol regulatory element-binding protein 1 and 2
SR-BI	as scavenger receptor BI
TLF	trypanosome lytic factor
VLDL	very low-density lipoprotein

1.Introuductio

n and literature review

1.1 Introduction

Smoking is a practice in which a substance is burned and the resulting smoke breathed in to be tasted and absorbed into the bloodstream. Most commonly the substance is the dried leaves of the tobacco plant which have been rolled into a small square of rice paper to create a small, round cylinder called a "cigarette". Smoking is primarily practiced as a route of administration for recreational drug use because the combustion of the dried plant leaves vaporizes and delivers active substances into the lungs where they are rapidly absorbed into the bloodstream and reach bodily tissue. In the case of cigarette smoking these substances are contained in a mixture (West et al, 2007). of aerosol particles and gasses and include the pharmacologically active alkaloid nicotine; the vaporization creates heated aerosol and gas to form that allows inhalation and deep penetration into the lungs where absorption into the bloodstream of the active substances occurs. In some cultures, smoking is also carried out as a part of various rituals, where participants use it to help induce trance-like states that, they believe, can lead them to "spiritual enlightenment" (West et al, 2007).Cigarette contains carcinogens (polycyclic aromatic hydrocarbons etc.), irritant substances,nicotine, carbon monoxide, and other gases. Cigarette smoke contains many oxidants,and free radicals which can harm lipids, proteins,DNA, carbohydrates, and other biomolecules (Al-Azzawy,2011).

Tobacco smoke contains many chemicals that are harmful to both smokers and nonsmokers. Breathing even a little tobacco smoke can be harmful .Of the more than 7,000 chemicals in tobacco smoke, at least 250 are known to be harmful, including hydrogen cyanide, carbon monoxide, and ammonia Smoking has been found to harm nearly every bodily organ and organ system in the body and diminishes a person’s overall health.Smoking is a leading cause of cancer and death from cancer. It causes cancers of the lung, esophagus, larynx, mouth, throat, kidney, bladder, liver, pancreas, stomach, cervix, colon, and rectum, as well as acute myeloid leukemia .Smoking causes heart disease, stroke, aortic aneurysm (a balloon-like bulge in an artery in the chest), chronic obstructive pulmonary disease (COPD) (chronic bronchitis and emphysema), diabetes, osteoporosis, rheumatoid arthritis, age-related macular degeneration,cataracts, and worsens asthma symptoms in adults. Smokers are at higher risk of developing pneumonia, tuberculosis, and

other airway infections . In addition, smoking causes inflammation and impairs immune function . Since the 1960s, a smoker's risk of developing lung cancer or COPD has actually increased compared with nonsmokers, even though the number of cigarettes consumed per smoker has decreased . There have also been changes in the type of lung cancer smokers develop – a decline in squamous cell carcinomas but a dramatic increase in adenocarcinomas. Both of these effects may be due to changes in the formulation of cigarettes (U.S. Department of Health and Human Services,1989).

Tobacco smoking is one of the most potent and prevalent addictive, influencing behavior of human beings for over four centuries. Smoking is now increasing rapidly throughout the developing world and is one of the biggest threats to current and future health.Tobacco continues to be the second major cause of death in the world. By 2030, if current trends continue smoking will kill over 9million people annually .Smoking is an important preventable cause of mortalityworldwide. The prevalence of pulmonary and cardiovascular disease, cataracts and some cancers is higher in smokers. Tobacco smoke contains some deadly carcinogenic chemicals formed from natural components of the tobacco plants and leads to the uptake of many hazardous compounds such as heavy metals and N-nitrosocompound. Apart from natural constituents of tobacco, many substances are added to cigarette by manufacturers to enhance the flavor or to make smoking more pleasant(Brischetto et al, 1983).

. Some of the compounds found in tobacco smoke include ammonia, tar and carbon monoxide. Exactly what effects these substances have on the cigarettes smokers' health is unknown, butthere is no evidence that lowering the tar content of cigarette lower the health risk .Cigarette smoking leads to the uptake of manyhazardous compounds and their metabolites extracted from burning tobacco. These substances may be electrophilic and react with biological molecules, give rise to oxidative stress through the formation of reactivespecies or the initiation of lipid peroxidation chain reactions in the membrane. Cigarette smoking has been found to alter the lipoprotein levels (Brischetto et al, 1983).

1.2 Literature review

1.2.1 Smoking

Cigarettes are primarily industrially manufactured but also can be hand-rolled from loose tobacco and rolling paper. Other smoking implements

include pipes, cigars, bidis, hookahs, vaporizers, and bongs. Smoking-related diseases have been shown to kill approximately half of long term smokers when compared to average mortality rates faced by non-smokers. A 2007 report states that, each year, about 4.9 million people worldwide die as a result of smoking.In recent years, it has become apparent that cigarette smoking is associatedwith excessive morbidity and mortality in various diseases prominentlycardiovascular and lung diseases (U.S. Department of Health,Education, and Welfare,1973).

Kidney is an important target organ of smoking induced damage (Orth et al,1997).There are numerous harmful substances found in tobacco and tobacco smoke. Nicotine is one of these substances that may be acquired through active and passive smoking (Halimi et al,2000).Blood stream and carbon monoxide binding to hemoglobin in red blood cells .In addition, the carcinogen benzo[a]pyrene binds to cells in the airways and major organs of smokers, and depresses immune function. Smoking results in an elevated incidence of chronic inflammation as a consequence of oxidative stress. Cigarette smoking increases the risk of developing numerous cancers (U.S. Department of Health and Human Services,1989)

There are elevated serum cadmium and lead levels in smokers resulting in glomerular dysfunction. Nephropathies are accelerated by nicotine with an increased incidence of microalbuminuria progressing to proteinuria (Satarug et al, 2004). Although tobacco has dangerous effect on human health, it still highly consumed throughout the world (Benowitzet al, 1988). Smoking is one of the most common addictions of modern times. It has been implicated as an etiological agent for various chronic diseases, including a variety of infection, cancers, heart diseases and respiratory illnesses (Mehta et al, 2008; Zhonget al, 2008). Cigarette smoke (CS) contains over 4000 compounds, including at least 200 toxicant, 80 known or suspected carcinogens, large quantities of oxidants and free radicals that induce oxidative stress (de Heenset al, 2008; Abel et al, 2005; Carel and Eviatar, 1985). Moreover, cigarette smoking generates many toxic and carcinogenic compounds harmful to the health, such as nicotine, nitrogen oxides, carbon monoxide, hydrogen cyanide and free radicals (Hoffmann et al., 2001).

Tobacco smoking is one of the most potent and prevalent addictive, influencing behavior of human beings for over four centuries. Smoking is now increasing rapidly throughout the developing world and is one of the biggest threats to current and future health (Edwards , 2004). Smoking is

an important preventable cause of mortality worldwide. The prevalence of pulmonary and cardiovascular disease, cataracts and some cancers is higher in smokers. Tobacco smoke contains some deadly carcinogenic chemicals formed from natural components of the tobacco plants and leads to the uptake of many hazardous compounds such as heavy metals and N-nitrosocompound (Godwin et al, 2010). Cigarette smoking leads to the uptake of many hazardous compounds and their metabolites extracted from burning tobacco. These substances may be electrophilic and react with biological molecules, give rise to oxidative stress through the formation of reactive species or the initiation of lipid peroxidation chain reactions in the membrane (Loeper et al, 1991). Cigarette smoking has been found to alter the lipoprotein levels (Brischetto et al,1983).

Smoking makes it harder for a woman to get pregnant. A pregnant smoker is at higher risk of miscarriage, having an ectopic pregnancy, having her baby born too early and with an abnormally low birth weight, and having her baby born with a cleft lip and/or cleft palate. A woman who smokes during or after pregnancy increases her infant's risk of death from Sudden Infant Death Syndrome (SIDS). Men who smoke are at greater risk of erectile dysfunction . Cigarette smoking and exposure to tobacco smoke cause about 480,000 premature deaths each year in the United States(U.S. Department of Health and Human Services,1989). Of these premature deaths, about 36 percent are from cancer, 39 percent are from heart disease and stroke, and 24 percent are from lung disease. Smoking is the leading cause of premature, preventable death in this country. Regardless of their age, smokers can substantially reduce their risk of disease, including cancer, by quitting.The immediate health benefits of quitting smoking are substantial:Heart rate and blood pressure, which are abnormally high while smoking, begin to return to normal.Within a few hours, the level of carbon monoxide in the blood begins to decline. (Carbon monoxide reduces the blood's ability to carry oxygen.).Within a few weeks, people who quit smoking have improved circulation, produce less phlegm, and don't cough or wheeze as often.Within several months of quitting, people can expect substantial improvements in lung function .Within a few years of quitting, people will have lower risks of cancer, heart disease, and other chronic diseases than if they had continued to smoke.In addition, people who quit smoking will have an improved sense of smell, and food will taste better. Smoking increases the risk of coronary artery disease in people who have high cholesterol and other diseases that increase the risk of heart disease, such

as high blood pressure and diabetes. Cigarette smoking lowers HDL ("good") cholesterol. It also injures the lining of the blood vessels and increases the risk of developing blood clots, which contributes to atherosclerosis (hardening of the arteries). Even inhaling others' cigarette smoke (secondhand smoke) has been shown to lower HDL cholesterol.Studies have shown that HDL levels often go up soon after a person quits smoking (U.S. Department of Health and Human Services,1989).

Cigarette smoking is generally considered as associated with increased risk of a variety of medical disorders. Several studies provide the evidence that tobacco is strongly associated with altering the normal status of the lipid profile (Cuesta et al, 1989; Guedeset al, 2007; Arslanet al, 2008). However, inspite of all that information, there is still much controversy about which part or parts in the lipid profile are mainly altered in response to cigarette smoking, and whether those lipidprofile components influence other parts directly or indirectly and vice versa. Differing results were obtained by various investigators,for example,(Siekmeieret al. 1996) concludes that HDL-C levels are same for smokers and non-smokers. Whereas other investigators obtained conflicting results wherein significant variations (low levels of HDL-C in cigarette smokers) were obtained (Criquiet al., 1980; Siekmeieret al., 1994; Ito et al., 1995). Ingredients of cigarette smoke such as nicotine and carbon monoxide have been found to be involved in causing hypoxia (Yokodeet al., 1988), and increased susceptibility of LDL to be oxidized (Scheffleret al., 1992). However, the precise mechanism of the harmful involvement of cigarette smoking is not clear (Moriguchiet al., 1990).Significantly higher serum concentrations of total cholesterol were obtained in smokers (Cuesta et al, 1989; Guedeset al, 2000).

1.2.2. Cholesterol

Cholesterol, from the Ancient Greekchole- (bile) and stereos (solid) followed by the chemicalsuffix-ol for an alcohol, is an organicmolecule. It is a sterol (Cholesterol at the US National Library of Medicine Medical Subject Headings).

It is lipid molecule and is biosynthesized by all animal cells because it is an essential structural component of all animal (not plant or bacterial) cell membranes that is required to maintain both membrane structural integrity and fluidity. Cholesterol enables animal cells to (a) not need a cell wall (like plants & bacteria) to protect membrane integrity/cell-

viability and thus be able to (b) change shape and (c) move about (unlike bacteria and plant cells which are restricted by their cell walls).In addition to its importance within cells, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D. Cholesterol is the principal sterol synthesized by animals. All kinds of cells in animals can produce it. In vertebrates the hepatic cells typically produce greater amounts than other cells. It is almost completely absent among prokaryotes (bacteria and archaea), although there are some exceptions such as Mycoplasma, which require cholesterol for growth (Razin et al, 1970).

Most ingested cholesterol is esterified, and esterified cholesterol is poorly absorbed. The body also compensates for any absorption of additional cholesterol by reducing cholesterol synthesis. For these reasons, seven to ten hours after ingestion, cholesterol will show little, if any, effect on total body cholesterol content or concentrations of cholesterol in the blood. However, during the first seven hours after ingestion of cholesterol, the levels significantly increase (Cholesterol is recycled). The liver excretes it in a non-esterified form (via bile) into the digestive tract. Typically about 50% of the excreted cholesterol is reabsorbed by the small bowel back into the bloodstream.Plants make cholesterol in very small amounts. Plants manufacture phytosterols (substances chemically similar to cholesterol produced within plants), which can compete with cholesterol for reabsorption in the intestinal tract, thus potentially reducing cholesterol reabsorption (John et al, 2007).

When intestinal lining cells absorb phytosterols, in place of cholesterol, they usually excrete the phytosterol molecules back into the GI tract, an important protective mechanism.Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. The hydroxyl group on cholesterol interacts with the polar head groups of the membranephospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolarfatty-acid chain of the other lipids. Through the interaction with the phospholipid fatty-acid chains, cholesterol increases membrane packing, which reduces membrane fluidity (Sadava et al, 2011). The structure of the tetracyclic ring of cholesterol contributes to the decreased fluidity of the cell membrane as the molecule is in a trans conformation making all but the side chain of cholesterol rigid and planar.In this structural role,

cholesterol reduces the permeability of the plasma membrane to neutral solutes, hydrogen ions, and sodium ions (Haines, 2001).

Within the cell membrane, cholesterol also functions in intracellular transport, cell signaling and nerve conduction. The role of cholesterol in such endocytosis can be investigated by using methyl beta cyclodextrin (MβCD) to remove cholesterol from the plasma membrane. Recent studies show that cholesterol is also implicated in cell signaling processes, assisting in the formation of lipid rafts in the plasma membrane. Lipid raft formation brings receptor proteins in close proximity with high concentrations of second messenger molecules. In many neurons, a myelin sheath, rich in cholesterol, since it is derived from compacted layers of Schwann cell membrane, provides insulation for more efficient conduction of impulses (Pawlina et al, 2006). Within cells, cholesterol is the precursor molecule in several biochemical pathways. In the liver, cholesterol is converted to bile, which is then stored in the gallbladder. Bile contains bile salts, which solubilize fats in the digestive tract and aid in the intestinal absorption of fat molecules as well as the fat-soluble vitamins, A, D, E, and K. Cholesterol is an important precursor molecule for the synthesis of vitamin D and the steroid hormones, including the adrenal gland hormones cortisol and aldosterone, as well as the sex hormones progesterone, estrogens, and testosterone, and their derivatives. Some research indicates cholesterol may act as an antioxidant(Smith, 1991).

All animal cells manufacture cholesterol for their use, with relative production rates varying by cell type and organ function. About 20–25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs. Synthesis within the body starts with one molecule of acetyl CoA and one molecule of acetoacetyl-CoA, which are hydrated to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). This molecule is then reduced to mevalonate by the enzyme HMG-CoA reductase. This is the regulated, rate-limiting and irreversible step in cholesterol synthesis and is the site of action for the statin drugs (HMG-CoA reductase competitive inhibitors).Mevalonate is then converted to 3-isopentenyl pyrophosphate in three reactions that require ATP. Mevalonate is decarboxylated to isopentenyl pyrophosphate, which is a key metabolite for various biological reactions. Three molecules of isopentenyl pyrophosphate condense to form farnesyl pyrophosphate through the action of geranyltransferase. Two molecules of farnesyl pyrophosphate

then condense to form squalene by the action of squalene synthase in the endoplasmic reticulum. Oxidosqualenecyclase then cyclizes squalene to form lanosterol. Finally, lanosterol is converted to cholesterol through a 19-step process (Berg, 2002).

Biosynthesis of cholesterol is directly regulated by the cholesterol levels present, though the homeostatic mechanisms involved are only partly understood. A higher intake from food leads to a net decrease in endogenous production, whereas lower intake from food has the opposite effect. The main regulatory mechanism is the sensing of intracellular cholesterol in the endoplasmic reticulum by the proteinSREBP (sterol regulatory element-binding protein 1 and 2). In the presence of cholesterol, SREBP is bound to two other proteins: SCAP (SREBP cleavage activating protein) and Insig1. When cholesterol levels fall, Insig-1 dissociates from the SREBP-SCAP complex, which allows the complex to migrate to the Golgi apparatus. Here SREBP is cleaved by S1P and S2P (site-1 and -2 protease), two enzymes that are activated by SCAP when cholesterol levels are low.The cleaved SREBP then migrates to the nucleus, and acts as a transcription factor to bind to the sterol regulatory element (SRE), which stimulates the transcription of many genes. Among these are the low-density lipoprotein (LDL) receptor and HMG-CoA reductase. The LDL receptor scavenges circulating LDL from the bloodstream, whereas HMG-CoA reductase leads to an increase of endogenous production of cholesterol.[Cholesterol synthesis can also be turned off when cholesterol levels are high. HMG-CoA reductase contains both a cytosolic domain (responsible for its catalytic function) and a membrane domain. The membrane domain senses signals for its degradation. Increasing concentrations of cholesterol (and other sterols) cause a change in this domain's oligomerization state, which makes it more susceptible to destruction by the proteosome. This enzyme's activity can also be reduced by phosphorylation by an AMP-activated protein kinase. Because this kinase is activated by AMP, which is produced when ATP is hydrolyzed, it follows that cholesterol synthesis is halted when ATP levels are low (Tymoczko et al, 2002).

1.2.2.1. Plasma transport and regulation of absorption of cholesterol

Cholesterol is only slightly soluble in water; it dissolves into the (water-based) bloodstream only at exceedingly small concentrations. Instead, cholesterol is transported inside lipoproteins, complex discoidal particles

with exterior amphiphilic proteins and lipids, whose outward-facing surfaces are water-soluble and inward-facing surfaces are lipid-soluble. Triglycerides and cholesterol esters are carried internally. Phospholipids and cholesterol, being amphipathic, are transported in the monolayer surface of the lipoprotein particle.There are several types of lipoproteins in the blood. In order of increasing density, they are chylomicrons, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), and high-density lipoprotein (HDL). Lower protein/lipid ratios make for less dense lipoproteins. Cholesterol within different lipoproteins is identical, although some is carried as "free" alcohol, while others as fatty acyl esters, known also as cholesterol esters.Lipoproteins contain apolipoproteins, which bind to specific receptors on cell membranes, directing their lipid payload to specific tissues. Lipoprotein particles thus include these molecular addresses, which determine the start- and end points of cholesterol transport.Chylomicrons, the least dense cholesterol transport molecules, contain apolipoprotein B-48, apolipoprotein C, and apolipoprotein E in their shells. Chylomicrons carry fats from the intestine to muscle and other tissues in need of fatty acids for energy or fat production. Unused cholesterol remains in more cholesterol-rich chylomicron remnants, and taken up from here to the bloodstream by the liver.VLDL molecules are produced by the liver from triacylglycerol and cholesterol which was not used in the synthesis of bile acids. These molecules contain apolipoprotein B100 and apolipoprotein E in their shells, and are degraded by lipoprotein lipase on the blood vessel wall to IDL.Blood vessels cleave and absorb triacylglycerol from IDL molecules, increasing the concentration of cholesterol. IDL molecules are then consumed in two processes: half is metabolized by HTGL and taken up by the LDL receptor on the liver cell surfaces, while the other half continues to lose triacylglycerols in the bloodstream until they become LDL molecules, with the highest concentration of cholesterol within them.LDL particles are the major blood cholesterol carriers. Each one contains approximately 1,500 molecules of cholesterol ester. LDL molecule shells contain just one molecule of apolipoprotein B100, recognized by LDL receptors in peripheral tissues. Upon binding of apolipoprotein B100, many LDL receptors concentrate in clathrin-coated pits. Both LDL and its receptor form vesicles within a cell via endocytosis. These vesicles then fuse with a lysosome, where the lysosomal acid lipase enzyme hydrolyzes the cholesterol esters. The cholesterol can then be used for membrane

biosynthesis or esterified and stored within the cell, so as to not interfere with the cell membranes (Weingartner et al, 2010).

LDL receptors are used up during cholesterol absorption, and its synthesis is regulated by SREBP, the same protein that controls the synthesis of cholesterol de novo, according to its presence inside the cell. A cell with abundant cholesterol will have its LDL receptor synthesis blocked, to prevent new cholesterol in LDL molecules from being taken up. Conversely, LDL receptor synthesis proceeds when a cell is deficient in cholesterol.When this process becomes unregulated, LDL molecules without receptors begin to appear in the blood. These LDL molecules are oxidized and taken up by macrophages, which become engorged and form foam cells. These foam cells often become trapped in the walls of blood vessels and contribute to atherosclerotic plaque formation. These plaques are the main causes of heart attacks, strokes, and other serious medical problems, leading to the association of so-called LDL cholesterol (actually a lipoprotein) with "bad" cholesterol.HDL particles are thought to transport cholesterol back to the liver, either for excretion or for other tissues that synthesize hormones, in a process known as reverse cholesterol transport (RCT). Large numbers of HDL particles correlates with better health outcomes., whereas low numbers of HDL particles is associated with atheromatous disease progression in the arteries (Lewis et al, 2005).

1.2.2.2. Metabolism, recycling and excretion of cholesterol

Cholesterol is susceptible to oxidation and easily forms oxygenated derivatives known as oxysterols. Three different mechanisms can form these; autoxidation, secondary oxidation to lipid peroxidation, and cholesterol-metabolizing enzyme oxidation. A great interest in oxysterols arose when they were shown to exert inhibitory actions on cholesterol biosynthesis. This finding became known as the “oxysterol hypothesis”. Additional roles for oxysterols in human physiology include their: participation in bile acid biosynthesis, function as transport forms of cholesterol, and regulation of gene transcription (Russell, 2000).

In biochemical experiments radiolabelled forms of cholesterol, such as tritiated-cholesterol are used. These derivatives undergo degradation upon storage and it is essential to purify cholesterol prior to use.Cholesterol is oxidized by the liver into a variety of bile acids. These, in turn, are conjugated with glycine, taurine, glucuronic acid, or sulfate. A mixture of conjugated and nonconjugated bile acids, along with cholesterol itself, is

excreted from the liver into the bile. Approximately 95% of the bile acids are reabsorbed from the intestines, and the remainder are lost in the feces. The excretion and reabsorption of bile acids forms the basis of the enterohepatic circulation, which is essential for the digestion and absorption of dietary fats. Under certain circumstances, when more concentrated, as in the gallbladder, cholesterol crystallises and is the major constituent of most gallstones. Although, lecithin and bilirubin gallstones also occur, but less frequently. Every day, up to 1 g of cholesterol enters the colon. This cholesterol originates from the diet, bile, and desquamated intestinal cells, and can be metabolized by the colonic bacteria. Cholesterol is converted mainly into coprostanol, a nonabsorbable sterol that is excreted in the feces. A cholesterol-reducing bacterium origin has been isolated from human feces (Wipperman et al, 2013)

1.2.2.3. Clinical significance of cholesterol

Hypercholesterolemia:is the lipid abnormality most closely linked to heart that predispose affected individuals to elevated cholesterol levels, is called familial hypercholesterolemia (FH).Homozygotes for FH are fortunately rare (1:1 million in the population) and can have total cholesterol concentrations as high as 800 to 1,000 mg/dL (20-26 mmol/L).These patients frequently have their first heart attack when still in their teenage years. Heterozygotes for the disease are seen much more frequently (1:500 in the population) because it is an autosomal codominant disorder; a defect in just one of the two copies of the LDL receptor can adversely affect lipid levels. Heterozygotes tend to have total cholesterol concentrations in the range of 300-600 mg/dL (8-15 mmol/L) and, if not treated, become symptomatic for heart disease in their 20s to50s.Approximately 5% of patients younger than age 50 with CAD are FH heterozygotes. Other symptoms associatedwith FH include tendinous and tuberous xanthomas,which are cholesterol deposits under the skin, and arcus,which are cholesterol deposits in the cornea. In both homozygotes and heterozygotes, the cholesterol elevation is primarily associated with an increase in LDL cholesterol. These individuals synthesize intracellular cholesterol normally but lack, or are deficient in, active LDL receptors. Consequently, LDL builds up in the circulation because there are insufficient receptors to bind the LDL and transfer the cholesterol into the cells.Cells, however, which require cholesterol for use in cell membrane and hormone production, synthesize cholesterol intracellularly at an increased rate to compensate for the lack of

cholesterol from the receptor mediated mechanism.In FH heterozygotes and other forms of hypercholesterolemia, reduction in the rate of internal cholesterol synthesis, by inhibition of HMG-CoA reductase with statin drugs, stimulates the production of additional LDL receptors, particularly in the liver, which removes LDL from the circulation. Homozygotes, however, do not usually benefit from this type of therapy, because they typically do not have enough functional receptors to stimulate. Homozygotes can be treated by a technique called LDL pheresis, a method similar to the dialysis treatment, in which blood is periodically drawn from the patient, processed to remove LDL, and returned to the patient.Most individuals with elevated LDL cholesterol levels do not have FH but are still at increased risk for premature CHD 59,66,76 and should be maintained on a low-fat,low cholesterol diet and receive statin treatment when necessary. Regular physical activity should also be incorporated, with drug therapy (Michael et al, 2010).

Hypocholesterolemia: Abnormally low levels of cholesterol are termed hypocholesterolemia. Research into the causes of this state is relatively limited, but some studies suggest a link with depression, cancer, and cerebral hemorrhage. In general, the low cholesterol levels seem to be a consequence, rather than a cause, of an underlying illness. A genetic defect in cholesterol synthesis causes Smith-Lemli-Opitz syndrome, which is often associated with low plasma cholesterol levels (Durrington, 2003).

1.2.3. High-density lipoprotein (HDL)

Is one of the five major groups of lipoproteins. Lipoproteins are complex particles composed of multiple proteins which transport all fat molecules (lipids) around the body within the water outside cells. They are typically composed of 80-100 proteins/particle (organized by one, two or three ApoA; more as the particles enlarge picking up and carrying more fat molecules) and transporting none to hundreds fat molecules/particle. Unlike the larger lipoprotein particles which deliver fat molecules to cells, HDL particles remove fat molecules from cells which want to export fat molecules. The fats carried include cholesterol, phospholipids, and triglycerides; amounts of each quite variable.Lipoproteins, in order of molecular size, largest to smallest, are chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and HDL. Lipoprotein molecules (all particles far smaller than human cells), enable the transportation of all lipids, such as

cholesterol, phospholipids, and triglycerides, within the water around cells (extracellular fluid), including the bloodstream (Lewington et al,2007).

HDL particles, unlike the larger particles, transfer fats away from cells, artery walls and tissues (around the body, body wide) through the bloodstream, back to both (a) LDL particles and (b) back to the liver for other disposition.Increasing concentrations of HDL particles are strongly associated with decreasing accumulation of atherosclerosis within the walls of arteries over weeks, years, decades. This is important because atherosclerosis, eventually, results in sudden plaque ruptures triggering clots within the artery opening, narrowing/closing the opening(s), i.e. cardiovascular disease, stroke(s) and other vascular disease complications body wide.HDL particles are sometimes referred to as good cholesterol because they can transport fat molecules (including cholesterol, triglycerides, etc.) out of artery walls, reduce macrophage accumulation, and thus help prevent, even regress atherosclerosis over weeks, years, decades, thereby helping prevent cardiovascular disease, stroke(s) and other vascular disease complications body wide. In contrast, LDL particles are often called bad cholesterol or unhealthy cholesterol, because they deliver fat molecules to macrophages in the wall of arteries (Toth, 2005).

1.2.3.1 Structure and function of HDL c

HDL is the smallest of the lipoprotein particles. It is the densest because it contains the highest proportion of protein to lipids. Its most abundant apolipoproteins are apo A-I and apo A-II. (A rare genetic variant, ApoA-1 Milano, has been documented to be far more effecitive in both protecting against and regressing arterial disease; atherosclerosis). The liver synthesizes these lipoproteins as complexes of apolipoproteins and phospholipid, which resemble cholesterol-free flattened spherical lipoprotein particles; the complexes are capable of picking up cholesterol, carried internally, from cells by interaction with the ATP-binding cassette transporter A1 (ABCA1)(Despres et al, 2009).

A plasma enzyme called lecithin-cholesterol acyltransferase (LCAT) converts the free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of the lipoprotein particle, eventually causing the newly synthesized HDL to

assume a spherical shape. HDL particles increase in size as they circulate through the bloodstream and incorporate more cholesterol and phospholipid molecules from cells and other lipoproteins, for example by the interaction with the ABCG1 transporter and the phospholipid transport protein (PLTP).HDL transports cholesterol mostly to the liver or steroidogenic organs such as adrenals, ovary, and testes by both direct and indirect pathways. HDL is removed by HDL receptors such as scavenger receptor BI (SR-BI), which mediate the selective uptake of cholesterol from HDL. In humans, probably the most relevant pathway is the indirect one, which is mediated by cholesteryl ester transfer protein (CETP). This protein exchanges triglycerides of VLDL against cholesteryl esters of HDL. As the result, VLDLs are processed to LDL, which are removed from the circulation by the LDL receptor pathway. The triglycerides are not stable in HDL, but are degraded by hepatic lipase so that, finally, small HDL particles are left, which restart the uptake of cholesterol from cells.The cholesterol delivered to the liver is excreted into the bile and, hence, intestine either directly or indirectly after conversion into bile acids. Delivery of HDL cholesterol to adrenals, ovaries, and testes is important for the synthesis of steroid hormones.Several steps in the metabolism of HDL can participate in the transport of cholesterol from lipid-laden macrophages of atherosclerotic arteries, termed foam cells, to the liver for secretion into the bile. This pathway has been termed reverse cholesterol transport and is considered as the classical protective function of HDL toward atherosclerosis.However, HDL carries many lipid and protein species, several of which have very low concentrations but are biologically very active. For example, HDL and its protein and lipid constituents help to inhibit oxidation, inflammation, activation of the endothelium, coagulation, and platelet aggregation. All these properties may contribute to the ability of HDL to protect from atherosclerosis, and it is not yet known which are the most important. In addition, a small subfraction of HDL lends protection against the protozoan parasite Trypanosomabruceibrucei. This HDL subfraction, termed trypanosome lytic factor (TLF), contains specialized proteins that, while very active, are unique to the TLF molecule (Huang et al, 2013).

In the stress response, serum amyloid A, which is one of the acute-phase proteins and an apolipoprotein, is under the stimulation of cytokines (IL-1, IL-6), and cortisol produced in the adrenal cortex and carried to the damaged tissue incorporated into HDL particles. At the inflammation

site, it attracts and activates leukocytes. In chronic inflammations, its deposition in the tissues manifests itself as amyloidosis.It has been postulated that the concentration of large HDL particles more accurately reflects protective action, as opposed to the concentration of total HDL particles. This ratio of large HDL to total HDL particles varies widely and is measured only by more sophisticated lipoprotein assays using either electrophoresis (the original method developed in the 1970s) or newer NMR spectroscopy methods, developed in the 1990s.Five subfractions of HDL have been identified. From largest (and most effective in cholesterol Recommended ranges.High LDL with low HDL level is an additional risk factor for cardiovascular disease (Hirano etal, 2008).

1.2.4. Low-density lipoprotein (LDL)

Is one of the five major groups of lipoproteins. These groups, from least dense to most dense, are: chylomicrons (ULDL), very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and High Density Lipoprotein (HDL), all of them, particles far smaller than human cells.Lipoproteins transfer fats through the bloodstream, then the intracellular (water) to all cells around the body (Dashti M et al 2011). Lipoproteins are complex particles composed of multiple proteins which transport all fat molecules (lipids) around the body within the water outside cells. They are typically composed of 80-100 proteins/particle (organized by a single ApoB for LDL and the larger particles) and transporting about 3,000 to 6,000 fat molecules/particle. The fats carried include cholesterol, phospholipids, and triglycerides; amounts of each vary considerably.LDL particles pose a risk for cardiovascular disease when they invade the endothelium and become oxidized, since the oxidized forms are more easily retained by the proteoglycans. A complex set of biochemical reactions regulates the oxidation of LDL particles, chiefly stimulated by presence of necrotic cell debris and free radicals in the endothelium. Increasing concentrations of LDL particles are strongly associated with increasing amounts of atherosclerosis within the walls of arteries over time, eventually resulting in sudden plaque ruptures and triggering clots within the artery opening, or a narrowing or closing of the opening, i.e.cardiovascular disease, stroke, and other vascular disease complications.LDL particles are sometimes referred to as bad cholesterol because they can transport their content of fat molecules into artery walls, attract macrophages, and thus drive atherosclerosis. In contrast, HDL particles are often called good cholesterol or healthy cholesterol because

they can remove fat molecules from macrophages in the wall of arteries. A hereditary form of high LDL is familial hypercholesterolemia (FH). High LDL is termed hyperlipoproteinemia type II (after the dated Fredrickson classification) (Dashtyet al, 2014)

1.2.4.1. Structure of LDL c

Each native LDL particle enables emulsification, i.e. surrounding/packaging all fatty acids being carried, enabling these fats to move around the body within the water outside cells. Each particle contains a single apolipoprotein B-100 molecule (Apo B-100, a protein that has 4536 amino acid residues and a mass of 514 kDa), along with 80 to 100 additional ancillary proteins. Each LDL has a highly hydrophobic core consisting of polyunsaturated fatty acid known as linoleate and hundreds to thousands (about 1500 commonly cited as an average) esterified and non-esterified cholesterol molecules. This core also carries varying numbers of triglycerides and other fats and is surrounded by a shell of phospholipids and unesterified cholesterol, as well as the single copy of Apo B-100. LDL particles are approximately 22 nm (0.00000087 in.) in diameter and have a mass of about 3 million daltons. Since LDL particles contain a variable and changing number of fatty acid molecules, there is a distribution of LDL particle mass and size(Determining the structure of LDL has been a tough task because of its heterogeneous structure. The structure of LDL at human body temperature in native condition, with a resolution of about 16 Angstroms using cryo-electron microscopy, has been recently described (Segrestetal, 2001).LDL particles are formed as VLDL lipoproteins lose triglyceride through the action of lipoprotein lipase (LPL) and they become smaller and denser (i.e. fewer fat molecules with same protein transport shell), containing a higher proportion of cholesterol esters (Friedewald et al, 1972).

1.2.4.2. Transport of LDLc into the cell

When a cell requires additional cholesterol (beyond its current internal HMGCoA production pathway), it synthesizes the necessary LDL receptors, and inserts them into the plasma membrane. The LDL receptors diffuse freely until they associate with clathrin-coated pits. LDL particles in the bloodstream bind to these extracellular LDL receptors. The clathrin-coated pits then form vesicles that are endocytosed into the cell.After the clathrin coat is shed, the vesicles deliver the LDL and their receptors to early endosomes, onto late endosomes to lysosomes. Here the

cholesterol esters in the LDL are hydrolysed. The LDL receptors are recycled back to the plasma membrane (Kumar et al, 2011).

1.2.4.3. LDL subtype patterns

LDL particles vary in size and density, and studies have shown that a pattern that has more small dense LDL particles, called Pattern B, equates to a higher risk factor for coronary heart disease (CHD) than does a pattern with more of the larger and less-dense LDL particles (Pattern A). This is thought to be because the smaller particles are more easily able to penetrate the endothelium. Pattern I, for intermediate, indicates that most LDL particles are very close in size to the normal gaps in the endothelium (26 nm). According to one study, sizes 19.0–20.5 nm were designated as pattern B and LDL sizes 20.6–22 nm were designated as pattern ASome in the medical community have suggested the correspondence between Pattern B and CHD is stronger than the correspondence between the LDL number measured in the standard lipid profile test. Tests to measure these LDL subtype patterns have been more expensive and not widely available, so the common lipid profile test is used more often (Warnick et al 1990). There has also been noted a correspondence between higher triglyceride levels and higher levels of smaller, denser LDL particles and alternately lower triglyceride levels and higher levels of the larger, less dense LDL. With continued research, decreasing cost, greater availability and wider acceptance of other lipoprotein subclass analysis assay methods, including NMR spectroscopy, research studies have continued to show a stronger correlation between human clinically obvious cardiovascular events and quantitatively measured particle concentrations (Otvos, 1999).

1.3 **Rationale**

Cigarette smokers have a high risk of coronary heart disease and smokers are at much greater risk of developing atherosclerotic plaques and different heart diseases than non-smokers (Godwin et al,2010) .

Several studies provide the evidence that tobacco is strongly associated with altering the normal status of the lipid profile (Cuesta et al., 1989; Guedeset al., 2007; Arslanet al., 2008) . There are limited local data about the effect of cigarette smoking on serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol levels,and few published data about this in Sudanese male smokers, that why we attempt to do this study.

1.4. Objectives

General objective:

To study the influence of cigarette smoking on serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol level among Sudanese male smokers.

Specific objectives:

1. To measure serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol concentrations in cigarette smokers in comparison to non smokers healthy individuals.

2. To correlate between the levels of serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol with age of smokers, number of cigarettes per day and duration of the smoking per year.

2. Materials and Methods

2.1. **Materials**:

2.1.1. Study design: This is a descriptive analytical comparative case control study.

2.1.2. Study area: The study was conducted in male cigarette smokers in Khartoum state.

2.1.3. Study population: This study included 60 smokers as test group and 40 apparently healthy non smokers as control group during February to March 2015.

2.1.4. Inclusion Criteria: Male cigarette smokers were included.

2.1.5. Exclusion criteria: Individual with heart disease , hypertension, DM, familial hyperlipidemia and thyroid gland disease were excluded.

2.1.6. Samples: About 5ml of venous blood were collected from each patient at the plain containers, and after clot formation samples were centrifuged for 3 minutes at 3000 RPM to obtain serum and analyzed.

2.1.7. Ethical consideration: Individuals who voluntarily participate in this study were included.

2.1.8. Data analysis: Data was analyzed by using the SPSS computer program

2.2. Methods

2.2.1. Estimation of cholesterol concentration using the enzymatic, liquid (CHOD/PAP) method:

Principle of method:

Free and esterified cholesterol in the sample originates by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry. (appendix II)

Cholesterol ester + H_2O chol.esterase. Cholesterol+fatty acid

Cholesterol +1/2 O_2 +H_2O chol. oxidase Chlestenone + H_2O_2

2H_2O_2 + 4- Aminoantipryine + phenol peroxidase Quinoneimine + 4 H_2O

2.2.2. Estimation of HDL after precipitation using PTA/mgcl method:

Principle of method:

Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the sample precipitate with phostungestate and magnesium ions. The supernatant contains HDL. The HDL cholesterol is then spectrophotometrically measured by means of the coupled reaction described below: (appendix III)

Cholesterol ester + H_2O chol.esterase. Cholesterol+fatty acid

Cholesterol +1/2 O_2 +H_2O chol. oxidase Chlestenone + H_2O_2

2H_2O_2 + 4- Aminoantipryine + phenol peroxidase Quinoneimine + 4 H_2O

2.2.3. Estimation of LDL after precipitation using polyvinyl sulphate method:

Principle of method:

Low density lipoprotein (LDL) in the sample precipitate with polyvinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in supernatant after centerfugation. The cholesterol is then spectrophotometrically measured by means of the coupled reaction described below: (appendix IV)

Cholesterol ester + H_2O chol.esterase. Cholesterol+fatty acid

Cholesterol +1/2 O_2 +H_2O chol. oxidase Chlestenone + H_2O_2

$2H_2O_2$ + 4- Aminoantipryine + phenol peroxidase Quinoneimine + 4 H_2O

2.2.4. Quality control:

Control sera were used during analysis and the values obtained fall within the defined limits.

3.Results

Agroup of 60 smokers involved in this study for measurement of cholesterol ,HDLc , and LDLc levels, other 40 apparently healthy subject (control) were inrolled in this study.

Enzymatic methods were used for estimation of serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol levels. Statistical analysis was done by using SPSS computer program and the results were as follow:

Table 3.1:shows insignificant increase in cholesterol concentration compared to control group(p=0.585), significant decrease in HDLc level compared to control group (p=0.000), and significant increase in LDLc level compared to control group(0.057).

Figure 3.1: a scatte plot shows insignificant posotive correlation between cholesterol concentration and age.

Figure 3.2 : a scatte plot shows insignificant negative correlation between HDLc concentration and age.

Figure 3.3: a scatte plot shows insignificant posotive correlation between LDLc concentration and age.

Figure 3.4: a scatte plot shows insignificant posotive correlation between cholesterol concentration and duration.

Figure 3.5: a scatte plot shows insignificant posotive correlation between HDLc concentration and duration .

Figure 3.6: a scatte plot shows insignificant posotive correlation between LDLc concentration and duration .

Figure 3.7:a scatte plot shows insignificant posotive correlation between cholesterol concentration and number of cigarette per day .

Figure 3.8: a scatte plot shows significant negative correlation between HDLc concentration and number of cigarette per day.

Figure 3.9 ; a scatte plot shows insignificant posotive correlation between LDLc concentration and number of cigarette per day.

Table 3.1: Comparison between the mean of cholesterol, HDLc, and LDLc level in the smokers (test groug) and control group.

Variables	**Test group N=60 Mean ± 2SD**	**Control group N=40 Mean ± 2SD**	**P.value**
Cholesterol mg/dl	245.9 ± 122.6	193.2±23.8	0.000
HDLc mg/dl	25.3 ± 35.0	91.4 ± 84.2	0.000
LDLc mg/dl	162.5 ± 162.4	138.9 ± 128.4	0.052

Independent sample T test was used for comparison, P value considered significant at levl ≤ 0.05

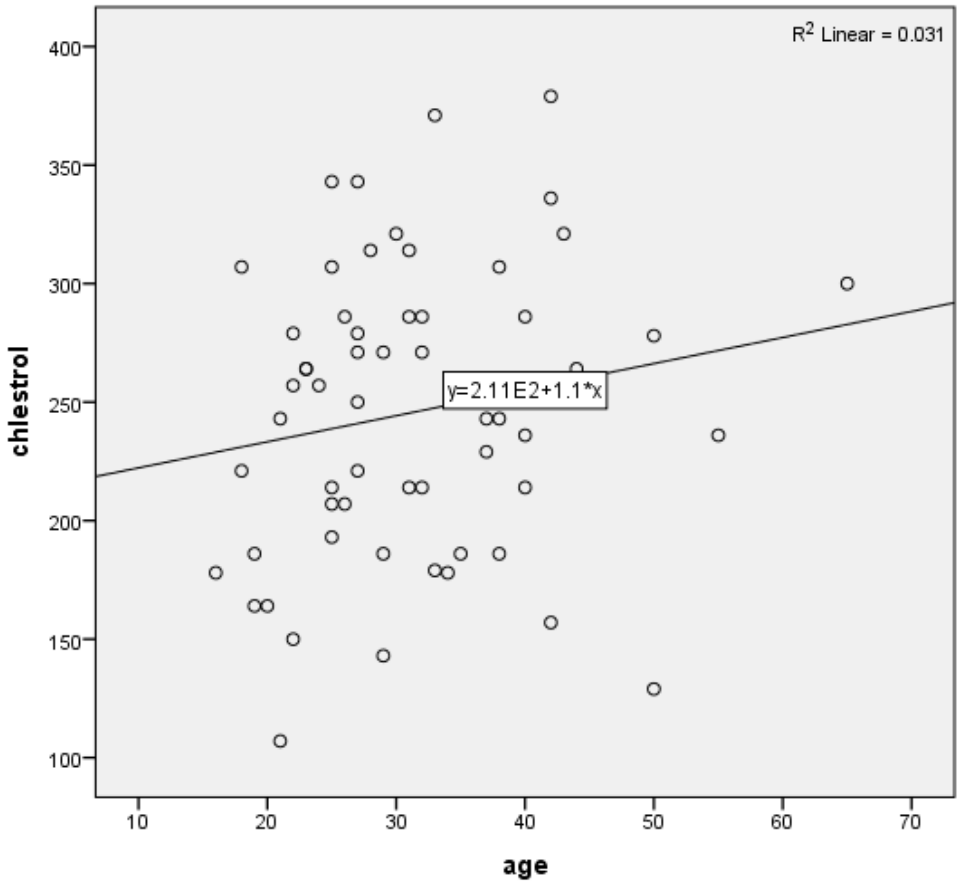

Figure 3.1: Correlations between cholesterol concentration and age of smokers

(p=0.179,r=0.176)

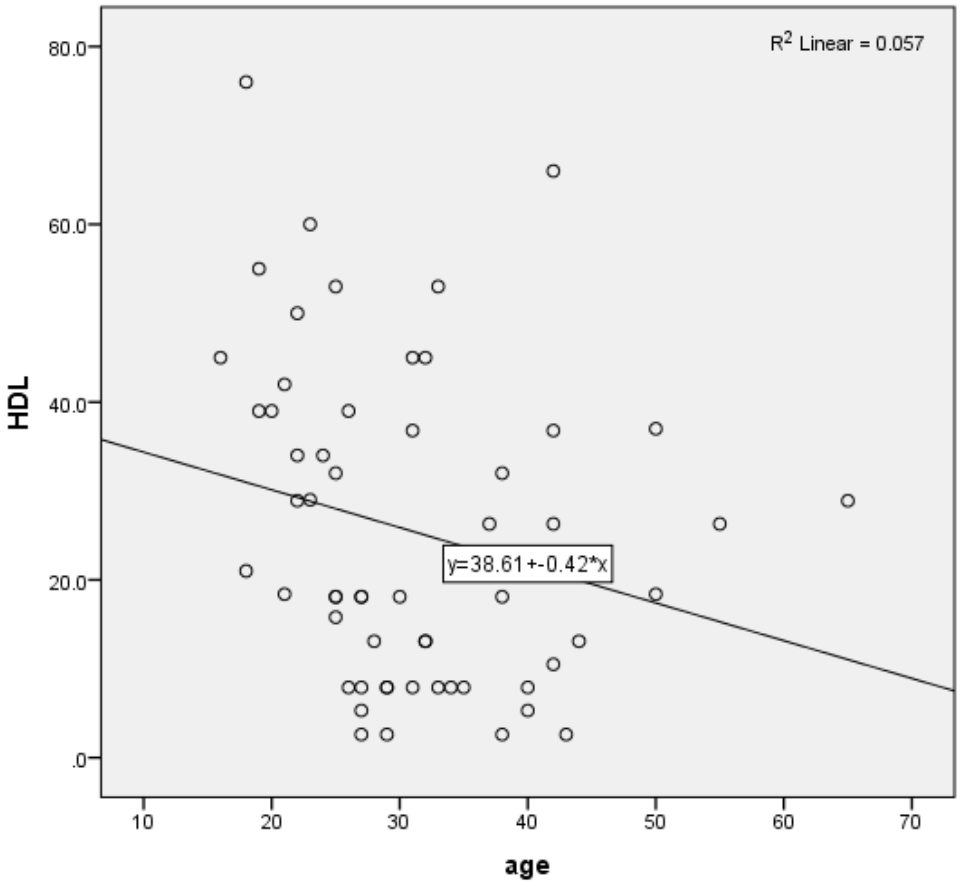

Figure 3.2: Correlation between HDLc and age of smokers

(p=0.067,r= -0.238)

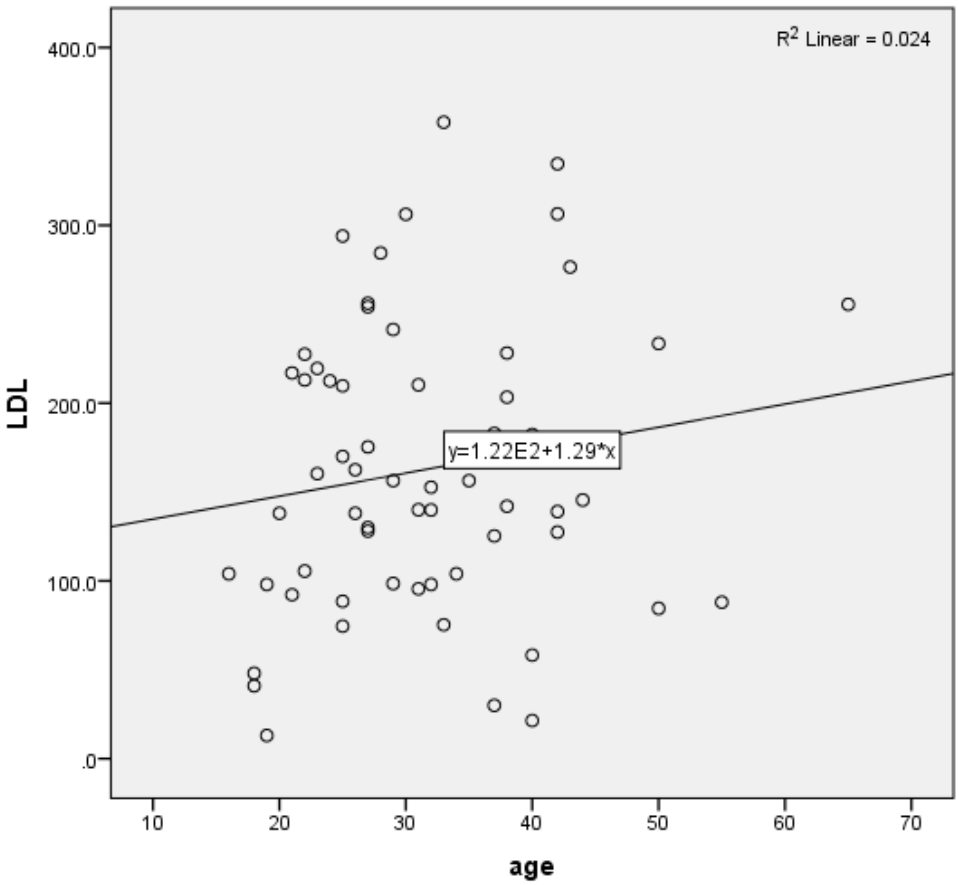

Figure 3.3: Correlation between LDLc and age of smokers .(*p*=0.233,r=0.156)

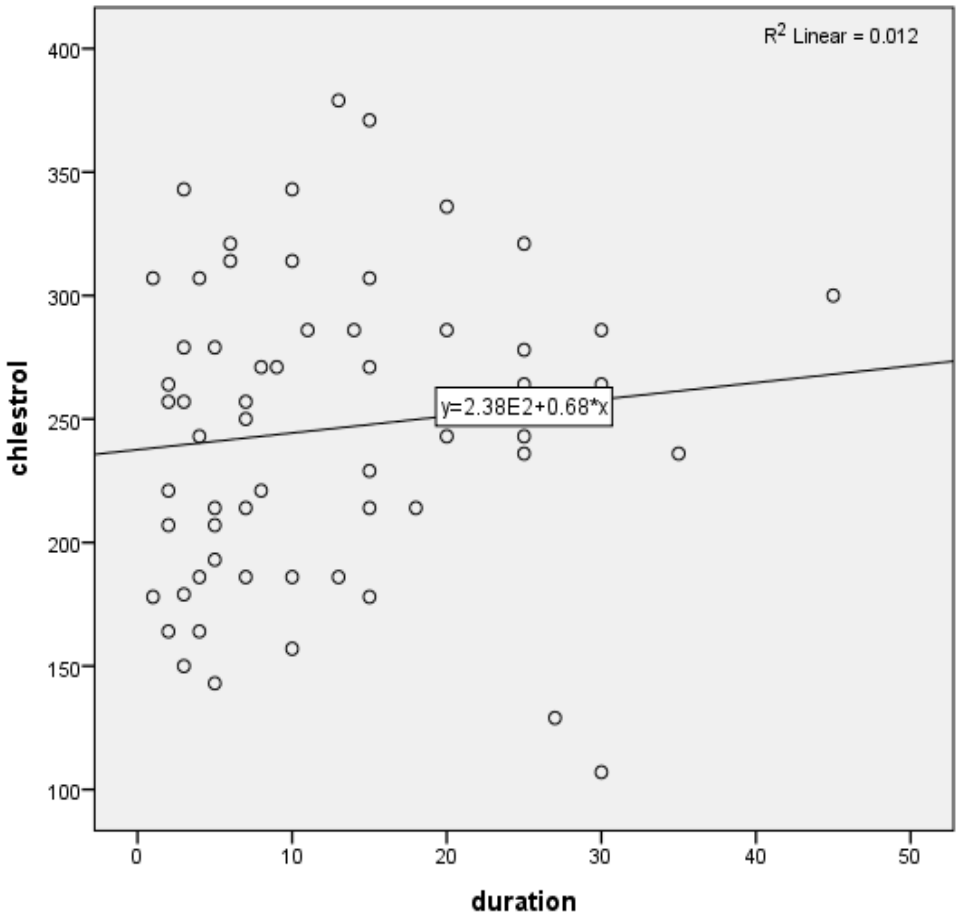

Figure 3.4: Correlation between cholesterol concentration and duration of smoking

(p=0.091,r=0.220)

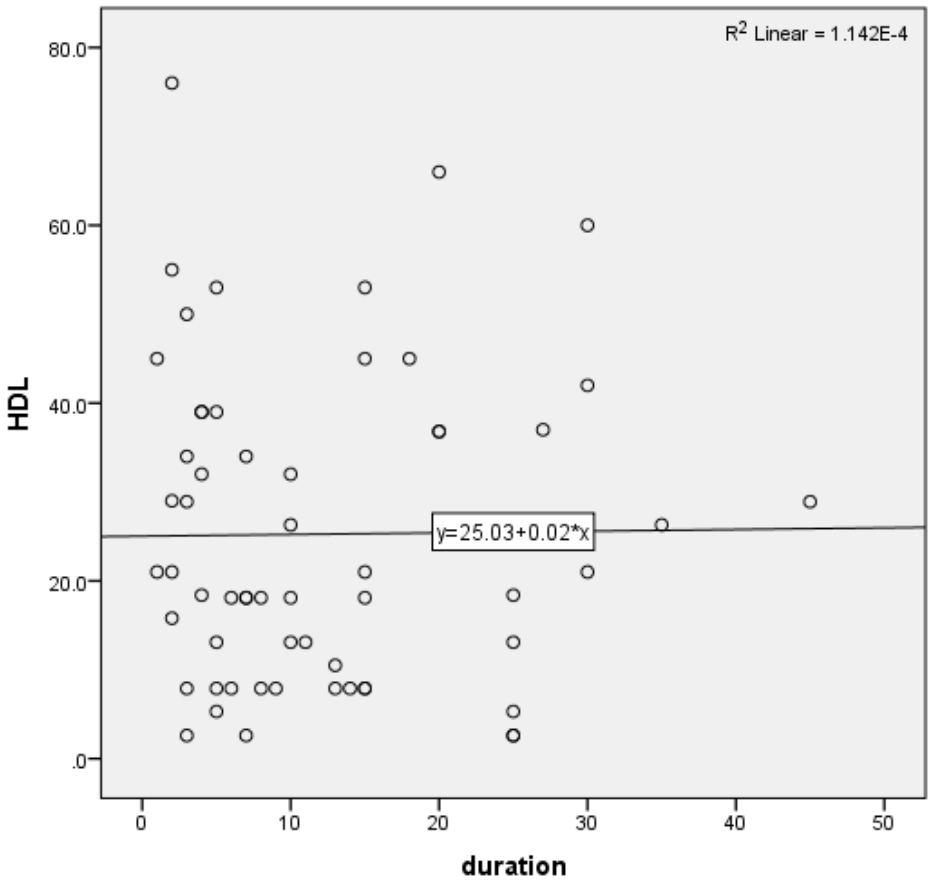

Figure 3.5: Correlation between HDLc and duration of smoking

(p=0.349,r= -0.123)

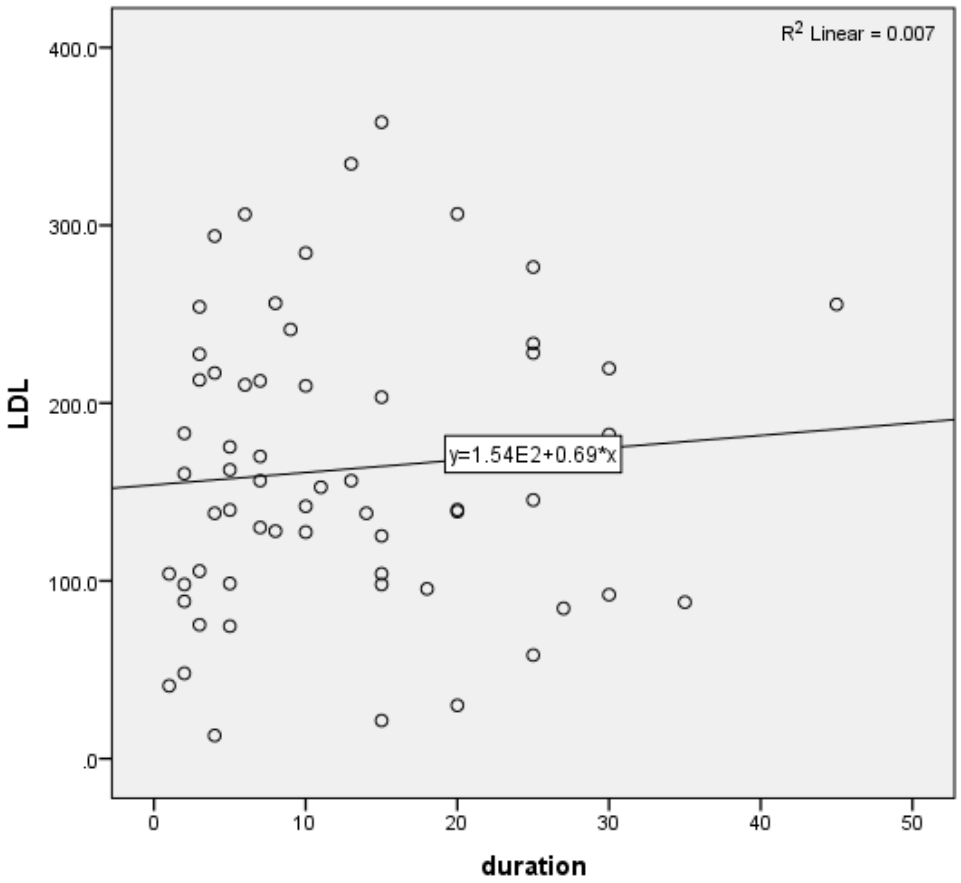

Figure 3.6: Correlation between LDLc andduration of smoking(p=0.463,r=0.096)

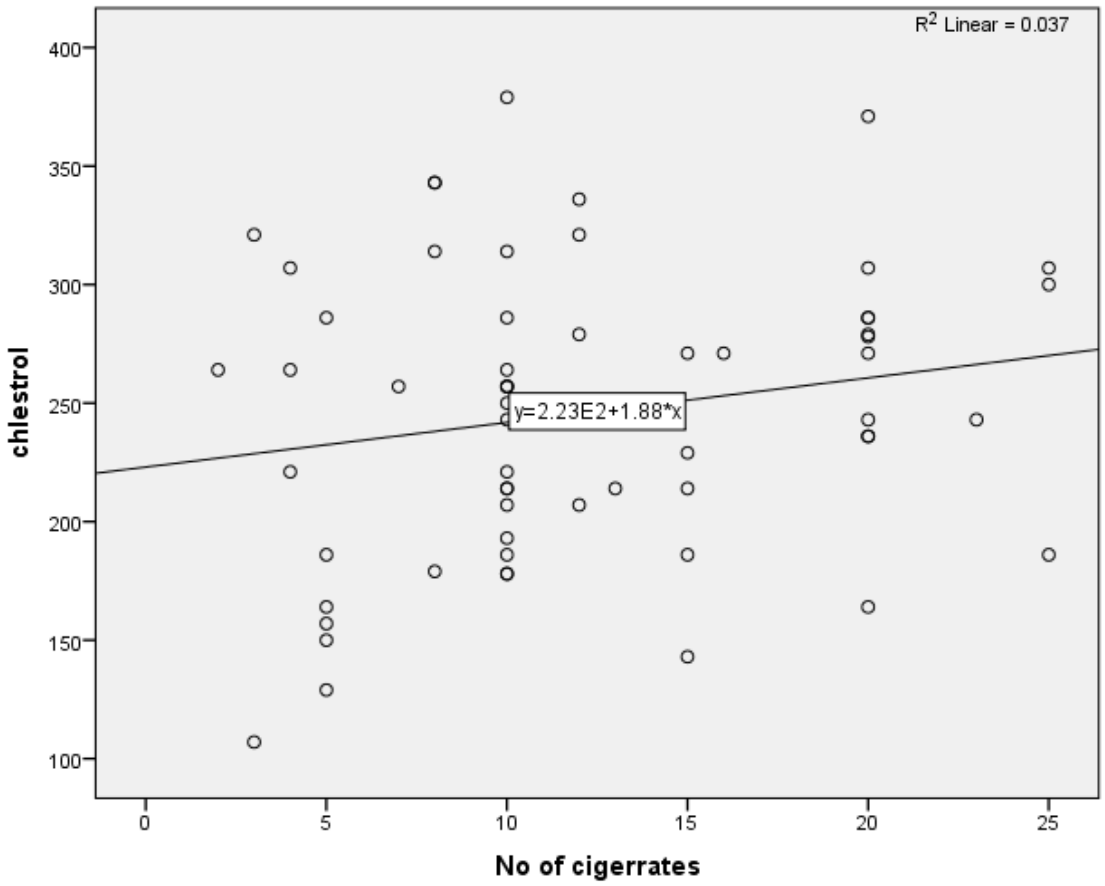

Figure 3.7: Correlation between Cholesterol concentration and number of cigarette per day.

(p=0.774,r=0.038)

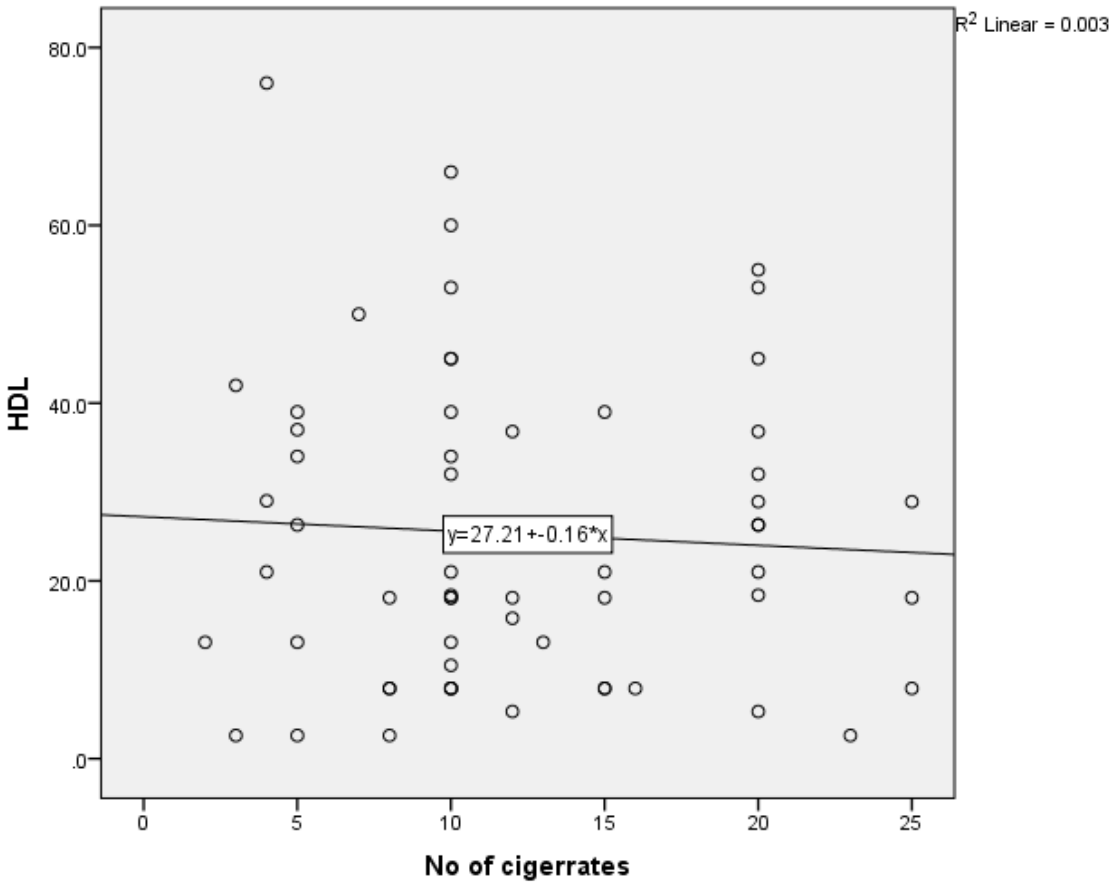

Figure 3.8: Correlation between HDLcconcentration and numberof cigarette per day.

(p=0.052,r= -0.253)

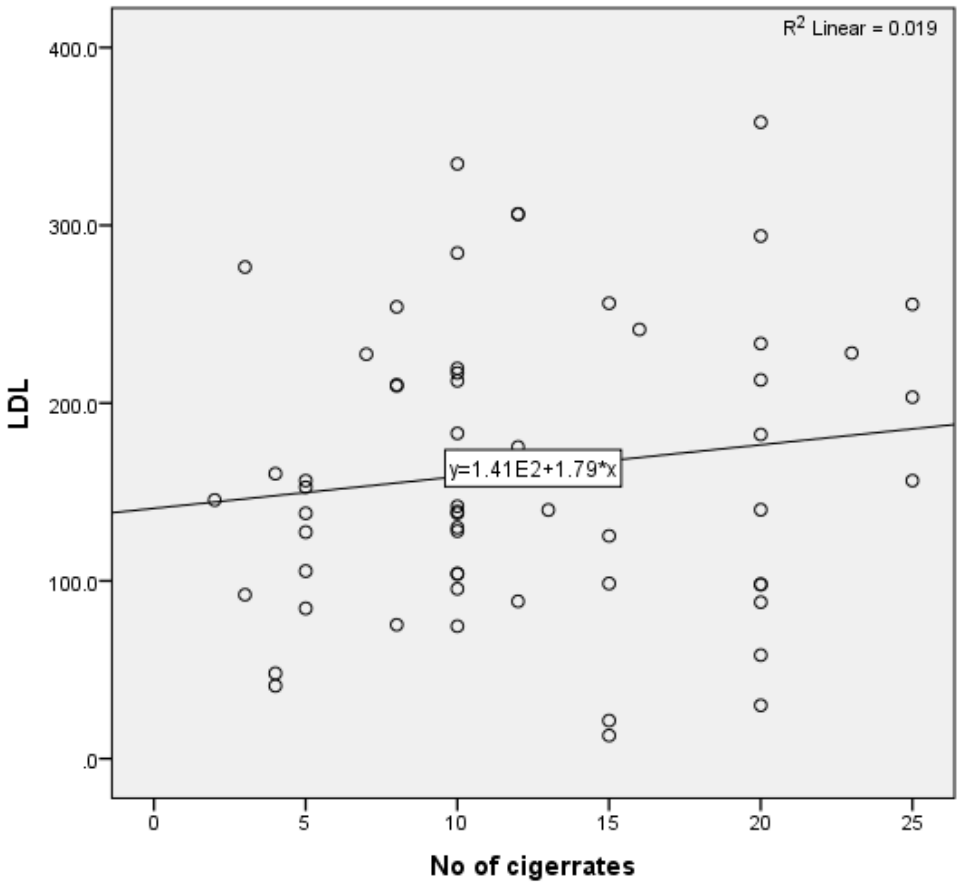

Figure 3.9: Correlation between LDLcconcentration and number of cigarette per day.

(p=0.774, r=0.038)

4. Discussion, conclusion and recommendations

4.1. Discussion

Smoking is one of the most common addictions of modern times. It has been implicated as an etiological agent for various chronic diseases, including a variety of infection, cancers, heart diseases and respiratory illnesses (Mehta *et al*, 2008; Zhong*et al*, 2008).Cigarette contains carcinogens (polycyclic aromatic hydrocarbons etc.), irritant substances,nicotine, carbon monoxide, and other gases. Cigarette smoke contains many oxidants,and free radicals which can harm lipids, proteins,DNA, carbohydrates, and other biomolecules(Al-Azzawy,2011). This is a case control study aimed to study the effect of smoking on serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol levels.One hundred Sudanese male (60 smokers,and 40 non smokers) were enrolled in this study. After evaluation of serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol levels by enzymatic methods, the statistical analysis was done by using SPSS computer program and results showed that cholesterol level was significantly higher in smoker group when compared to non smoker group, HDLc level was significantly lower in smoker group when compared to non smoker group, and LDLc level was significantly higher in smoker group when compared to non smoker group (mean ± 2SD=245.9 ± 122.6, P=0.000); (mean ± 2SD =25.3 ± 35.0, P=0.000); (mean ± 2SD =162.5 ± 162.4, P= 0.057) respectively (table 3-1) .This result agreed withstudy done in in Umm Al-Qura University and related institutions in Makkah, Saudi Arabia by Waheeb d. M. Alharbi and his team to show influence of cigarette smoking on lipid profile in male university students,showed that the smokers hadLDLc level significantly increased for smokers compared to

the healthy control non-smoking subjects (p=0.0420), mean ± SD;(126.87 ± 39.09) vs (107.40 ± 39.24) ,but disagreed with cholesterol level which showedinsignificantlyincreased for smokers compared to the healthy control non-smoking subjects(p=0.5980) and mean ± SD (210.52 ± 35.34)Vs (205.23 ± 48.05) (Waheeb, 2011).Also the results showed that HDLc level was significantly lower in smoker group when compared to non smoker group((table 3-1) and This result agreed with result of study done byOyedeji Samuel Oyewole and his team in Nigeria. conducted in Lipid Profile of Cigarette Smokers in an Ancient City showed that High density lipoprotein of smokers was significantly lower when compared with non-smokers (p value < 0.05)(mean ± standard deviation (SD) 1.03±0.10) vs 1.49±0.13) (Oyedeji etal,2013) . The results also showed insignificant weak positive correlation between cholesterol concentration and age, insignificantweak negativecorrelation between HDLc concentration and age,and insignificant weak positive correlation between LDLc concentration and age (p=0.179,r=0.176); (p=0.067,r=-0.238); (p =0.233 ,r=0.156) respectively.

Statistical analysis also showed insignificant weak positive correlation between cholesterolwith duration of smoking per year ,insignificantvery weak positive correlation between LDLc concentration with duration of smoking per year, and insignificant very weak negative correlation between HDLc concentration with duration of smoking per year (p=0.091, r=0.220); (p=0.463, r=0.096); (p=0.349, r=-0.123) respectively. Statistical analysis also showed insignificant very weak positivecorrelation between cholesterol concentration and LDLc concentration with number of cigarette per day,but showed significant weak negativecorrelation between HDLc concentration and number of

cigarette per day (p=0.774, r=0.038); (p=0.774, r=0.038); (p=0.052, r=-0.253) respectively

4.2.Conclusion

Fromstudy results it's concluded that:

1. The level of serum cholesterol is significantly increase in cigarette smokers compared to non smokers, the level of serum HDLc is significantly decrease in cigarette smokers compared to non smokers, and the level of serum LDLc is significantly increase in cigarette smokers compared to non smokers.
2. The level of HDLc is sinificantly negative correlated with number of cigarette per day, while total cholesterol and LDLcare insignificantly positive correlated with number of cigarette per day.
3. Total cholesterol and LDLc are insignificantly positive correlated with age of smokers and duration of smoking but HDLc isinsignificantly negative correlated with age of smokers and duration of smoking .

4.3. Recommendation

1. Smokers should estimate total cholesterol, HDLc, and LDLc regularly to detect heart disease earlier.
2. Further studies by evaluation of C. reactive protein as a predictive marker of atherosclerosis is recommend to be done on smokers.

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AppendixI

Sudan University of Science and Technology

College of Graduate Studies

Influence of smoking on serum total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol levels among Sudanese male smokers (Khartoum state 2015)

Questionnaire

Name:…….. NO. of sample ( )

Age:…….

Duration of smoking / years:……

Number of cigarettes / day :……

History of other disease:……

Results :

Total cholesterol = …….. .mg/dl

HDLc =…… mg/dl

LDL c= ……. mg/dl