Sudan University of Science and Technology

Faculty of Graduate Studies

Assessment of C - Reactive Protein and High Density Lipoprotein Cholesterol in Sudanese Cigarette Smokers in khartoum State

تقييم مستويات بروتين سي التفاعلي وكلسترول الدهون عالية الكثافة لدى المدخنين السودانيين فى ولاية الخرطوم

A dissertation submitted in partial fulfillment of the requirement for the Master Degree in clinical chemistry

By:

Amira Alhadi Gar Elnabi

Supervised by:

Dr. Nuha Eljaili Abubaker

Assistant professor of clinical biochemistry

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الآية

بِسْمِ اللهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى :

(يَرْفَعِ اللهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا العِلْمَ دَرَجَاتٍ وَاللهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

صدق الله العظيم

سورة المجادلة الآية (11)

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Dedication

To my family …….with love

Amira …….

Acknowledgments

Thanks first and last to(ALLAH) who enabled me to conduct this study by the grace of him and give me strength and patience.

Special thanks to **Dr.NuhaEljaili Abu Baker**- my supervisor for her continuoussupervisor,patience ,critical moments, valuable advice and careful guidance .

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Words can never help to express my feelings towards every one stand beside me to carry work

Abstract

This is a case – control study ,conducted to determine the effect of cigarette smoking on CRP and HDL-c levels in Sudanese male cigarette smokers in period from March 2017 to April 2017 .

Fifty samples were collected from cigarette smokers without disease and thirty from former smoker (who quit smoking) without disease also and fifty non – smokers people as control were informed .

Mindray (semi automated) was used for measurement of HDL-c and

Quikread apparatus was used for the measurment of CRP , (SPSS)was used for the analysis of results .

The study showed that there was a significant increase in CRP level and a significant decrease in HDL-c level of cigarette smokers compared to non-cigarette smokers.(mean±SD cases versus control; 6.6±1.96 versus 4.96±0.6 mg/l P . value 0.000),(45.6±13.5 versus 66.1±3.4 mg/dl P . value 0.000) respectively.

The study showed that there was significant increase in CRP level and significant decrease in HDL-c of smokers compared to former smokers (mean ±SD smokers versus former smokers : 7.4±2 versus 5.3±0.85 mg/l . P .value 0.000),(38.3±11.4 versus 58.1±4 mg/dl . P .value 0.000) respectively.

Pearson correlation showed that there were no correlation between CRP ,HDL-c and age (r=0.05 p. value =0.7), (r=0.15 p. value =0.27) respectively.

Pearson correlation showed that there were a significant positive correlation between CRPand number of cigarette smoked per day and significant negative correlation between HDL-c and number of cigarette smoked per day (r=0.48 p. value =0.000), (r= - 0.6 p.value 0.000) respectively .

Also there were no correlation between CRP,HDL-c and duration of smoking (r=0.08p. value =0.57), (r=0.02 p. value =0.86) respectively.

The study concluded that ,there was significant increase in CRP and significant decrease in HDL-c in cigarette smoker

مستخلص الدراسة

أجريت هذه الدراسة التوقعية لقياس مدي تأثيرالتدخين علي مستويات بروتين سي التفاعلي ومعدل كلسترول الدهون العالية الكثافة في المدخنين السودانين في الفترة مابين مارس حتي أبريل 2017م

خمسون عينة أخذت من مدخنين للسجائر الأصحاء الذين لا يعانون من مرض مع ثلاثون عينة أخذت من مدخنين سابقين وخمسون عينة أخذت من غير المدخنين كمجموعة تحكم (مجموعة ضابطة) تم أخبارهم بغرض الدراسة وأخذ موافقتهم .

تم قياس مستويات كلسترول الدهون العالية الكثافة بإستخدام (جهاز مندري شبه أوتوماتيكي للتحليل) وجهاز (كويك ريد لقياس مستويات بروتين سي التفاعلي) وبرنامج الحزمة الأحصائية للعلوم الإجتماعية لمعالجة البيانات .

أظهرت الدراسة ازدياد فى مستويات بروتين سي التفاعلى ونقصان فى معدل كلسترول الدهون العالية الكثافة لدى المدخنين مقارنة بغير المدخنين

المتوسط + الانحراف المعياري للمدحنين مقارنة بغير المدخنين(6.6+1.96 مل جم / ليتر مقابل 4.96+0.6مل جم / ليتر ، القيمة المعنوية =0.000)و(45.6+13.5مقابل 66.1+3.4 مل جم/ديسيليتر,القيمة المعنوية =0.000)على التوالى.

أظهرت الدراسة ازدياد فى مستويات بروتين سي التفاعلى ونقصان فى معدل كلسترول الدهون العالية الكثافة لدى المدخنين مقارنة بالمدخنين السابقين .

المتوسط + الانحراف المعياري للمدحنين مقارنة بالمدخنين السابقين (7.4+2 مقابل 5.3+0.85 مل جم /ليتر,القيمة المعنوية = 0.000)و(38.3+11.4مقابل 58.1+4 مل جم/ديسيليتر,القيمة المعنوية =0.000)على التوالى .

تحليل ارتباط بيرسون وجد انه لا توجد علاقة ذات دلالة إحصائية بين بروتين سي التفاعلى,معدل كلسترول الدهون العالية الكثافة والعمر (معدل ارتباط بيرسون =0.05, القيمة المعنوية =0.7)و (معدل ارتباط بيرسون =0.15 ,القيمة المعنوية =0.27) على التوالى.

تحليل ارتباط بيرسون أظهر علاقة ايجابية ذات دلالة إحصائية بين بروتين سي التفاعلى وعدد تدخين السجاير فى اليوم وعلاقة سلبية ذات دلالة إحصائية بين معدل كلسترول الدهون العالية الكثافة وعدد تدخين السجاير فى اليوم (معدل ارتباط بيرسون=0.48 ,القيمة المعنوية=0.000) و(معدل ارتباط بيرسون 0.6- ,القيمة المعنوية =0.000) على التوالى .

ايضا اظهرت الدراسة انه لاتوجد علاقة ذات دلالة إحصائية بين بروتين سي التفاعلى,معدل كلسترول الدهون العالية الكثافة ومدة التدخين (معدل ارتباط بيرسون =0.08 ,القيمة المعنوية = 0.57) و (معدل ارتباط بيرسون =0.02 ,القيمة المعنوية =0.86) على التوالى .

وعليه خلصت الدراسة الى انه يوجد ازدياد فى مستويات بروتين سي التفاعلى ونقصان فى معدل كلسترول الدهون العالية الكثافة لدى المدخنين .

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List of abbreviations

ABCA1	Adenosine tri-phosphate binding cassette transporter A1
ABCG1	Adenosine tri-phosphate binding cassette transporter G1
ADHD	Attention deficit hyperactivity disorder
CETP	Cholesterol ester transfer protein
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CV	Cardiovascular
HDL	High density lipoprotein
IBD	Inflammatory bowel disease
IL 1	Interleukine 1
IL 6	Interleukine 6
LCAT	Leccithine –cholesterol acyltranseferase
LDL	Low density lipoprotein
OSA	Obstructive sleep apnea
PLTP	Phospholipid transport protein
POD	Peroxidase
SR-BI	Scavenger receptor –BI
TLF	Trypanosome lytic factor
TNF	Tumor necrosis factor
VLDL	Very low density lipoprotein
4AAP	4 Amino antipyrine

Chapter One

Introduction

Rationale

Objectives

Chapter One

1. Introduction

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 generally has negative health effects, because smoke inhalation inherently poses challenges to various physiologic processes such as respiration. Diseases related to tobacco smoking 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. (West*et al*., 2007)

Smoking causes a variety of adverse effects on organs that have no direct contact with the smoke itself such as the liver. It increases the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) that would be involved in liver cell injury also increases serum and hepatic iron which induce oxidative stress and lipid peroxidation that lead to activation of stellate cells and development of fibrosis.(Abdel-Rahman, 2006).

CRP is elevated in smokers compared with nonsmokers (Bermudez*et al*., 2002) smoker also have significantly lower HDL-c levels. This effect of cigarette smoking is dose-dependent. In contrast, smoking cessation can elevate HDL-c, up to levels seen in non-smokers. (He *et al*., 2013)

1.2 Rationale

Every year hundreds of thousands of people around the world die from disease caused by cigarette smoking .Smoking has much serious effect on human body and cause different diseases such as cardiac disease. Lighting a cigarette creates over 4000 harmful chemicals with hazardous adverse effects on almost every organ in the body. Smoking yields chemical substances with cytotoxic potentials,These chemicals created by smoking induce oxidative stress associated with lipid peroxidation ,which leads to activation of stellate cells and development of fibrosis. It, also, affects on intravascular remodeling of HDL. In addition, smoking increases the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) involved in liver cell injury.

CRP is an acute phase protein of hepatic origin, which increase in response to (IL-1, IL-6 and TNF-α). measure of CRP and HDL-c may help to provide more information to reduce the risk of smoking among sudanese population .

1.3 Objectives

1.3.1 General objective

To assess C - reactive protein and high density lipoprotein cholesterolin Sudanese smokers in Khartoum state.

1.3.2 Specific objectives

1. To measure C - reactive protein and high density lipoprotein cholesterol and compare mean concenteration in cigarette smokers.

2. To correlate between C - reactive protein, high density lipoprotein cholesterol and age, number of cigarette per day and duration of smoking in smokers .

Chapter Two

Literature Review

Chapter Two

2. Literature Review

2.1 Smoking

2.1.1Definition

Is a practice in which a substance is burned and the resulting smoke breathed in to be tasted and absorbed into the bloodstreammost 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 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 inducetrance-like states that, they believe, can lead them to "spiritual enlightenment".(Emmanuela*et al*.,2015)

Smoking generally has negative health effects, because smoke inhalation inherently poses challenges to various physiologic processes such as respiration. Diseases related to tobacco smoking have been shown to kill approximately half of long-term smokers when compared to average mortality rates faced by non-smokers. According to a 2017 report

in TheLancet, smoking has caused over five million deaths every year from 1990 to 2015.(Reitsma*et al*.,2017)

Smoking is one of the most common forms of recreational drug use. Tobacco smoking is the most popular form, being practiced by over one billion people globally, of whom the majority are in the developing world. Less common drugs for smoking include cannabis andopium. Some of the substances are classified as hard narcotics, like heroin, but the use of these is very limited as they are usually not commercially available. 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, and bongs. (WHO, 2016)

Smoking can be dated to as early as 5000 BC, and has been recorded in many different cultures across the world. Early smoking evolved in association with religious ceremonies; as offerings to deities, in cleansing rituals or to allow shamans and priests to alter their minds for purposes of divination or spiritual enlightenment. After the European exploration and conquest of the Americas, the practice of smoking tobacco quickly spread to the rest of the world. In regions like India and Sub-Saharan Africa, it merged with existing practices of smoking (mostly of cannabis). In Europe, it introduced a new type of social activity and a form of drug intake which previously had been unknown.(WHO, 2016)

2.1.2 Physiology

Inhaling the vaporized gas form of substances into the lungs is a quick and very effective way of delivering drugs into the bloodstream (as the gas diffuses directly into the pulmonary vein, then into the heart and from there to the brain) and affects the user within less than a second of the first inhalation. The lungs consist of several million tiny bulbs

called alveoli that altogether have an area of over 70 m² (about the area of a tennis court). This can be used to administer useful medical as well as recreational drugs such as aerosols, consisting of tiny droplets of a medication, or as gas produced by burning plant material with a psychoactive substance or pure forms of the substance itself. Not all drugs can be smoked, for example the sulphate derivative that is most commonly inhaled through the nose, though purer free base forms of substances can, but often requires considerable skill in administering the drug properly. The method is also somewhat inefficient since not all of the smoke will be inhaled.(Leslie, 2004).

The inhaled substances trigger chemical reactions in nerve endings in the brain due to being similar to naturally occurring substances such as endorphins and dopamine, which are associated with sensations of pleasure. The result is what is usually referred to as a "high" that ranges between the mild stimulus caused by nicotine to the intense euphoria caused by heroin, cocaine and methamphetamines.(Leslie, 2004)

The incomplete combustion produced by burning plant material, like tobacco or cannabis, produces carbon monoxide, which impairs the ability of blood to carry oxygen when inhaled into the lungs. There are several other toxic compounds in tobacco that constitute serious health hazards to long-term smokers from a whole range of causes; vascular abnormalities such as stenosis, lung cancer, heart attacks, strokes, impotence, low birth weight of infants born by smoking mothers. 8% of long-term smokers develop the characteristic set of facial changes known to doctors asmoker's face.(Model,1985)

2.1.3 Component of cigarette smoking

More than 4000 different chemicals have been identified in cigarette smoke .most of us have a very basic idea these chemicals can be harmful to health and that the mechanism whereby this complex mixture of toxins contained in tobacco smoke leads to specific diseases are complex. (Jonathan, 2004)

The simplest categorization of the components if cigarette smoking identifies 3 major components:tar, nicotine,and carbon –monoxide (CO).Tar is the black sticky mass that coats the lung and airways.

There are many hundreds of different chemicals within the tar, some of which have been shown to be carcinogenic in animals and/or humans.

The deposition of particles of tar in the lungs and upper airways lead to the blocking of the airways and to serious breathing problems, including chronic obstructive pulmonary disease (COPD). The toxic chemicals also cause inflammation and reduce of the elasticity of the lung and hence the ability to inhale and exhale normally .the carbon monoxide in smoke replace the oxygen in the hemoglobin (a component of blood),adversely affecting oxygen transport and energy supply, and requiring the heart to do more work to supply the same amount of oxygen to the body. A large number of smoke constitute and particularly components of the gaseouse phase of tobacco smoke, cause immunologic responses and inflammation in the cells .this causes increased stickness of the blood, which increase the risk of clots (Jonathan, 2004) .these processes increase the likelihood of aheart attack ,stroke or other problems with the cardiovascular system. Irritants such as nitric oxide cause hyper-secretion of mucus and substance such as acrolein, acetone and acetaldehyde cause damage to the small hair–

like strands that line the airways (cilia). This damage to the cilia impairs the ability of the cilia to clear mucus, causing breathing difficulties

Years of smoking and daily coating of the lungs and airways in tar leads to irreversible lung damage and ultimately death from CPOD .A cute nicotine (critical for the development addiction), increase heart rate,blood pressure and causes peripheral vasoconstriction (i.e., impairs peripheral circulation and thus exacerbates Reynauds disease and erectile dysfunction). However, studies of smokeless tobacco users (who have high nicotine exposure like smokers,but without the smoke) compared with smokers, suggest that most of cardio vascular disease are not caused by nicotine. It therefore appears that it is the thrombogenic effects of tobacco smoke exposure (primarily oxidant gases), combined with reduced oxygen supply (carbon monoxide) and increased myocardial oxygen demand (nicotine) that cause the cardiovascular harms from smoking, include formaldehyde, acetaldehyde, Acetone, Acrolein, Propionaldehyde, Crotonaldehyde, Methyl-Ethyl-Ketone, Butyraldehyde Hydroquinone, Resorcinol, Catechol, Phenol, Cresol, O-toluidine, O-anisidine NO, Benzene, Toluene (Jhonathan, 2004)

2.1.4 Health effects and regulation

Smoking is one of the leading causes of preventable death globally. In the United States about 500,000 deaths per year are attributed to smoking-related diseases and a recent study estimated that as much as 1/3 of China's male population will have significantly shortened life-spans due to smoking(Leslie,2004). Male and female smokers lose an average of 13.2 and 14.5 years of life, respectively(CDC, 2002). At least half of all lifelong smokers die earlier as a result of smoking(Doll*et al*., 2004;Thu*et al*., 1995)

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The risk of dying from lung cancer before age 85 is 22.1% for a male smoker and 11.9% for a female current smoker, in the absence of competing causes of death. The corresponding estimates for lifelong nonsmokers are a 1.1% probability of dying from lung cancer before age 85 for a man of European descent, and a 0.8% probability for a woman(Thu *et al.*,2008) Smoking one cigarette a day results in a risk of heart disease that is halfway between that of a smoker and a non-smoker. The non-linear dose response relationship may be explained by smoking's effect on platelet aggregation(Law*et al.*, 1997).

Among the diseases that can be caused by smoking are vascular stenosis, lung cancer, heart attacks(Nyboe*et al.*,1989) and chronic obstructive pulmonary disease(Devereux, 2006) Smoking during pregnancy may cause ADHD to a fetus(Braun*et al.*, 2006).

Smoking is a risk factor in Alzheimer's disease(Cataldo*et al.*, 2010). While smoking more than 15 cigarettes per day has been shown to worsen the symptoms of Crohn's disease,(Cosnes*et al.*, 1999) smoking has been shown to actually lower the prevalence of ulcerative colitis (Calkins, 1989;Lakatos*et al.*,2007).

2.2 C-reactive protein (**CRP**)

2.2.1 Definition

Is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion bymacrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells

(and some types of bacteria) in order to activate the complement system via the C1Q complex.(Thompson*et al.*, 1999)

Is synthesized by the liver(Pepys and Hirschfield,2003) in response to factors released by macrophages and fat cells (adipocytes).(Lau*et al.*, 2005) It is a member of the pentraxin family of proteins.(Pepys and Hirschfield,2003) It is not related to C-peptide (insulin) or protein C (blood coagulation). It was the first pattern recognition receptor (PRR) to be identified.(Mantovani*et al.*,2008)

The *CRP* gene is located on the first chromosome (1q21-q23). It has 224 amino acids,andithas a monomer molecular mass of 25,106 Da, and has an annular pentameric discoid shape.

Binding to the phosphocholine expressed on the surface of dead or dying cells and some bacteria. This activates thecomplement system, promoting phagocytosis by macrophages, which clears necrotic, and apoptotic cells and bacteria. This acute phase response occurs as a result of a rise in the concentration of IL-6, which is produced by macrophages(Pepys and Hirschfield,2003)as well as adipocytes(Lau et al., 2005), in response to a wide range of acute and chronic inflammatory conditions such as bacterial, viral, or fungal infections; rheumatic and other inflammatory diseases; malignancy; and tissue injury and necrosis. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver.(Lau et al., 2005).

It rises within two hours of the onset of inflammation, up to a 50,000-fold, and peaks at 48 hours. Its half-life of 18 hours is constant, and therefore its level is determined by the rate of production and hence the severity of the

precipitating cause. CRP is thus a marker for inflammation that can be used to screen for inflammation.(Enocsson *et al*., 2009)

2.2.2 ClinicalSignificance

2.2.2.1 Diagnostic use

CRP is used mainly as a marker of inflammation. Apart from liver failure, there are few known factors that interfere with CRP production.(Pepys and Hirschfield, 2003) Interferon alpha inhibits CRP production from liver cells which may explain the relatively low levels of CRP found during viral infections compared to bacterial infections (Enocsson*et al*.,2009)

It is more sensitive and accurate reflection of the acute phase response than the ESR (Erythrocyte Sedimentation Rate). ESR may be normal while CRP is elevated. CRP returns to normal more quickly than ESR in response to therapy.

The utility of CRP in differentiating inflammatory diseases (including inflammatory bowel disease, intestinal lymphoma, intestinal tuberculosis, and Behcet's syndrome) has been investigated and compared to other inflammatory biomarkers, such as ESR and WBC.(Liu*et al*.,2013)

2.2.2.2 Cancer

The role of inflammation in cancer is not well understood. Some organs of the body show greater risk of cancer when they are chronically inflamed.(Liu*et al*.,2006) While there is an association between increased levels of C-reactive protein and risk of developing cancer, there is no association between genetic polymorphisms influencing circulating levels of CRP and cancer risk.(Allin,2011)

In a 2004 prospective cohort study on colon cancer risk associated with CRP levels, people with colon cancer had higher average CRP

concentrations than people without colon cancer.(Erlinger*et al.*, 2004) It can be noted that the average CRP levels in both groups were well within the range of CRP levels usually found in healthy people. However, these findings may suggest that low inflammation level can be associated with a lower risk of colon cancer, concurring with previous studies that indicate anti-inflammatory drugs could lower colon cancer risk.(Baron*et al.*,2003)

2.2.2.3 Cardiovascular disease

Recent research suggests that patients with elevated basal levels of CRP are at an increased risk of diabetes,(Pradhan*et al.*,2001;Dehghan*et al.*,2007) hypertension and cardiovascular disease.

2.2.2.4 Fibrosis and inflammation:

Scleroderma, polymyositis, and dermatomyositis elicit little or no CRP response. CRP levels also tend not to be elevated in SLE unless serositis or synovitis is present. Elevations of CRP in the absence of clinically significant inflammation can occur in renal failure. Patients with high CRP concentrations are more likely to develop stroke, myocardial infarction, and severe peripheral vascular disease. (Clearfield,2005) Elevated level of CRP can also be observed in inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis.(Liu*et al.*,2013) Obstructive sleep apnea

C-reactive protein (CRP), a marker of systemic inflammation, is also increased in obstructive sleep apnea (OSA) (Latina*et al.*,2013)

2.2.2.5 Rheumatoid arthritis

It has previously been speculated that single-nucleotide polymorphisms in the CRP gene may affect clinical decision-making based on CRP in rheumatoid arthritis.

2.2.3 Relationship between CRP and cigarette smoking

Cigarette smoking is an important, independent and modifiable cardiovascular risk factor. CRP is elevated in smokers compared with nonsmokers ,Elevated CRP concentrations in smokers could at least partially explain the effect of smoking on cardiovascular risk.(Bermudez*et al.*,2002)

2.3 High-density lipoproteins (HDL)

Is one of the five major groups of lipoproteins. Lipoproteins are complex particles composed of multiple proteins which transport all fatmolecules (lipids) around the body within the water outside cells. They are typically composed of 80-100 proteins per particle (organized by one, two or three ApoA; more as the particles enlarge picking up and carrying more fat molecules) and transporting up to hundreds of fat molecules per particle. Unlike the larger lipoprotein particles which deliver fat molecules to cells, HDL particles remove fat molecules from cells which need to export fat molecules. The fats carried include cholesterol, phospholipids, and triglycerides; amounts of each are quite variable.

Increasing concentrations of HDL particles are strongly associated with decreasing accumulation of atherosclerosis within the walls of arteries. This is important because atherosclerosis eventually results in sudden plaque ruptures, cardiovascular disease, stroke and other vascular diseases. HDL particles are sometimes referred to as "good cholesterol" because they

can transport fat molecules out of artery walls, reduce macrophage accumulation, and thus help prevent or even regress atherosclerosis. However, studies have shown that HDL-lacking mice still have the ability to transport cholesterol to bile, suggesting that there are alternative mechanisms for cholesterol removal.(Betteridge*et al.*, 2008)

2.3.1 Structure and function

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 andapo A-II(Despres, 2009) A rare genetic variant, ApoA-1 Milano, has been documented to be far more effective 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).(Huang and Zhang,2013)

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). (Stephens*et al.*, 2012)

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 cholesterol ester transfer protein (CETP). This protein exchanges triglycerides of VLDL against cholesterol 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.(Kieft*et al.*,2012)

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-ladenmacrophages 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.(Macleod*et al.*,2012)

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.(Stephens*et al.*,2012)

In the stress response, serum amyloid A, which is one of the acute-phase proteins and an apolipoprotein, is under the stimulation of cytokines (interleukin 1, interleukin 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.(Kwiterovich, 2000)

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.(Kwiterovich, 2000) This ratio of large HDL to total HDL particles varies widely and is measured only by more sophisticated lipoprotein assays using either electrophoresis. (Ashwood*et al.*, 2008).

2.3.2 Subfractions

Five subfractions of HDL have been identified. From largest (and most effective in cholesterol removal) to smallest (and least effective), the types are 2a, 2b, 3a, 3b, and 3c.

2.3.3 Relationship between HDL-c and cigarette smoking

It has long been established that cigarette smoking is an independent risk factor for atherosclerosis and coronary heart disease. The underlying mechanisms are however unclear, although evidence points in the direction of endothelial dysfunction, platelet activation, increasing oxidative stress and inflammation. Cigarette smoking also appears to disrupt lipid and lipoprotein metabolism; smokers have lower HDL-c levels as compared to non-smokers. In particular the effects of cigarette smoking on HDL-c appear to contribute to the increased risk of CV disease in smokers. (Jcell, 2013).

Chapter Three

Materials and Methods

Chapter Three

3. Materials and Methods

3.1 Materials

3.1.1 Study approach

A quantitative method was used to measure plasma C-reactive protein and high density lipoprotein cholesterol levels in Sudanese cigarette smoker and former smoker in Khartoum state,during period from March to April 2017.

3.1.2 Study design and area

This is case control study andwas conducted in Khartoum state

3.1.3Target population and sample size

Fifty cigarette smokers and 30 former smokers enrolled in this studyin additionto 50 normal healthy non-smokers(age matched with test group)were involved as a control group.

3.1.4Inclusion criteria

Sudanese cigarette smokers, former smokers and healthy volunteer were included.

3.1.5Exclusion criteria

Cigarette smoker with diabetes, cardiovascular disease, inflammation, cancer, burn and any recent injury when the sample is collected were excluded.

3.1.6Ethical consideration

The aim and benefits of this study were explained to participants and an informed consent was obtained from each participants.

3.1.7Data collection and analysis

A questionnaire was used to obtain clinical data from each participant.

3.1.8Blood samples collection

After informed consent, local antiseptic 70% ethanol was used to clean the skin. Venous blood (2mls) were taken from each participant divided into one heparin anti-coagulant container (for plasma C-reactive protein and high density lipoprotein), and then centrifuged at 3000rpm for 5 minutes and obtained plasma for CRP and HDL-c was separated in eppendorf tube, and kept in refrigerator until used.

3.2 Methods

3.2.1 Estimation of CRP

3.2.1.1 Principle of the method

The Orion DiagnosticQuikread CRP is an immunoturbidimetric test based on microparticles coated with anti-human CRP .the CRP present in the samples reacts with the microparticles, and the resultant change in the turbidity of the solution is measured by the Quikread101Instrument .

3.2.1.2 Procedure

1. The card reader in instrument was prepared.
2. The cuvette was opened.
3. 14ul of plasma sample was added.
4. The cap was put on and mixed gently (not upside –down), The cuvette was put into the measurement well.
5. Blank measurement, The CRP –reagent was added by pressing down the inner part of the cap.
6. The cuvette was taked out and mixed vigorously (back and forth) to dissolve the reagent.
7. The cuvette was put back into the measurement well the CRP result is displayed within 1 minute.

3.2.2 Estimation of HDL-c

Mindray –semi automated was used for estimation

High density lipoprotein measurement in conjunction with other lipid, precipitation of LDL (low density lipoprotein) and VLDL (very low density lipoprotein)

3.2.2.1 Principle

Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) in sample precipitate with phosphotungestate and magnesium ions,aftercentrifugation,the cholesterol concentration in HDL-c fraction, which remains in supernatant is determined

3.2.2.2Procedure

Reagent	0.5 ml
Specimen	0.2ml

Mixed and incubated for 10 minutes at room temperature,then centrifuge for 10 minute and collect the supernatant for the determination of cholesterol

Method

Enzymatic colorimetric method

Principle

cholesterol esters are enzymatically by cholesterol esterase (CE) to cholesterol and free fatty acids .free cholesterol is then oxidized by cholesterol oxidase (CO) to cholesterene 3 one and peroxide, the hydrogen peroxide combines with phenol and 4-amino-antipyrine (4AAP) in the presence of peroxidase (POD) to form a chromophore (quinoneimine dye) which may be quantitated at 500-550nm

Procedure

The reagents were first brought to room temperature, and then the following amounts were pipetted according to the table below:

	Blank	**Standard**	**Sample**
Reagent	1.0ml	1.0ml	1.0ml
Cholesterol standard	-	10ul	-
Sample	-	-	10ul

Mixed and incubated for 5 minutes at 37 c or 10 minutes at 15-25 c

The absorbance (A) of standard and sample was read against the blank

3.2.2.3 Calculation

$$\text{Plasma cholesterol conc.} \left(\frac{mg}{dl}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

3.3 Quality control

The precision and accuracy of amethod in the estimation of HDL-c was checked each time batch was analyzed by commercially prepared control sera.

3.4 Statistical analysis

Statistical package for social science (SPSS, version 16) was used for data analysis.

The mean and standard deviation of plasma level of CRP and HDL-c was calculated and t-test was used for comparison (significant level was set at $p \leq 0.05$).

Linear regression analysis was used to assess correlation between the duration, number of cigarette per day, age and plasma level of CRP and HDL-c result presented in the form of tables and figures.

Chapter Four

Results

Chapter Four

4. Results

4.1 Results

The levels of biochemical parameter of plasma CRP and HDL-c in cigarette smokers and also compared to non-cigarette smokers, the result presented as follows:

Table (4.1) represent the mean of the levels of plasma CRP (mg/l) and HDL –c (mg/dl) in cigarette smokers and control group there were significantly increas in CRP levels of cigarette smokers compared to the non –smokers and significantly decreas in HDL-c levels of smokers compared to non- smokers

(mean ±SD:7.44±2.03 mg/l versus 4.96±0.6 mg/l .p.value=0.000) for CRP

(mean ±SD:38.2±11.4 mg/dl versus 66.1±3.4 mg/dl .p.value=0.000) for HDL-c

Table (4.2) represent the mean of plasma levels of CRP and HDL-c in smokers and former smokers there were significantly increase in CRP level of smokers compared to former smokers andsignificantly decrease of HDL-c level in smokers compared to former smokers .

(mean± SD:7.4 ± 2 versus 5.3 ± 0.85 mg/l. P.value= 0.000). For CRP

(mean± SD:38.3 ± 11.4 versus 58.1± 4 mg/dl. P.value = 0.000). For HDL-c

Table (4.3) represent the mean of the levels of plasma CRP (mg/l) and HDL-c (mg/dl) in cigarette smokers and control subjects there were significantly increase in CRP levels of cigarette smoker compared to the control group andsignificantly decrease in HDL-c levels of cigarette smokers compared to control group.

(mean± SD: 6.6 ± 1.96   versus 4.96 ± 0.6 mg/l. P.value =0.000). For CRP

(mean± SD: 45.6 ± 13.5 versus 66.1 ± 3.4 mg/dl. P.value= 0.000). For HDL-c

Figure (4.1) shows the correlation between age and CRP level (mg/l) among Sudanesesmokers. (r=0.05 P.value =0.7)there was no correlation.
Figure (4.2) shows the correlation between number of cigarette and CRP level (mg/l) among Sudanese smokers.(r=0.48 P.value =0.000), there was significant positive correlation.

Figure (4.3) shows the correlation between the duration of smoking and CRP level (mg/l) among Sudanese smokers. (r=0.08 P.value =0.57), there was no correlation.

Figure (4.4) shows the correlation between age and HDL-c level (mg/dl)amongSudanese smokers.(r= 0.15 P.value =0.27), there was no correlation.

Figure (4.5) shows the correlation between number of cigarette of smoking and HDL-c level (mg/dl) among Sudanese smokers.(r= -0.6 P.value = 0.000), there was significant negative correlation.
Figure (4.6) shows the correlation between duration of smoking and HDL-c level among Sudanese smokers (r=0.02 P.value =0.86), there was no correlation.

Table (4.1) mean of CRP and HDL-c levels in sudanese smokers and non-smokers

variable	Smokers N=(50) Mean ±SD	Non-smokers N=(50) mean±SD	P.value
CRP mg/l	7.44±2.03	4.96±0.6	0.000
HDL-c mg/dl	38.2±11.4	66.1±3.4	0.000

Result given mean ± SD,P.value ≤ 0.05,consideredsignificant.

Table (4.2) mean of CRP and HDL-c levels in Sudanese smokers and former smokers.

Variable	smokers N=(80) Mean ± SD	Former smokers N=(30) Mean ± SD	P.value
CRP (mg/l)	7.4 ± 2	5.3 ± 0.85	0.000
HDL-c (mg/dl)	38.3 ± 11.4	58.1± 4	0.000

Result given mean ± SD,P.value ≤0.05,considered significant.

Table (4.3) mean of CRP and HDL-c levels in Sudanese smokers and control group

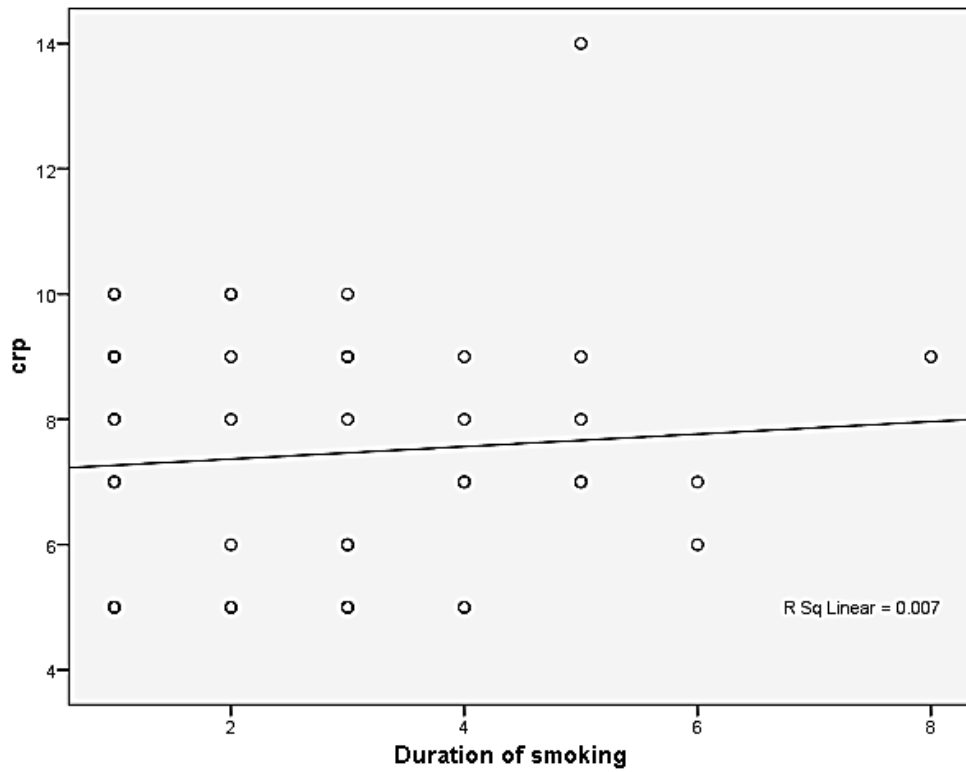| Variable	Smokers N=(50) Mean ± SD	control N=(50) Mean± SD	P.value
CRP (mg/l)	6.6±1.96	4.96 ± 0.6	0.000
HDL-c (mg/dl)	45.6 ± 13.5	66.1± 3.4	0.000

Result given mean ± SD,P.value ≤ 0.05,consideredsignificant.
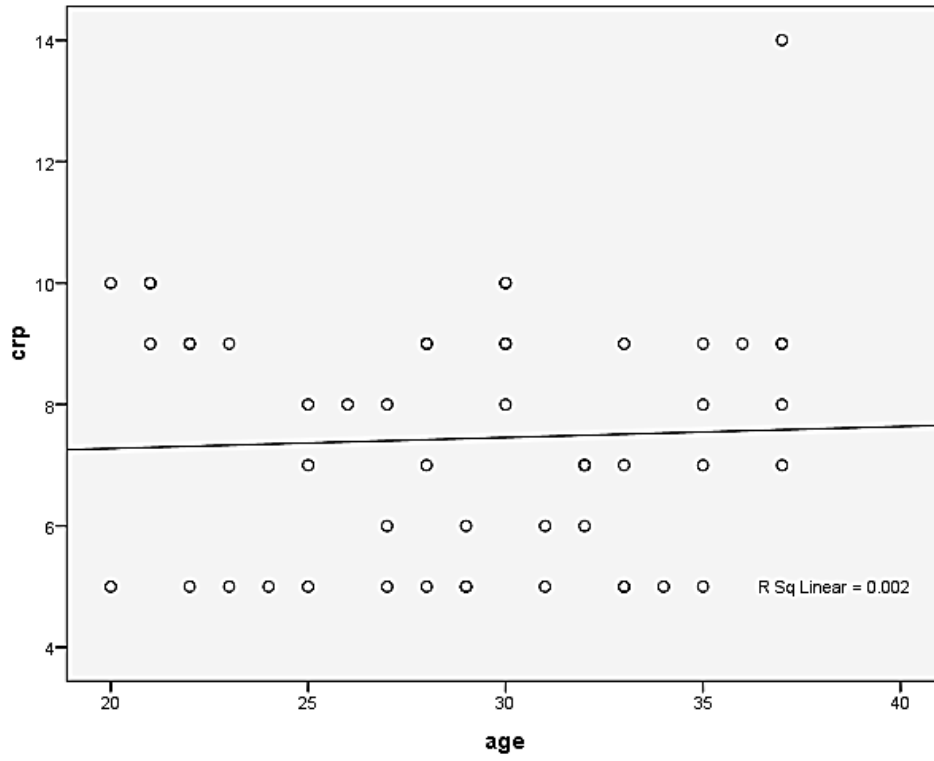
Figure (4.1): Correlation between CRP among Sudanese smokers and age (r=0.05 P.value=0.7)
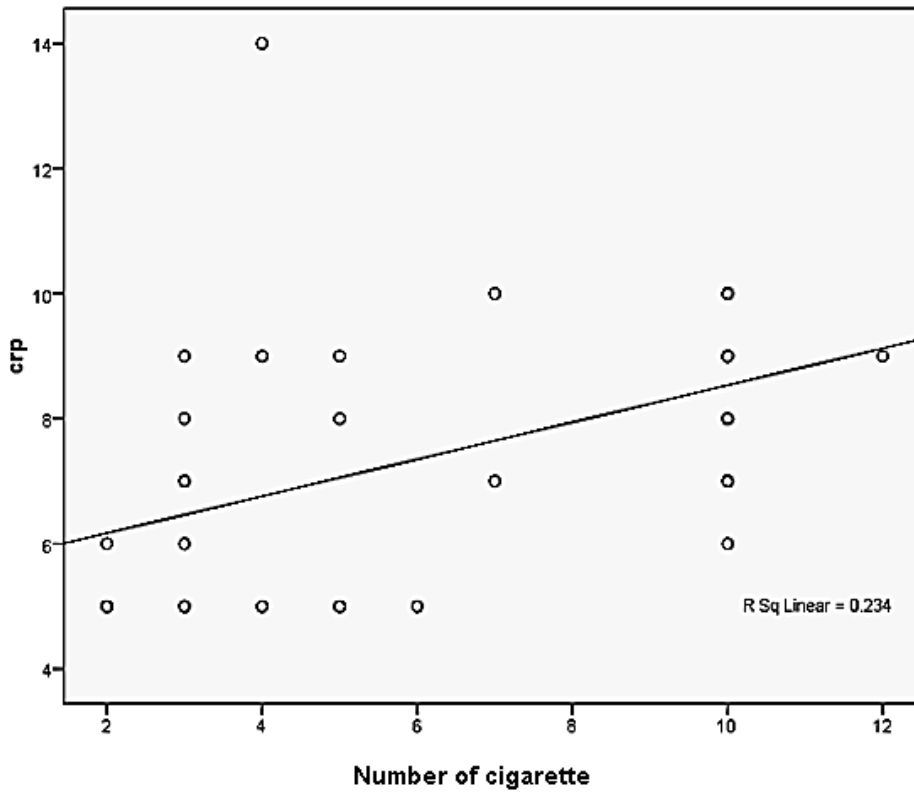
Figure (4.2):Correlation between CRP level and number of cigarette (r=0.48 P.value =0.000)

Figure (4.3):Correlation between CRP and duration of smoking (r=0.08 P.value=0.57)
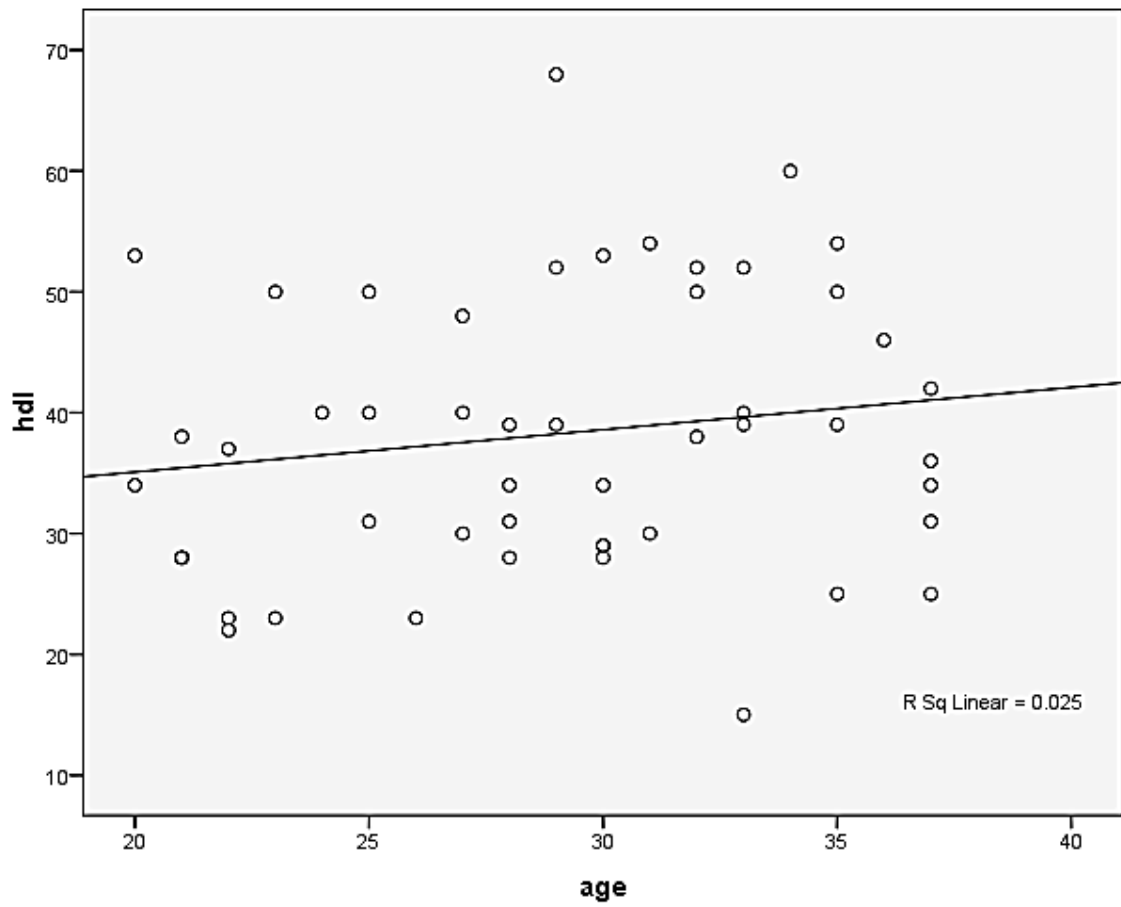
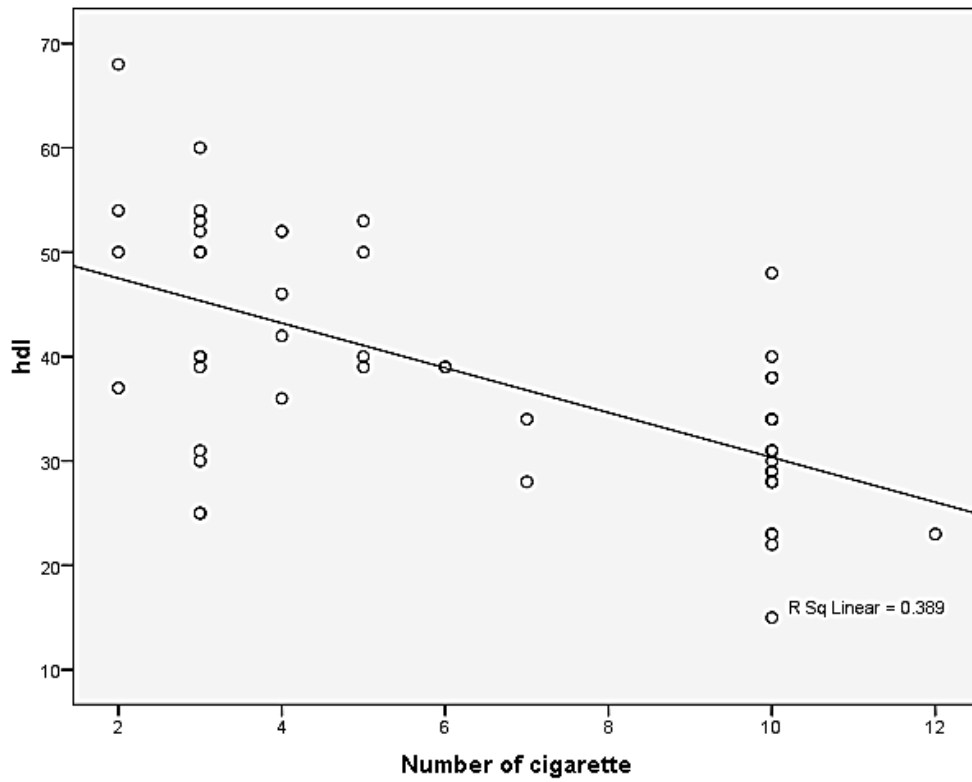**Figure (4.4): Correlation between HDL-c level among Sudanese smokers and age (r=0.15 P.value=0.27)**

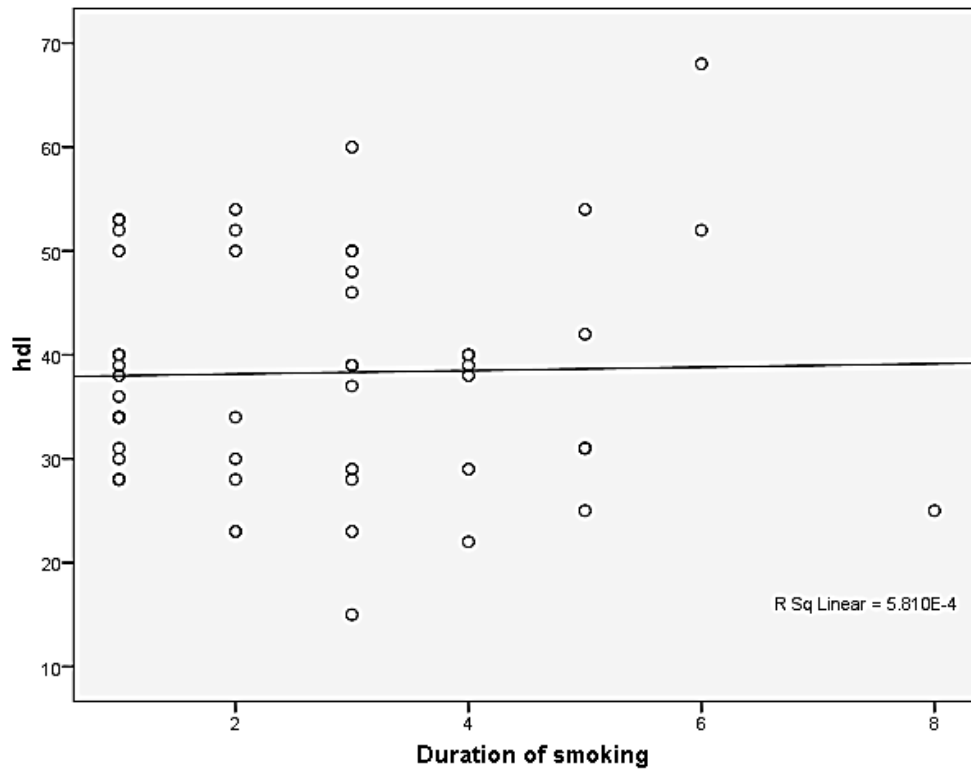**Figure (4.5): correlation between HDL-c level and number of cigarette (r=-0.6 P.value=0.000)**

Figure (4.6): Correlation between HDL-c level and duration of smoking (r=0.02 P.value=0.86)

Chapter Five

Discussion, Conclusion and Recommendations

Chapter Five

5. Discussion

5.1Discussion

There are many evidences that smoking is harmful and lead to death worldwide the effects of smoking are estimated to kill about 3 million per year (Tsuchiya*et al*.,2002)

This study is conducted to study the effect of smoking on the level of CRP and HDL-c in male.

The result obtained from this study indicated that, there was significantly increase of CRP in blood of smokers when compared with control group. (P. value =0.000). The result obtained from this study also indicated that, there was significantly increased of CRP in blood of smokers when compared with former smoker (p. value =0.000).

This result in agreement with another result done by (Tonstad andCowan,2009) which showed that there was significantly increased level of CRP in smokers compared to non –smokers subject and former smokers the cause of these results can be explained as cigarette smoking increase white blood cell mainly (polymorphonuclear which released from bone marrow and recruited to inflammed tissue) and IL-6 ,and CRP   It is an acute-phase   protein of   hepatic   origin   that   increases following interleukin-6 secretion which are increased in response to lung inflammation and are implicated in induction of CRP gene expression .

The results obtained from this study indicated that there was significantly decrease of HDL-cin smokers compared to non –smokers (P.value =0.000)and former smoker (P.value= 0.000).

This result in agreement with another result done by (He *et al*.,2013),who showed that there was significantly decreased level of HDL-camong smokers compared to the non-smokers and former smokersthe cause of these results can be explained as smoking affects on the biosynthesis and maturation of HDLit is effect on the synthesis of nascent HDL particles by decreasing the level of APO A-1 which involve in synthesis of nascent HDLalso smoking reduces LCAT activity an enzyme crucial for the maintenance of normal HDL metabolismalsoaffects intravascular remodeling of HDL,HDL subfraction and catabolism.

This study showed that, there was asignificant positive correlation betweenCRP level and number of cigarette smoked per day .the result was in agreement with result carried by (Lowe*et al*.,2001),who found there was significant positive correlation between CRP level and the number of cigarette smoked per day .also our result in agreement with previous one carried by (Shamima*et al*.,2015) ,whose findings confirmed that there was significant positive correlation between CRP level and number of cigarette smoked per day ,while as the result disagreed with result carried by (Ohsawa*et al*.,2005), they found no correlation between CRP level and number of cigarette smoked per day.

This study showed, there was no correlation between CRP level and age the result was in agreement with result carried by (Feldman and Sbong, 2014) who reported, there was no correlation between age and CRP level ,while our resultdisagreed with result carried by (Zhiqiang*et al*.,2005),which found that CRP level change with age.

The study showed that there was no correlation between CRP level and duration of smoking, the result is disagreed with result carried by (Shamima*et al.*, 2015)

Regarding correlation between HDL-c level and number of cigarette smoked per day, there was significant negative correlation that is similar to result carried by (Anandha*et al.*,2014),

While correlation   between HDL-c and duration of smokingwas not significant that disagreed with result carried by (Anandha*et al.*, 2014),who found positive correlation between HDL-c and duration of smoking.

Our result donot obtain positive correlation between HDL and age this result in agreement with another result carried by (Michael*et al.*, 2013),and(Tarig*et al.*,2013), they found there is no significant correlation.

5.2 Conclusion

The results and findings of this study, concluded that:

Plasma CRP level is significantly increased in Sudanese smokers,while plasma HDL-c level is significantly decreased and plasma CRP and HDL were significantly affected by number of cigarette smoked per day and not affected by age and duration of smoking .

5.3 Recommendations

1. CRP and HDL should be regularly monitor in blood of heavy smokers
2. In order to get more informative data, larger sample size should be used for future research.
3. Health education for the community to increase awairnesswith hazard and complications of cigarette smoking.

4. The government must play an obvious role in the war against cigarette smoking

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Appendices

<div align="center">بسم الله الرحمن الرحيم</div>

Sudan University of Science and technology

C - reactive protein& High Density Lipoprotein cholesterol in Sudanese Smokers

Instructions:

Please! Put a tick in the box next to the answer of your choice.

Name: ……………………………………………………..

Age (years):

16-21 ☐ 22-27 ☐ 28-34 ☐

Chronic disease:

Diabetes ☐ Cardiovascular ☐ Cancer ☐

Smoking status:

Smoker ☐ nonsmoker ☐ former smoker ☐

For how long do you smoke?

1-5 years ☐ 6-10 years ☐ 11-15 years ☐

How many cigarettes do you smoke at day?

Less than one box ☐ one box ☐ more than two ☐

Do you have any recent injury

Yes ☐ No ☐