بسم الله الرحمن الرحيم

Sudan University of Science and Technology
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High Sensitive C-reactive Protein, Magnesium and Lipid Profile Among non-diabetic, non-hypertensive Sudanese Smokers. (A study in Khartoum State)

بروتين سي التفاعلي ومستوى المغنسيوم والدهون لدي المدخنين السودانيون غير المصابين بمرض السكري وضغط الدم في ولاية الخرطوم

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الآية

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قال تعالى: (وَيُحِلُّ لَهُمُ الطَّيِّبَاتِ وَيُحَرِّمُ عَلَيْهِمُ الْخَبَائِثَ وَيَضَعُ عَنْهُمْ إِصْرَهُمْ وَالْأَغْلَالَ الَّتِي كَانَتْ عَلَيْهِمْ ۚ فَالَّذِينَ آمَنُوا بِهِ وَعَزَّرُوهُ وَنَصَرُوهُ وَاتَّبَعُوا النُّورَ الَّذِي أُنْزِلَ مَعَهُ ۙ أُولَٰئِكَ هُمُ الْمُفْلِحُونَ)

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Dedication

I dedicate this work to those who were the causes

of my existence and then my success after god Allah, for my parents,

affectionate mother and my father the savior.

to my loving older brother almughirah

to my dear sisters sarah and brothers

to my friends and all those supported me

and finally to you, my dear reader

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who gave me the ability and success to accomplish this work
and to meet all the challenge.
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and
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all who helped me did not mention his name

Abstract

Background and Aim: Cigarette smoking is a classical and a major risk factor in the development of several diseases with an inflammatory component, including cardiovascular disease, chronic obstructive pulmonary disease, and dyslipidemia. The **aim** of this study to evaluate the plasma levels of high sensitive c-reactive protein, magnesium and lipid profile in smoker's subjects.

Materials and methods: This was descriptive cross-sectional study conducted during the period from April to December 2018, eighty Sudanese individual were included in this study as case, plasma High sensitive c-reactive protein (HS-CRP) was measured by using latex immune turbidymetric method, plasma lipid profile and plasma magnesium were estimated by chemical detection method using photometer, the data obtained was analysis by using SPSS.

Results: study showed mean of plasma high sensitive c-reactive protein (HS-CRP), was insignificant increase in smokers when compared to reference value with p value > 0.05, where means of plasma magnesium, Total cholesterol (T.C), triglyceride (TG), High density lipoprotein (HDL-C) and low density lipoprotein cholesterol (LDL-C) were significantly decrease among smokers' subject when compared to reference values with p value < 0.05.

According to duration of smoking, study showed there were significant increase in means of HS-CRP, Total cholesterol, Triglyceride, and LDL-c where significant decrease in means of magnesium and HDL-c with p value < 0.05.

Conclusion:

The study concludes that, smokers had increase level of HS-CRP, total cholesterol and LDL-C, and decrease in both magnesium and HDL-C levels when increase duration of smoking.

المستلخّص

خلفية الدراسة: تدخين السجائر هو كلاسيكي و عامل خطرٍ رئيسيٍ في تنميةِ عِدّة أمراض مع مواد التهابية، مثل مرض الأوعية القلبيةِ ومرض الإعاقة الرئوية المزمنِ، وارتفاع نسبة الدهون في الدم. هدف هذه الدراسةِ لتَقييم مستويات البلازما للبروتين عالي الكثافة شديد الحساسية، المغنيسيوم ،وملف الدهون لدى المدخّنِين.

المواد والطرق: هذه كَانتْ دراسةً عرضيةً وصفيةً أجرتْ أثناء الفترةِ مِنْ مايو/مايسِ إلى نوفمبر/تشرين الثّاني 2018، ثمانون فرد سوداني تُضمّنَ في هذه الدراسةِ، مستويات البلازما للبروتين عالي الكثافة شديد الحساسية كَانَت مُقاسة بإستعمال طريقةِ لاتكس اميون تيربدمترك ,بلازما ملف الدهون والمغنسيوم قيستا بإستعمال طريقةِ الكشفِ الكيميائي باستخدام الفوتوميتر.

النَتائِج: اظهرت هذه الدراسةِ ان متوسط البلازما للبروتين عالي الكثافة شديد الحساسية مرتفع ارتفاعا غير معنوي عند مقارنتها مع المعدلات الطبيعية مع قيمة إحتمال > من (0.05) ، بينما متوسطات البلازما للمغنويوم ،الكولستيرول الكلي ، الدهون الثلاثية ،الدهون مرتفعة الكثافة والدهون منخفضة الكثافة كَانتْ منخفضة بشكل ملحوظ بين مجموعة المدخّنينِ مقابل المعدلات الطبيعية مع قيم إحتمال < 0.05.

وايضآ أظهرت الدراسة ، أنه على حسب الفترة الزمنية للتدخين أن هنالك زيادة ذات دلالة معنوية في متوسطات كلآ من بروتين سي التفاعلي عالي الكثافة شديد الحساسية، الكولستيرول الكلي ، الدهون الثلاثية والدهون منخفضة الكثافة بينما يوجد إنخفاض ذا دلالة معنوية في متوسطات كلآ من المغنزيوم والدهون مرتفعة الكثافة.

الخلاصة: الخلاصة أن مستويات البلازما لدى المدخنين في كلآ من البروتين عالي الكثافة شديد الحساسية الكولستيرول الكليّ ، الدهون الثلاثية والدهون منخفضة الكثافة ، تزيد بزيادة الفترة الزمنية للتدخين بينما كلآ من المغنزيوم والدهون مرتفعة الكثافة تنخفض بزيادة الفترة الزمنية للتدخين.

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List of Abbreviation

Abbreviation	Full term
HS-CRP	High sensitive c-reactive protein
TC	Total cholesterol
BMI	Body mass index
TG	Triglyceride
CRP	C-reactive protein
ATP	Adenine triphosphate
HDL-C	High density lipoprotein lipase cholesterol
Kg	Kilogram
LDL-C	Low density lipoprotein lipase cholesterol
Mg^{2+}	Magnesium
CV	Cardiovascular
Mg/dL	Milligram per deciliter
Mg/L	Milligram per liter
CDC	Center for disease control
AHA	American heart association
PCT	Proximal convoluted tubule
PTH	Parathyroid hormone
VLDL	Very low density lipoprotein
SLE	Systemic loupes erythromatosis
WHO	World health organization

Chapter one

Introduction, Rationale Objectives

1.Introduction, Rationale and Objectives:

1.1Introduction:

Smoking is the inhalation of the smoke of burned tobacco that may occur occasionally or habitually as a consequence of a physical addiction to nicotine **(Leone A *et al.*, 2010).** Cigarette smoking is a classical and a major risk factor in the development of several diseases with an inflammatory component including cardiovascular disease and chronic obstructive pulmonary disease **(Tonstad *et al.*, 2009)**, Serum high sensitive c-reactive protein, the main acute phase protein, is a sensitive marker for systemic inflammation in humans. It is produced mainly by the liver and to some extent by the adipose tissue in response to proinflammatory cytokine induced by inflammatory stimuli **(Masood A *et al.*, 2011).** Cigarette smoking is a classical and a major risk factor for development of inflammatory condition which can be assessed by serum high sensitive C reactive protein (HS-CRP) level **(Ambrose JA and Barua RS, 2004)**. a positive association between smoking status and elevated CRP in adolescents, and in particular among heavier past-month smokers**(Gray-Donald *et al.*,2008).**

Dyslipidemia among smokers indicates greater risk of atherogenic disorder, which may be higher as the number of cigarettes and duration of smoking increases **(Ega, 2016)**. Magnesium is the second most abundant intracellular bivalent cation, This cation plays an important role in central nervous system, The magnesium misbalances are involved in various pathological states such as attention deficit hyperactivity disorders, ischemic brain injury, seizures and others, There are a lot of magnesium dependent enzymes, increase of intracellular and extracellular magnesium concentration can reduce the development of nicotine addiction and tobacco smoking**(Nechifor., 2012).**

1.2Rationale:

Every hundred of thousand around the world die from disease caused by smoking cigarette. Number of researches indicated that smoking has numerous immediate health effects on the liver, respiratory, cardiovascular, gastrointestinal, immune and metabolic system. Lung cancer, other cancer, heart disease, and stroke typically do not occur until years after person's first cigarette.

Many study have shown the plasma levels of High sensitive c-reactive protein, Total cholesterol, Triglyceride and LDL-C, HDL-C and magnesium associated with cardiovascular disease.

In the Sudan there was no clear studies about the relationship between atherogenic marker in smokers, therefore This study was conducted to assess the levels of plasma HS-CRP, Total cholesterol, Triglyceride, LDL-c, HDL-c and magnesium among smokers.

1.3 Objectives:

1.3.1 General objective:

To study the association of smoking with the plasma levels of high sensitivity C-reactive protein, lipid profile and magnesium among Sudanese smoker's population.

1.3.2 Specific objectives:

1-To estimate and compare means of plasma levels HS-CRP, lipid profile and magnesium in smoker's groups versus normal levels.
2-To compare between the study variables (duration, number of cigarette/day and, exercise) and the levels of plasma HS-CRP, lipid profile and magnesium in smoker.
3-To correlate between the (BMI) and the levels of plasma HS-CRP and HDL-c.
4-To correlate between the plasma HS-CRP, T.C, triglyceride, HDL-C, LDL-C, and magnesium levels in smoker.

Chapter two

Literature review

2. Literature Review

2.1 Smoking:

Smoking is the inhalation of the smoke of burned tobacco that may occur occasionally or habitually as a consequence of a physical addiction to nicotine **(Leone A *et al.*, 2010)**. Person, who smoked at least 100 cigarettes in his entire life is known as current smoker **(Ryan H *et al.*, 2012)**. More than 4000 compounds have been identified in tobacco smoke **(Nnorom IC et al., 2005).** Among them, major components are nicotine, tar, carbon monoxide and certain other poisonous substances like hydrogen cyanide, nitrogen oxide and ammonia etc. **(Chalouhi N, *et.al* 2012).** Nicotine is a natural alkaloid which is obtained from the dried leaves and stems of tobacco plants. It is considered as the main component of cigarette which causes addiction **(Benowitz NL.,2010)**. About 6 to 18 mg of nicotine is present per gram of cigarette. At the time of smoking, from each cigarette about1.6 mg of nicotine is delivered to air, which is very dangerous for passive smokers **(Zbancioc G *et al.*,2012).** Prevalence of smoker in the world is increasing day by day According to WHO, current prevalence of smoker in the world is about 22.20% and in Bangladesh current prevalence of smoker is about 23% **(WHO.,2011).**

2.1.1 History of smoking:

The history of tobacco smoking dates back to as early as 5000 BC in shamanistic rituals. Many ancient civilizations, such as the Babylonians, Indians and Chinese, burnt incense as a part of religious rituals, as did the Israelites and the later Catholic and Orthodox Christian churches. Smoking in the Americas probably had its origins in the incense-burning ceremonies of shamans but was later adopted for pleasure, or as a social tool. The smoking of tobacco, as well as various hallucinogenic drugs

was used to achieve trances and to come into contact with the spirit world **(Gately *et al.*, 2001).**

2.1.2 Physical and biochemical properties of smoking:

Conventially, cigarette smoke is divided into two phases: a tar phase and a gas phase. The tar or particulate phase is defined as material that trapped when the smoke stream is passed through the Cambridge glass fiber filter that retains 99.9% of all particulate material with a size>0.1µm. The gas phase is the material that passes through the filter. The particulate (tar) phase of cigarette smoke contain> 10^{17} free radicals/g, and the gas phase contain>10^{15} free radicals/puff. The radical associated with the tar phase are long- lived (hours to months), where as the radicals associated with gas phase have a shorter life span (seconds). Cigarette smoke that is drawn through the tobacco into an active smoker's mouth is known as mainstream smoke. Side stream cigarette smoke emitted from the burning end of cigarette. Mainstream cigarette smoke comprises 8% of tar and 92%of gaseous component **(Tylor *et al.*, 1992).** Environmental tobacco smoke result from the combination of side stream smoke (85%) and small fraction of exhaled mainstream smoke(15%)from smokers **(Glantz.,1991).** Side stream cigarette smoke contain a relatively higher concentration of the toxic gaseous component than main stream cigarettesmoke of the entire known constituent, nicotine, a component of the tar phase, is the addictive substance of cigarette smoke **(Powell.,1998).**

2.1.3 Epidemiology of smoking:

According to the World Health Organization (WHO), smoking is currently responsible for approximately 3.5 million deaths worldwide each year. Smoking is the leading preventable cause of death in the United States, and it kills more than 400,000 U.S. citizens each year. The World Health Organization predicts that by 2020, the worldwide death toll from smoking will reach 10 million each year,

causing nearly 18 percent of all deaths in the developed world **(Jahan and Akhter., 2015).**

2.1.4 Disease associated with smoking:

Smoking accelerates atherosclerosis in different arteries **(Venn A and Britton J. ,2007).** It is well known that, active smoking causes micro vascular complications, acute myocardial infarction, stroke, a range of cancers and sudden death **(Benowitz NL.,and Gourlay SG,1997)**. Cigarette smoking is a prominent risk factor for coronary artery disease, atherosclerosis and peripheral vascular disorders.**(Craig WY *et al.*, 1989).** Passive smokers may also suffer from these diseases **(Schroeder SA, 2013).** Smoking is one of the most potent and prevalent addictive habits, influencing behavior of human beings. Smoking is now increasing rapidly throughout the developing world and is one of the biggest threats to current and future world health. Nearly 20% of all coronary heart disease deaths can be attributed to smoking **(Singh., 2016)** Cigarette smoking is a prominent risk factor for coronary artery disease, atherosclerosis and peripheral vascular disorders.**(Gossett LK, *et al,* 2009)**.

2.2 High sensitive c-reactive protein:

Serum hs-CRP, the main acute phase protein, is a sensitive marker for systemic inflammation in humans. It is produced mainly by the liver and to some extent by the adipose tissue in response to" proinflammatory cytokine induced by inflammatory stimuli. **(Masood A *et al.*, 2011)**. Is an annular ring-shaped, pentameric protein found in the blood plasma, the levels of which rise in response to inflammation (i.e., C-reactive protein is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion from macrophages 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).**

2.2.1Structure of c-reactive protein:

C - reactive protein (CRP) is a member of the hepatic pentraxin family of proteins, a group so named because they are composed of five identical subunits. Each subunit is composed of 206 amino acids with molecular weight of 23027 Daltons arranged around a central pore as shown in Figure (2.1). **(Danesh J *et al.*, 2000)**

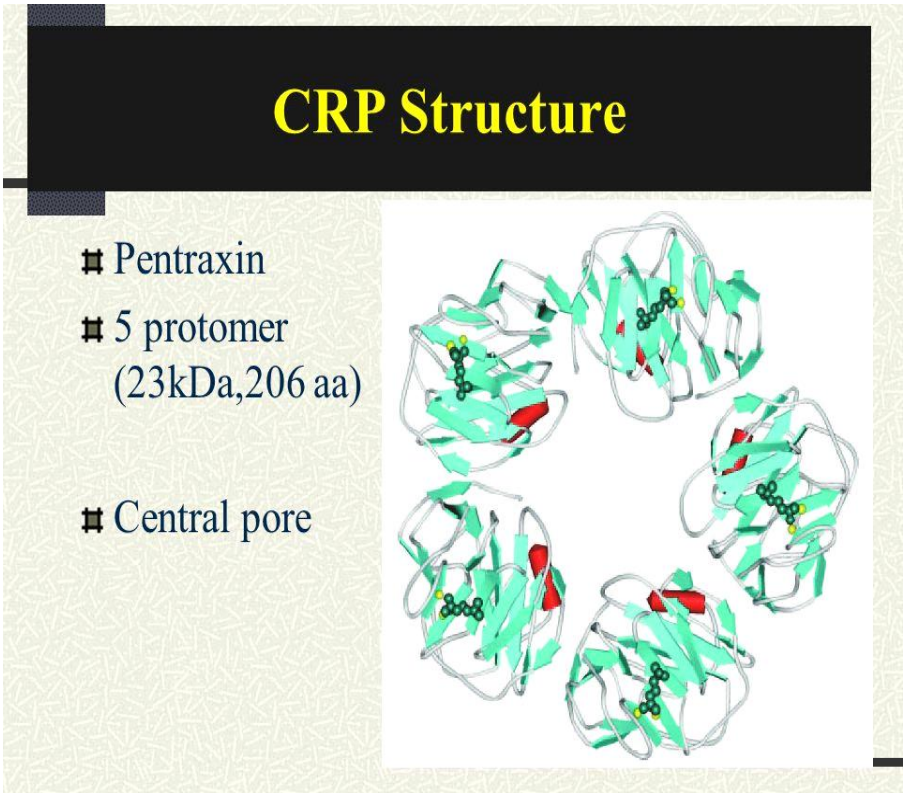

Figure (2.1). structure of CRP protein **(Ridker PM and Rifai N, 2006)**

2.2.2 History of c-reactive protein:

CRP is an acute phase plasma protein, it was discovered in 1930 William Tillet and Thomas Francis define CRP as a substance present in the serum of patients with acute inflammation and reacted with the 'C' polysaccharide of pneumococcus **(Albert MA *et al.*, 2001).**

2.2.3 C-reactive protein and atherosclerosis:

atherosclerosis is an inflammatory process, several markers of inflammation have been evaluated for this purpose. Among them, high-sensitive C-reactive protein (hs-CRP) has emerged as the most important CV risk marker. More than a simple marker of inflammation, hs-CRP may influence vascular vulnerability directly through several mechanisms including, enhanced expression of adhesive molecules, reduced nitric oxide, increased expression of endothelial PAI-1 and altered LDL uptake by macrophages. A scientific statement issued by Centre for Disease Control (CDC) and American Heart Association (AHA) has mentioned hs-CRP as the only inflammatory marker that can be used for risk prediction both for primary and secondary prevention of cardiovascular events **(Karadeniz *et al.*, 2010).**

2.2.4 Relationship between c-reactive protein level and training or physical activity:

Elevated plasma levels of C-reactive protein have been associated with an increased risk of coronary heart disease, ischemic stroke, peripheral artery disease, hypertension, and any cardiovascular disease, Regular physical activity and good cardiorespiratory fitness have been associated with a reduced risk of coronary heart disease, ischemic stroke, and premature cardiovascular and total mortality in people who have no prior cardiovascular disease. Exercise training has also been shown to reduce cardiac and total mortality in patients with coronary heart disease and to be

effective in the treatment of peripheral artery disease. Moreover, exercise has been found to decrease body adiposity.**(Lakka *et al.*, 2018).**

2.2.5 C-reactive protein and smoking:

The association between smoking and inflammation has been examined mainly in the context of cardiovascular disease. It was demonstrated that cigarette tobacco smoke causes direct vascular injury, subsequently leading to an immunological response. These immunological responses involve the release of potent cytokines that stimulate hepatic synthesis of acute phase reactants, proteins that are thought to be anti-infective, anti-proliferative, and pro-coagulative**.(Chung *et al.*, 2014).**

2.3 Magnesium:

Magnesium (Mg^{2+}) is the fourth most abundant cation in the body and second most abundant intracellular ion, the role of Mg^{2+} in the body is widespread. It is an essential cofactor of more than 300 enzymes, including those important in glycolysis, transcellular ion transport, neuromuscular transmission, synthesis of carbohydrates, proteins, lipids, and nucleic acids, and release of and response to certain hormones.**(Bishop *et al.*, 2010),** It also has important role in cell cycle, mitochondrial integrity, modulating ion transport **(Romani., 2011).** The binding of magnesium with important intracellular anionic –ligand especially ATP and the competition with calcium for binding sites on protein membranes are the most important role of magnesium that help to perform those function **(Swaminathan.,2003).** It is also important for normal neurological and muscular function, and cardiac excitability. A study done in human volunteers showed that consuming magnesium deficient diet resulted in negative magnesium balance but did not affect the serum magnesium concentration. This also affected the calcium, potassium and cholesterol metabolism **(Nielsen., 2004).**

2.3.1 Body content and distribution of magnesium:

Adult human body contains approximately 1mol (21-28g) Mg. Of total magnesium content in human body, less than 1% is found in serum and red blood cells. About 53% that is one half of the magnesium is found between bones, 27% in intracellular compartment of muscles, and 19% in soft tissues, 0.5%in erythrocyte and 0.3% in serum **(Fawcett *et al.*,1999)**, It stays in equilibrium with ionized magnesium of extra cellular fluid and antagonizes calcium during muscle contraction **(de Baaij *et al.*, 2012),** The availability of magnesium in bone decreases with age and therefore might not be completely available when there is magnesium deficiency **(Maguire and Cowan 2002)**, Serum magnesium is present in three different states; free, complexes to anions and bound to protein, Approximately 62% of the plasma magnesium is found circulating in ionized state, approximately 33% are protein bound mostly to albumin and approximately 5% are complexes to anions, citrates and phosphate **(Elin., 1987),** However ionized magnesium is the one which is most involved in biological activity, The reference value for magnesium concentration in blood plasma ranges from 0.65 to 1.05 mmol/l for adults **(Saris *et al.*, 2000)** and for ionized magnesium reference value ranges from 0.53 to 0.67 mmol/L in normal healthy people **(Altura *et al.*, 1991).**

2.3.2 Magnesium intake:

Magnesium concentration depends on the magnesium intake from food and drinking water, Whole seeds, unmilled grains, green leafy vegetables (rich in magnesium containing chlorophyll) legumes and nuts are the most important sources of dietary magnesium, Meat, fish, fruits are also good sources of magnesium. Drinking water especially hard water is also one of the sources of magnesium which might account for almost 10% of daily magnesium intake, the absorption of magnesium is influenced by various dietary factors either promoting or inhibiting the absorption,

Absorption of magnesium can be inhibited by phytate, fibre, alcohol or excess of calcium and phosphate. Processing and refining of food leads to loss of magnesium content in food, the effect of vitamin D in magnesium absorption is still unclear, some studies have shown that vitamin D and its active metabolites increases intestinal magnesium absorption in normal human beings and also in patients with chronic renal failure **(Shils *et al.*, 2006),** The normal serum magnesium concentration or (Mg2+) ranges between 0.75 and 0.95mmol/l **(Weisinger and Bellorin-Font 1998).**

2.3.3 Magnesium homeostasis and regulation:

Magnesium homeostasis is maintained by intestine, the bone and the kidney, In brief magnesium is absorbed through gut, stored in bones and excreted through kidney if excess, Intestinal absorption of magnesium was inversely related to magnesium intake in a healthy volunteer which ranged from 65% at low intake and 11% at high intake **(Fine *et al.*,1991),** Two pathways that is paracellular and transcellular pathways are involved in the absorption of Mg^{2+}, Paracellular pathway which is a passive mechanism absorbs Mg^{2+} through small spaces between epithelial, The transcellular pathway involves movement of Mg^{2+} to the blood through the interior of epithelial cell, Around 30 to 40% of normal dietary intake of magnesium is absorbed through intestine, Jejunum and ileum are the important sites where magnesium absorption takes place, After 1 hour of ingestion the absorption begins and continues for 2 to 8 hours, After 12 hours the ingested material reaches large bowel in human where little or no absorption takes place **(de Baaij et al., 2012)**, regulation of body Mg^{2+} is controlled largely by the kidney, which can reabsorb Mg^{2+} in deficiency states or readily excrete excess Mg^{2+} in overload states, Of the non-protein-bound Mg^{2+} that gets filtered by the glomerulus, 25%–30% is reabsorbed by the proximal convoluted tubule (PCT), unlike Na^{+} in which 60%–

75% is absorbed in the PCT, Henle's loop is the major renal regulatory site, where 50%–60% of filtered Mg^{2+} is reabsorbed in the ascending limb, In addition, 2%–5% is reabsorbed in the distal convoluted tubule, The renal threshold for Mg^{2+} is approximately 0.60–0.85 mmol/L (1.46–2.07 mg/dL), Because this is close to normal serum concentration, slight excesses of Mg^{2+} in serum are rapidly excreted by the kidneys, Normally, only about 6% of filtered Mg^{2+} is excreted in the urine per day, Mg^{2+} regulation appears to be related to that of $Ca^{2+}$and Na^{+}, Parathyroid hormone (PTH) increases the renal reabsorption of Mg^{2+} and enhances the absorption of Mg^{2+} in the intestine, However, changes in ionized Ca^{2+} have a far greater effect on PTH secretion, Aldosterone and thyroxine apparently have the opposite effect of PTH in the kidney, increasing the renal excretion of Mg^{2+}**(Bishop *et al.*, 2010).**

2.3.4 Hypomagnesia:

The level of magnesium in our body might not always be the same., Hypomagnesia indicates depletion of body magnesium. It is defined as hypomagnesia when the serum magnesium is less than 1.8mg/dl (<0.74mmol/l). Most of the cases of hypomagnesia are asymptomatic., Symptomatic cases are seen only when serum magnesium falls below 1.2mg/dl **(Assadi.,2010)**, Magnesium deficiency or hypomagnesia can occur due to various reasons and mechanisms, some of the reasons for magnesium deficiency are redistribution of magnesium, reduction in dietary intake and intestinal absorption, renal loss, endocrine causes, diabetes mellitus, alcohol, drugs **(Swaminathan., 2003).**

2.3.5 Hypermagnsia:

Hypermagnesia, the excess of magnesium in body may be the result of high intake of magnesium salt or magnesium containing drugs which is mostly seen in people with renal failure or reduced renal function. Occurrence of hyper magnesia is very

rare but it may result in various neuromuscular, cardiovascular manifestation and hypocalcaemia. Higher level of magnesium also leads to cardio toxicity **(Swaminathan 2003)**. Hypermagnesia has been associated with several endocrine disorders, Thyroxine and growth hormone cause a decrease in tubular reabsorption of Mg^{2+}.**(Bishop *et al.*, 2010)**, Chronic kidney disease or end stage kidney disease is the only strong clinical predictor for hyper magnesia and net positive magnesium balance. Dialysis patients have higher magnesium level **(Spiegel 2011).**

2.3.6 Magnesium and Smoking:

Smoking causes magnesium deficiency due to decreased supply (lesser appetite) and reduced absorption caused by disturbances in the digestive system functions. Minerals disturbances may lead to sever and even life- threatening metabolic abnormalities such as coronary heart disease, liver disease, lung infection, kidney failure, and disorders of endocrine system.**(Ali *et al.*, 2013)**

2.4 Lipids:

Is heterogeneous group of fat and fat like substances characterized by using water insoluble and soluble in non-polar solvents such as alcohol ,ether and chloroform **(Burits CA, 2008)**,The most important roles of lipid are serving as hormones ,serving as energy source , aiding in digestion , components of cell membrane and many cell structures , provide stability of cell membrane**(Bishop *et al.*, 2010).**

2.4.1 Classification of plasma Lipids:

2.4.1.1 Triglycerides:

Triglycerides constitute 95% of tissue storage fat and are the predominant form of glyceryl esters found in plasma, the fatty acid residues found in monoglycerides, diglycerides, or triglycerides vary considerably and usually include different combinations of long-chain fatty acids, in general, triglycerides from plant sources, such as corn, sunflower, and safflower, tend to be enriched in unsaturated fatty acids

and are liquid oils at room temperature, triglycerides from animals, especially ruminants, tend to have saturated acids and are solids at room temperature, triglycerides are the main metabolic fuel carried by chylomicrons; they are delivered to the liver and peripherals after they have been hydrolyzed to fatty acids by lipases. **(Burits *et al.*, 2008)**

2.4.1.2 Lipid Cholesterol:

is found a most exclusively in animals and is a key membrane component of all cells **(Burits *et al.*, 2008)**, Cholesterol is an unsaturated steroid alcohol containing four rings (A, B, C, and D), and it has a single C-H side chain tail similar to a fatty acid in its physical properties, the only hydrophilic part of cholesterol is the hydroxyl group in the A-ring. Cholesterol is, therefore, also an amphipathic lipid and is found on the surface of lipid layers along with phospholipids. Cholesterol is oriented in lipid layers so that the four rings and the side chain tail are buried in the membrane in a parallel orientation to the fatty acid acyl chains on adjacent phospholipid molecules. The polar hydroxyl group on the cholesterol A-ring faces outward, away from the lipid layer, allowing it to interact with water by noncovalent hydrogen bonding. Cholesterol can also exist in an esterified form called cholesteryl ester, with the hydroxyl group conjugated by an ester bond to a fatty acid, in the same way as in triglycerides. In contrast to free cholesterol, there are no polar groups on cholesteryl esters, making them very hydrophobic. Cholesterol is almost exclusively synthesized by animals, but plants do contain other sterols similar in structure to cholesterol. **(Bishop *et al.*, 2010).**

2.4.1.3 Chylomicron:

Chylomicrons, which contain apo B-48, are the largest and the least dense of the lipoprotein particles, having diameters as large as 1200 nm, Because of their large size, they reflect light and account for the turbidity of postprandial plasma,

Chylomicrons are produced by the intestine, where they are packaged with absorbed dietary lipids. Once they enter the circulation, triglycerides and cholesteryl esters in chylomicrons are rapidly hydrolyzed by lipases and, within a few hours, they are transformed into chylomicron remnant particles, which are recognized by proteoglycans and remnant receptors in the liver, facilitating their uptake.The principal role of chylomicrons is the delivery of dietary lipids to hepatic and peripheral cells**(Bishop *et al.*, 2010).**

2.4.1.4 Very Low Density Lipoproteins:

VLDL is produced by the liver and contains apo B-100, apo E, and apo Cs; like chylomicrons, they are also rich in triglycerides, They are the major carriers of endogenous (hepatic-derived) triglycerides and transfer triglycerides from the liver to peripheral tissue Like chylomicrons, they also reflect light and account for most of the turbidity observed in fasting hyperlipidemic plasma specimens, although they do not form a creamy top layer like chylomicrons, because they are smaller and less buoyant, Excess dietary intake of carbohydrate, saturated fatty acids, and trans fatty acids enhances the hepatic synthesis of triglycerides, which in turn increases VLDL production **(Bishop *et al.*, 2010)**.

2.4.1.5 Low-Density Lipoproteins:

LDL primarily contains apo B-100 and is more cholesterol rich than other apo B–containing lipoproteins They form as a consequence of the lipolysis of VLDL. LDL is readily taken up by cells via the LDL receptor in the liver and peripheral cells , because LDL particles are significantly smaller than VLDL particles and chylomicrons, they can infiltrate into the extracellular space of the vessel wall, where they can be oxidized and taken up by macrophages through various scavenger receptors, Macrophages that take up too much lipid become filled with intracellular lipid drops and turn into foam cells, which is the predominant cell type of fatty

streaks, an early precursor of atherosclerotic plaques. LDL particles increase the risk of atherosclerotic cardiovascular events **(Bishop et al.,2010).**

2.4.1.6 High-Density Lipoproteins:

HDL the smallest and densest lipoprotein particle, is synthesized by both the liver and intestine, HDL can exist as either disk-shaped particles or, more commonly, spherical particles. Discoidal HDL typically contains two molecules of apo A-I, which form a ring around a central lipid bilayer of phospholipid and cholesterol, Discoidal HDL is believed to represent nascent or newly secreted HDL and is the most active form in removing excess cholesterol from peripheral cells. The ability of HDL to remove cholesterol from cells, called reverse cholesterol transport, is one of the main mechanisms proposed to explain the antiatherogenic property of HDL. There are two major types of spherical HDL based on density differences: HDL2 and HDL3, HDL2 particles are larger in size and richer in lipid than HDL3 and may reflect better efficiency in delivering lipids to the liver. **(Bishop *et al.*, 2010).**

2.4.2 Body Mass Index:

Mass Index is a number calculated from a person's weight and height. BMI is a fairly reliable indicator of body fatness for most people. By WHO criteria, based on the international classification of adults, a person with a BMI between 18.5 and 25 kg/m2 is considered as healthy. A person with a BMI over 25 kg/m2 but less than 30kg/m2 is considered overweight and a person with BMI over 30kg/m2 is considered obese **(Donoghue., 1985).**

2.4.3 Lipid profile and smoking:

Nicotine is one of the toxins present in tobacco smoke, it is found to have effect on person’s catecholamine & cortisol secretion, Elevated catecholamine and cortisol can alter carbohydrate and lipid metabolism in such person. Alteration in lipid metabolism may lead to dyslipidemia changes which may become predisposing

factor for atherosclerosis and ischemic heart disease leading to increased morbidity and mortality in smokers**(Sonagra *et al.*, 2017).**

Classification of total cholesterol, LDL-c and LDL-c(mg/dl): (Burits CA., *et al*)

Total cholesterol	< 200	desirable
	200 – 239	Borderline high
	≥ 240	high
LDL-c	< 100	Optimum
	100 – 129	Near optimum
	130 – 159	Borderline high
	160 – 189	High
	≥ 190	Very high
HDL-c	< 40	low
	≥ 60	high

Chapter three
Materials and Methods

3.Materials and methods

3.1. Study design

Descriptive cross-sectional study.

3.2 Study Area and time

This study carried out in Khartoum state, during the period of April to October 2018.

3.3 Inclusion criteria

Specimens were collected from cigarette smoker's people (plasma specimens collected from these smokers)

3.4 Exclusion criteria

Persons with chronic infection, coronary heart disease, surgery, neoplastic proliferation, SLE ,hypertension, diabetes mellitus, alcoholism, liver, bone or renal diseases or any other major illness were excluded from the study. Subjects who are on medications which can affect plasma HS-CRP, magnesium and lipid profile levels were also excluded from study.

3.5 Ethical Considerations

Study was approved from local ethical committee of the Sudan University of Science and Technology; verbal informed consent was obtained from all participants after informed by the aims of the study.

3.6 Data collection and Samples

direct interviewing of patients using standardized questionnaire to collect the data, concerning determination of plasma high sensitive c-reactive protein, magnesium and lipid profile levels. Samples were collected by using dry, plastic syringes, tourniquet was used to make the veins more prominent, fasting blood samples (5ml) was collected in lithium heparin containers from each volunteer under septic

condition, then they were centrifuged at 4000 rpm to obtain the plasma samples, and stored in -20° until the analyzed.

3.7 Estimation of plasma HS-CRP

3.7.1 Principle of method:

plasma c-reactive protein (CRP) causes agglutination of latex particles coated with anti-human c-reactive protein. The agglutination of the latex particles is proportional and can be measured by turbidymetric **(price CP *et al.*,1987)**

3.7.2 Procedure: Appendix (III)

3.8 Estimation of plasma magnesium

3.8.1 Principle of method:

Magnesium forms colored complex when reacts with magon sulfonate in alkaline solution. The intensity of the color formed is proportional to the magnesium concentration in the sample **(Farrell EC,1984).**

3.8.2 Procedure: Appendix(IV)

3.9 Estimation of plasma total cholesterol

3.9.1 Principle of method:

Ester cholesterol hydrolyzed in present of cholesterol esterase to free fatty acid and free cholesterol which oxidized by atmospheric oxygen in presence of cholesterol oxidize to cholestene-3,1 and hydrogen peroxide, which converted by peroxidase to H2O and oxygen then oxygen accepted by para amino phenazone in presence of phenol to produce quinoninmine pink color measured by spectrophotometry. **(Allain *et al.*, 1974).**

3.9.2 Procedure: Appendix (V)

3.10 Estimation of Triglycerides:

3.10.1 Principle of method:

Triglycerides hydrolyzed enzymatically in the presence of lipase to 3fatty acid and glycerol, which phosphorylated in the presence of ATP and glycerol kinase to glycerol-3-phosphate that oxidized in presence of glycerol-3-phospate oxidase to dihydroxyacetone phosphate and hydrogen peroxide which converted by peroxidase to H2O and oxygen then oxygen accepted by para-amino phenazone in presence of phenol to produce quinoninmine pink color measured by spectrophotometry. **(Fossati and Prencipe., 1982).**

3.10.2 Procedure: Appendix (VI)

3.11 Estimation of high density lipoprotein cholesterol (HDL-c):

3.11.1 Principle of method:

Very low density lipoproteins, chylomicrons and low density lipoproteins in the sample precipitate with phosphotungstate and magnesium ions, after centrifugation the supernatant contains high density lipoproteins which measured by cholesterol oxidase method spectrophotometrically. **(Burstein *et al.*, 1980).**

3.11.2 Procedure: Appendix(VII)

3.12 Calculation of low density lipoprotein cholesterol (LDL-c):

LDL-c calculated from Fried-Wald's equation:

LDL-c = Total cholesterol – HDL-c –Triglyceride\5. **(Bishop *et al*.,2010)**

3.13 BMI calculation:

BMI obtained by calculation according to formula:

weight(kg) ÷ height (m^2) (Who ,2000)

3.14 Quality control:

To ensure adequate quality control, to verify the performance of measurement procedures were monitored by use biochemistry control serum normal level I and control serum abnormal level II.

3.15 statistical analysis:

The data was analyzed using statistical package of social science (SPSS) version 16. computer program using one sample t.test, independent sample t.test, one way a nova and Pearson correlation, results was expressed as (mean ± SD), and significance difference was considering as (P-value <0.05).

Table (3.1) Reference rang of Hs-crp: **(Bishop *et al.*, 2010)**

Hs-crp Reference value	Risk to CVD
< 1 mg/l	Low risk
1 – 3 mg/l	Moderate risk
>3 mg/l	High risk

Table (3.2) Reference rang of Mg^{2+}: **(Burits, 2008)**

Analyte	Reference value
Mg^{2+}	1.7 – 2.4 mg/dl

Table (3.5) Reference rang of lipid profile: **(Bishop *et al.*, 2010)**

Analyte	Reference value
T. cholesterol	140 – 200 mg/dl
Triglyceride	60 – 150 mg/dl
HDL-c	40 – 75 mg/dl
LDL-c	50 – 130 mg/dl

Chapter four

Results

4.Result

The study was conducted on 80 subjects healthy smokers. samples were collected from non-diabetics no hypertension person, and participants average age is (34.0± 11.6)years ,average BMI (22.9 ± 3.9) kg/m^2 to evaluate the plasma levels of high sensitive c-reactive protein ,lipid profile and magnesium of study subjects.

Figure (4.1) distribution of study population according to the age grouping by year.

show distribution of smokers according to the age, out of 80 smokers 42.5% was (19 – 30), 31.25% was (31 – 40) and 26.25% was (more than 40).

Table (4.1) means of study variables and parameters among study population.

Show means ± SD of parameters and variables, (age, BMI, duration, HS-CRP, Mg, TC, HDL-C, and LDL-C)

Table (4.2) Frequency and percentage of parameters.

Frequency analysis of High sensitive c-reactive protein, Total cholesterol, High density lipoprotein and Low density lipoprotein.

Table (4.3) comparison of study parameters with normal range.

Show high sensitive c-reactive protein level in smokers was insignificant increase when compared to normal, Magnesium level was significant increase in smokers group when compared to normal, Total cholesterol, triglyceride, HDL-C and LDL-C levels were significant decrease in smokers when compared to normal values.

Table (4.4) Comparison of study parameters according to the smoking duration.

Show the comparison of mean ± SD values of plasma hs-crp, magnesium, Total cholesterol TG, HDL-C and LDL-C according to smoking duration (0.1 – 10), (more than 10) by year.

Table (4.5) Comparison of study parameters according to number of cigarette/day .

show the comparison of mean ± SD values of plasma hs-crp, Magnesium, Total cholesterol, Triglyceride, HDL-C and LDL-C according to number of cigarette/day (mild, moderate and heavy).

Table (4.6) Comparison of study parameters according to physical activity.

show the comparison of mean ± SD values of plasma hs-crp, Magnesium, Total cholesterol, Triglyceride, HDL and LDL-C according to physical activity (exercise active, exercise inactive).

Figures (4.2), (4.3) correlation between (HS-CRP, HDL-C) levels and BMI. showed personal correlation between BMI (Kg/m^2) in smoker group and plasma levels of hs-crp, HDL-C.

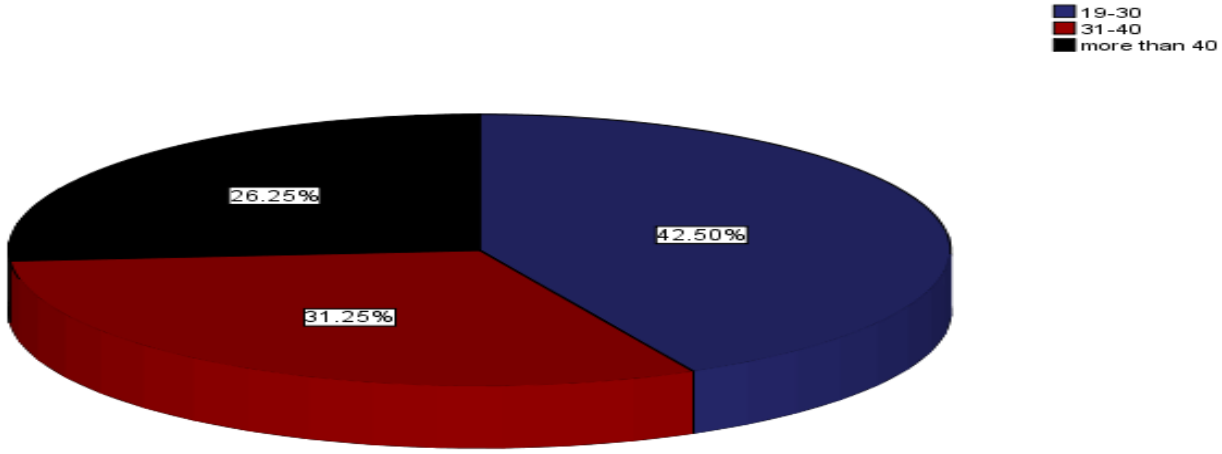

Figure (4.1) distribution of study population according to the age grouping

Table (4.1) means of study variables and parameters among study population

Parameter	Minimum	Maximum	Mean ±SD
Age (year)	19	62	34.0 ± 11.6
BMI (kg/m^2)	17	35	22.9 ± 3.90
Duration (year)	0.1	40.0	14.7 ± 11.2
HS-CRP (mg/L)	0.001	6.6	1.35 ± 1.80
Mg (mg/dL)	1.1	3.2	2.10 ± 0.40
TC (mg/dL)	98	386	164 ± 52.0
TG (mg/dL)	29	452	110 ± 81.0
HDL-C (mg/dL)	13	48	32.2 ± 8.2
LDL-C (mg/dL)	50	301	110 ± 47.1

Table (4.2) Frequency and percentage of HS-CRP, TC, HDL-C and LDL-C

Parameters	Frequency	Percentage (%)
HS-CRP		
Low Risk (< 1.0)	48	60
Moderate Risk (1.0 – 3.0)	16	20.0
High Risk (>3.0)	16	20.0
TC		
Optimal (<200)	66	82.0
Border line high (200 – 239)	9	11.0
High (≥ 240)	5	7.0
HDL-C		
No Risk (≥ 40)	16	20.0
Moderate Risk (35 – 39)	19	23.8
High Risk (< 35)	45	58.2
LDL-C		
Optimal (< 100)	40	50
Near Optimum (100 – 129)	23	28.8
Border Line High (130 – 159)	11	13.7
High (160 – 189)	1	1.3
Very high (≥ 190)	5	6.2
Total	80	100

Table (4.3): Comparison between study variables and reference value among Sudanese smoker.

Parameter	Mean ± SD	R.V	P.value
HS-CRP	1.35 ± 1.8	UP to 1.0	0.08
Mg	2.13 ± 0.45	(1.7 – 2.4)	0.00
TC	164 ± 52.0	(140 – 199)	0.00
TG	110 ± 81.0	(60 – 150)	0.00
HDL-C	32.2 ± 8.2	(40 – 75)	0.00
LDL-C	110 ± 47.0	(50 – 130)	0.00

*One sample T test was used to compared between means

*P value considered significant at level 0.05

Table (4.4) Comparison of study parameters according to duration (year) of smoking.

Variable	0.1 – 10 n = 43	≥ 10 n = 37	p.value
	Mean ± SD		
Plasma HS-CRP (mg/L)	0.92 ± 1.41	1.85± 2.09	0.02*
Plasma (mg/dL)	2.26 ± 0.50	1.97 ± 0.32	0.00**
Plasma T.Cholesterol (mg/dl)	146 ± 30.2	185 ± 64.0	0.00**
Plasma Triglyceride (mg/dL)	83.0 ± 42.4	141 ± 102	0.00**
Plasma HDL-C (mg/dL)	34.0 ± 8.0	30.1 ± 8.1	0.03*
Plasma LDL-C (mg/dL)	95.9 ± 29.1	128 ± 57.4	0.00**

*Independent T test was used to compared between means

*P value considered significant at level 0.05

Table (4.5) Comparison of study parameters according to number of cigarette/day

Variable	Mild* n=25	Moderate* n=27	Heavy* n=28	p.value
	Means ± SD			
Plasma HS-CRP (mg/L)	1.02 ± 1.84	1.09 ± 1.23	1.90 ± 2.1	0.14
Plasma Magnesium (mg/dL)	2.33 ± 0.44	2.05 ± 0.48	2.02 ± 0.36	0.02*
p.T.cholesterol (mg/dL)	156 ± 45.3	159 ± 69.4	177 ± 35.0	0.29
Triglyceride (mg/dL)	76.9 ± 38.9	94.3 ± 57.9	155 ± 106	0.00**
HDL-C (mg/dL)	34.3 ± 9.0	30.3 ± 8.5	32.1 ± 6.9	0.20
LDL-C (mg/dL)	106 ± 45.3	110 ± 58.9	115 ± 35.8	0.78

-mild*(1 - 10 cigarette/day), moderate*(11 – 20 cigarette/day), heavy (< 20).

*One way anova was used to compared between means.

*P value considered significant at level 0.05.

Table (4.6) Comparison of study parameters according to physical activity

Variable	Exercise "YES" n=59	Exercise "NO" n=21	p.value
	Means ± SD		
Hs-crp	1.12 ± 1.7	2.00 ± 1.9	0.05*
Mg^{2+}	2.14 ± 0.37	2.11 ± 0.61	0.830
T.cholesterol	164 ± 58.4	166.9 ± 29.5	0.829
Triglyceride	106 ± 68.5	121.7 ± 110.6	0.451
HDL-C	34.1 ± 7.3	26.7 ± 8.5	0.000**
LDL-C	109.1 ± 52.9	115.3 ± 24.7	0.481

*Independent T test was used to compared between means

*P value considered significant at level 0.05

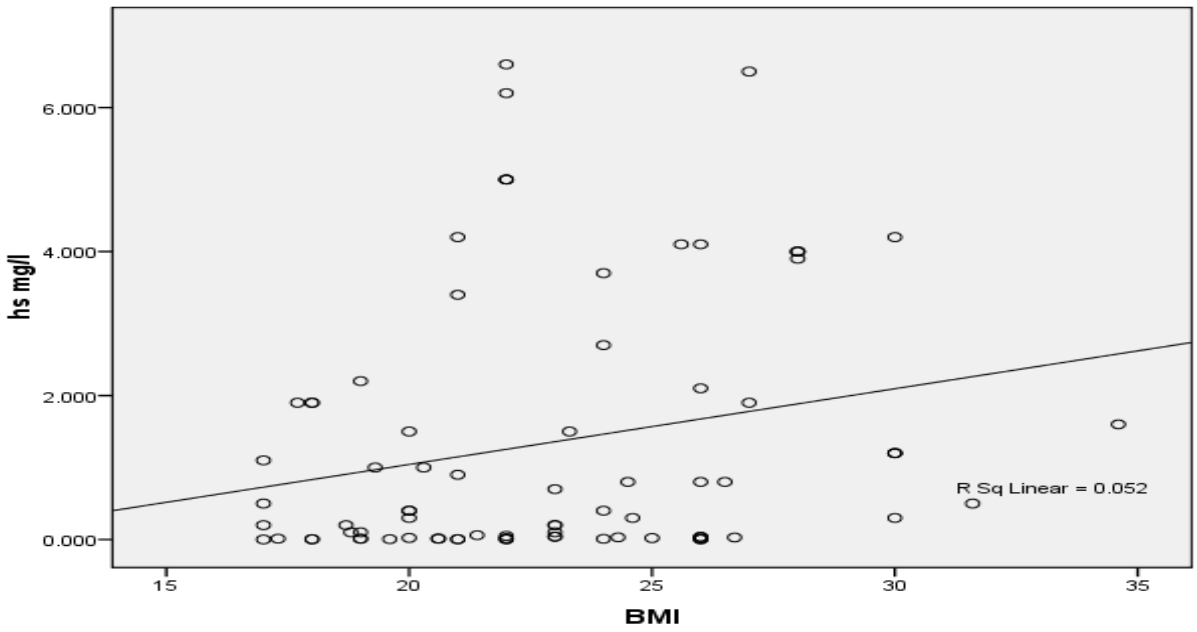

Figure (4.2) scatter shows significant positive correlation (r= 0.229, PV=0.041) between the plasma levels of hs-crp and BMI in smoker .

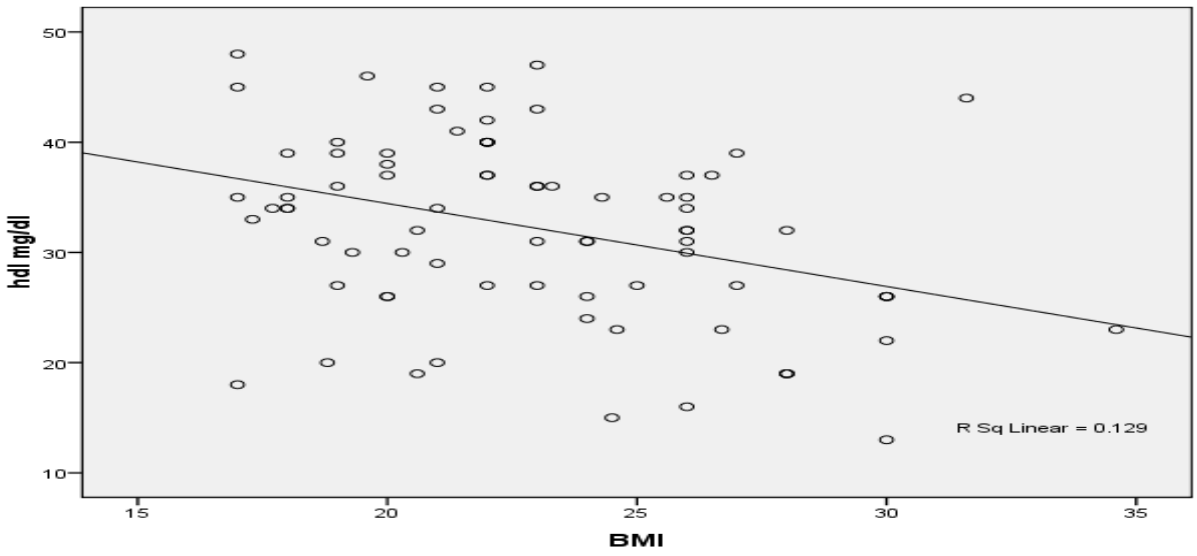

Figure (4.3) scatter shows highly significant negative correlation (r=0.360, PV= 0.001) between the plasma levels of HDL-c and BMI in smokers.

Chapter five

Discussion

Conclusion, Recommendation

5.Discussion, Conclusion and Recommendation:

5.1 Discussion

Cigarette smoking is a classical and a major risk factor in the development of several diseases with an inflammatory component, including cardiovascular disease and chronic obstructive pulmonary disease.**(Tonstad and Cowan, 2009)**, CRP is an acute-phase reactant produced mainly by the hepatocytes in response to inflammatory stimuli. It has been shown to be a sensitive nonspecific biomarker of systematic inflammation. **(Pepys, 2003)**

In the present study showed, the mean of plasma hs-CRP level was insignificantly higher in smokers when compared to the normal value, plasma levels of magnesium total cholesterol, triglyceride, HDL-C and LDL-C were significantly decrease when compared to the normal values. The previous study showed significant increase in plasma levels of HS-CRP, Total cholesterol, LDL-C and significant decrease in magnesium and HDL-C when compared to non-smokers subjects. **(Jahan and Akhter,2015), (Ali *et al.*, 2013) and (Ega, 2016)** this results may be explaining by the environmental condition, diet habit in sudan may effect on the levels of lipid profile when compared to another country or reference values.

study showed, highly significant increase in the means of plasma level of HS-CRP with increase the duration of smoking, this finding were agreement with **(Jahan and Akhter., 2015).** also showed significant decrease in the mean of plasma magnesium with increase of smoking duration, this finding was confirmed by**(Ali *et al.*, 2013).** study also showed highly significant increase in means of the plasma levels of total cholesterol, triglyceride and LDL-C according to duration of smoking, this study was in agree with **(Hassan *et al.*, 2013, Ega., 2016),** that lead to conclusion of inflammatory changes were more pronounced in the subjects who are smoking for a long time.

in this study also found insignificant increase of plasma level HS-CRP with increase number of cigarette stick/day(intensity) this result was in agree with **(Aldaham *et al.*, 2015)**, and significant decrease plasma level magnesium with increase number of cigarette stick/day (intensity) of smoking**,** because cigarette smoking causes decreased supply of magnesium caused due to lesser appetite and reduced absorption due to digestive system disturbances depleted magnesium leads to hypertension and cardiovascular diseases **(Ali *et al.*, 2013).**

In the previous studies showed there were significant positive correlation between the intensity of smoking with Total cholesterol, Triglyceride and LDL-C and significant negative correlation with HDL-c, in this study showed significant positive correlation between the intensity of smoking with the plasma level of triglyceride, insignificant increase with Total cholesterol, LDL-C and insignificant decrease HDL-C, this finding is partially deal with **(Elamin and Osamn, 2016).**

study showed, significant positive correlation between HS-CRP and BMI, this result was agree with **(Ryu *et al.*, 2005, Aldaham *et al.*, 2015**,**Chung *et al.*, 2014),** also found significant negative correlation between HDL-C and BMI, this finding was in agree **(Vol., 2012),** In this study More precisely, central obesity and the presence of visceral adipose tissue might be a key promoter of low-grade chronic inflammation Fat cells produce cytokines, in particular IL-6 that induces the synthesis of CRP by the liver **(Ryu *et al.*, 2005).**

According to physical exercise study showed, the significant decrease in the mean of hs-crp in physically active exercise group when compared with inactive exercise group , This finding may partly explain the effectiveness of regular physical activity in the prevention and treatment of cardiovascular and metabolic diseases**(Lakka *et al.*, 2018).**

5.2 Conclusion:

The study concludes, plasma among smokers have increase in the levels of HS-CRP, total cholesterol and LDL-C with increase duration of smoking where decrease levels of magnesium and HDL-C with duration of smoking.

5.3 Recommendation:

From finding of this study it is recommended:

1.More studies compare the results with more variable such as gender, body mass index, age, exercise, life style and diet habits

2. Further exploration of the effect of smoking on other parameter.

3. further research is needed with large sample size to study other related parameters.

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Appendix (1)

Sudan university of sciences and technology

Faculty of medical laboratory sciences

Department of clinical chemistry

High Sensitive C-reactive Protein, Magnesium and Lipid Profile Among non-diabetic, non-hypertensive Sudanese Smokers (A study in Khartoum State)

QUESTIONAIRE

Serial number:……

Name:……

Age:……

Duration:…….. (years)…….. (months)

weight:……(kg) Height:…….....(m)

BMI:……..(kg/m^2)

No. of cigarettes/day:……

Intensity of smoking: light ( ) moderate ( ) heavy ( )

Other type of smoking: yes ( )…….. No ( )

Other diseases :……

Exercise: yes ( ) NO ( )

Mobile No:……
signature…….

Appendix (II)

COD 13927 1 x 50 mL
STORE AT 2-8°C
Reagents for measurement of CRP concentration Only for *in vitro* use in the clinical laboratory

C-REACTIVE PROTEIN-hs (CRP-hs)

Automated Systems

BioSystems REAGENTS & INSTRUMENTS

CE

C-REACTIVE PROTEIN (CRP-hs)
LATEX-HIGH SENSITIVITY

PRINCIPLE OF THE METHOD

Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with anti-human C-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry[1].

CONTENTS AND COMPOSITION

A. Reagent: 1 x 40 mL. Glycine buffer 0.1 mol/L, sodium azide 0.95 g/L, pH 8.6.
B. Reagent: 1 x 10 mL. Suspension of latex particles coated with anti-human CRP antibodies, sodium azide 0.95 g/L.

STORAGE

Store at 2-8°C.
Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.
Indications of deterioration:
– Reagents: Absorbance of the blank over the limit indicated in "Assay Parameters".

AUXILIARY REAGENTS

S. CRP-hs Standard: For 1 x 5 mL (BioSystems Cod. 31113). Human serum. C-reactive protein concentration is stated on the vial label. The concentration value of the CRP-hs Standard is traceable to the Reference Material ERM-DA472/IFCC (Institute for Reference Materials and Measurements, IRMM).
Human serum used in the preparation of the standard has been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the standard should be handled cautiously as potentially infectious.
Reconstitute with 5.0 mL of distilled water. Stable for 1 month at 2-8°C.
Calibration curve: Prepare dilutions of the CRP-hs Standard using 9 g/L saline as diluent. Multiply the concentration of the CRP-hs Standard by the corresponding factor indicated below to obtain the CRP-hs concentration of the dilutions.

DILUTION	1	2	3	4	5
CRP-hs Standard (µL)	30	60	120	180	240
Saline (µL)	210	180	120	60	—
Factor	0.125	0.25	0.5	0.75	1.0

REAGENT PREPARATION

Working Reagent: Pour the contents of a Reagent B vial into a Reagent A bottle (Note 1). Mix thoroughly. Stable for 60 days at 2-8°C.
Smaller Working Reagent volumes can be prepared by mixing: 1 mL of Reagent B + 4 mL of Reagent A. Shake the Reagent B vial before pipetting.
Reagent open and kept in the refrigerated compartment of the analyzer is stable 1 month.

SAMPLES

Serum collected by standard procedures.
CRP in serum is stable for 7 days at 2-8°C.

REFERENCE VALUES

Serum[2,3]:

	Men
5-13 years	< 1.45 mg/L
14-18 years	< 2.13 mg/L
19-39 years	< 2.68 mg/L
40-49 years	< 4.80 mg/L
50-64 years	< 7.90 mg/L
65-99 years	< 6.80 mg/L

	Women
5-18 years	< 1.90 mg/L
19-49 years	< 3.33 mg/L
50-64 years	< 8.50 mg/L
65-99 years	< 6.60 mg/L

This range is given for orientation only; each laboratory should establish its own reference range.

CALIBRATION

It is recommended to do a reagent blank every day and a calibration at least every 1 month, after reagent lot change or as required by quality control procedures.

ASSAY PARAMETERS

		A25	A15
GENERAL	Test name	CRP-hs	CRP-hs
	Analysis mode	mono. fixed time	mono. fixed time
	Sample type	SER	SER
	Units	mg/l	mg/l
	Reaction type	increasing	increasing
	Turbidimetry test	yes	yes
	Decimals	1	1
	No. of replicates	1	1
	Test name in patient report	-	-
PROCEDURE	Reading	monochromatic	monochromatic
Volumes	Sample	4	4
	Reagent 1	300	300
	Reagent 2	-	-
	Washing	1.2	1.2
	Predilution factor	-	-
	Postdilution factor	2	2
Filters	Main	535	535
	Reference	-	-
Times	Reading 1	30 s	48 s
	Reading 2	330 s	360 s
	Reagent 2	-	-
CALIBRATION	Calibration type	specific	specific
	No. of calibrators	5	5
	Calibrator replicates	3	3
	Blank replicates	3	3
	Calibration curve	increasiong polygonal	increasiong polygonal
OPTIONS	Blank absorbance limit	1.600	1.600
	Kinetic blank limit	-	-
	Linearity limit	-	-

QUALITY CONTROL

It is recommended to use the Protein Control Serum level I (Cod. 31211) and II (Cod. 31212) to verify the performance of the measurement procedure.
Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

The following data were obtained using an A25 analyser. Results are similar with A15. Details on evaluation data are available on request.

– Detection limit: 0.15 mg/L.
– Measurement interval: 0.15-15 mg/L. For higher values dilute sample 1/5 with distilled water and repeat measurement.
– Repeatibility (within run):

Mean concentration	CV	n
2.52 mg/L	1.9 %	20
4.85 mg/L	1.3 %	20

– Reproducibility (run to run):

Mean concentration	CV	n
2.52 mg/L	2.6 %	25
4.85 mg/L	2 %	25

– Trueness: Results obtained with this procedure did not show systematic differences when compared with a reference procedure. Details of the comparison experiments are available on request.
– Zone effect: This method has not zone effect (< 500 mg/L).
– Interferences: Hemoglobin (10 g/L) and lipemia (triglycerides 10 g/L) do not interfere. Bilirubin (>10 mg/dL) and rheumatoid factors (>75 IU/mL) may interfere. Other drugs and substances may interfere[4].

DIAGNOSTIC CHARACTERISTICS

C-Reactive Protein (CRP), which is synthesized in the liver, is one of the the most sensitive acute phase reactants after tissue damage or inflammation. CRP activates the classical complement pathway as a response to the inflammatory reaction.
CRP levels in serum can rise dramatically after myocardial infarction, stress, trauma, infection, inflammation, surgery or neoplastic proliferation. The increase occurs within 24 to 48 hours and the level may be up to 2000 times normal. An elevation can be expected in virtually all diseases involving tissue damages so the finding is nonspecific[5].
Although traditionally used to monitor or detect major inflammatory conditions, elevations of CRP levels within the conventional reference range have been reported in several studies. These studies have shown that high sensitivity CRP (CRP-hs) is of interest in predicting the risk for future cardiovascular events and peripheral vascular diseases. Concentrations greater than 10 mg/L generally have a significant other inflamatory process occurring [6-9].
Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Shake the Reagent B vial gently before pouring its contents into the Reagent A bottle. It is advisable to wash the Reagent B vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.

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Appendix (III)

COD 11805	COD 11505	COD 11506	COD 11539
1 x 50 mL	1 x 200 mL	1 x 500 mL	1 x 1 L
STORE AT 2-8°C			
Reagents for measurement of cholesterol concentration Only for *in vitro* use in the clinical laboratory			

CHOLESTEROL

BioSystems
REAGENTS & INSTRUMENTS

CE

CHOLESTEROL
CHOLESTEROL OXIDASE/PEROXIDASE

PRINCIPLE OF THE 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[1,2].

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{chol. esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \text{4 – Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS

	COD 11805	COD 11505	COD 11506	COD 11539
A. Reagent	1 x 50 mL	1 x 200 mL	1 x 500 mL	1 x 1 L
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Pipes 35 mmol/L, sodium cholate 0.5 mmol/L, phenol 28 mmol/L, cholesterol esterase > 0.2 U/mL, cholesterol oxidase > 0.1 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.0.

S. Cholesterol Standard. Cholesterol 200 mg/dL (5,18 mmol/L). Aqueous primary standard.

STORAGE

Store at 2-8°C.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37°C
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm

SAMPLES

Serum or plasma collected by standard procedures.

Cholesterol is stable for 7 days at 2-8°C. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Cholesterol Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
4. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

The cholesterol concentration in the sample is calculated using the following general formula:

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = C_{\text{Sample}}$$

If the Cholesterol Standard provided has been used to calibrate (Note 2):

$\frac{A_{\text{Sample}}}{A_{\text{Standard}}}$	x 200 = mg/dL cholesterol x 5.18 = mmol/L cholesterol

REFERENCE VALUES

The following uniform cut-off points have been established by the US National Cholesterol Education Program and have also been adopted in many other countries for the evaluation of coronary artery disease risk[3].

Up to 200 mg/dL = 5.2 mmol/L	Desirable
200-239 mg/dL = 5.2-6.21 mmol/L	Borderline High
> 240 mg/dL = > 6.24 mmol/L	High

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.3 mg/dL = 0.008 mmol/L
- Linearity limit: 1000 mg/dL = 26 mmol/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration		
121 mg/dL = 3.13 mmol/L	1.1 %	20
257 mg/dL = 6.66 mmol/L	0.9 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
121 mg/dL = 3.13 mmol/L	1.9 %	25
257 mg/dL= 6.66 mmol/L	1.0 %	25

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides 10 g/L) does not interfere. Bilirubin (>10 mg/dL) and hemoglobin (>5 g/L) may affect the results. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Cholesterol is a steroid of high molecular weight and possesses the cyclopentanophenanthrene skeleton. Dietary cholesterol is partially absorbed and it is also synthesized by the liver and other tissues. Cholesterol is transported in plasma by lipoproteins. It is excreted unchanged into bile or after transformation to bile acids.

Increased total cholesterol values are associated with a progressively escalating risk of atherosclerosis and coronary artery disease[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

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Appendix (IV)

COD 11828 1 x 50 mL	COD 11528 4 x 50 mL	COD 11529 2 x 250 mL
STORE AT 2-8ºC		
Reagents for measurement of triglycerides concentration Only for *in vitro* use in the clinical laboratory		

TRIGLYCERIDES

BioSystems
REAGENTS & INSTRUMENTS

CE

TRIGLYCERIDES
GLYCEROL PHOSPHATE OXIDASE/PEROXIDASE

PRINCIPLE OF THE METHOD

Triglycerides in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1,2].

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{glycerol kinase}} \text{Glycerol} - 3 - P + ADP$$

$$\text{Glycerol} - 3 - P + O_2 \xrightarrow{\text{G-3-P-oxidase}} \text{Dihidroxyacetone} - P + H_2O_2$$

$$2\ H_2O_2 + 4 - \text{Aminoantipyrine} + 4 - \text{Chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

CONTENTS

	COD 11828	COD 11528	COD 11529
A. Reagent	1 x 50 mL	4 x 50 mL	2 x 250 mL
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent: Pipes 45 mmol/L, magnesium chloride 5 mmol/L, 4-chlorophenol 6 mmol/L, lipase > 100 U/mL, glycerol kinase > 1.5 U/mL, glycerol-3-phosphate oxidase > 4 U/mL, peroxidase > 0.8 U/mL,4-aminoantipyrine 0.75 mmol/L, ATP 0.9 mmol/L, pH 7.0.

S. Triglycerides Standard: Glycerol equivalent to 200 mg/dL (2.26 mmol/L) triolein. Aqueous primary standard.

STORAGE

Store at 2-8ºC.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC.
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum or plasma collected by standard procedures.

Triglycerides in serum or plasma are stable for 5 days at 2-8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Triglycerides Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and incubate the tubes for 15 minutes at room temperature (16-25ºC) or for 5 minutes at 37ºC.
4. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

The triglycerides concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

If the Triglycerides Standard provided has been used to calibrate (Note 2):

$\frac{A_{Sample}}{A_{Standard}}$	x 200 = mg/dL triglycerides x 2.26 = mmol/L triglycerides

REFERENCE VALUES

The following uniform cut-off points have been established by the US National Institutes of Health and have also been adopted in many other countries for the evaluation of risk[3].

Up to 150 mg/dL = 1.7 mmol/L	Normal
150-199 mg/dL = 1.70-2.25 mmol/L	Borderline-high
200-499 mg/dL = 2.26-5.64 mmol/L	High
> 500 mg/dL = > 5.65 mmol/L	Very high

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.6 mg/dL = 0.018 mmol/L
- Linearity limit: 600 mg/dL = 6.78 mmol/L. For higher values dilute sample 1/ 4 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration	CV	n
100 mg/dL = 1.13 mmol/L	1.7 %	20
245 mg/dL = 2.77 mmol/L	0.7 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
100 mg/dL = 1.13 mmol/L	2.6 %	25
245 mg/dL = 2.77 mmol/L	1.7 %	25

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (10 g/L) does not interfere. Bilirubin (2.5 mg/dL) may intefere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Triglycerides are esters of glycerol and fatty acids coming from the diet or obtained by synthesis mainly in the liver. Triglycerides are transported in plasma by lipoproteins and used by adipose tissue, muscle and other. Their primary function is to provide energy to the cell.

Elevated serum triglycerides levels can be caused by liver disease, diabetes mellitus, nephrosis, hypothyroididsm, alcoholism, familial hyperlipoproteinemia IV and V, and other[3,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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Appendix (V)

COD 11648 50 mL
STORE AT 2-8°C
Reagents for measurement of HDL cholesterol concentration Only for *in vitro* use in the clinical laboratory

CHOLESTEROL HDL PRECIPITATING REAGENT

CE

BioSystems REAGENTS & INSTRUMENTS

CHOLESTEROL HDL PRECIPITATING REAGENT

PRINCIPLE OF THE METHOD

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample precipitate with phosphotungstate and magnesium ions. The supernatant contains high density lipoproteins (HDL). The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below[1,2].

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{chol. esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + 4 - \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS AND COMPOSITION

A. Reagent: 1 x 50 mL. Phosphotungstate 0.4 mmol/L, magnesium chloride 20 mmol/L.

S. HDL Cholesterol Standard: 1 x 5 mL. Cholesterol 15 mg/dL. Aqueous primary standard.

STORAGE

Store at 2-8°C.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity.
- Standard: Presence of particulate material, turbidity.

ADDITIONAL REAGENTS

These auxiliary reagents are to be used together with the Cholesterol Reagent contained in any of the BioSystems Cholesterol kits (cod. 11805, 11505, 11506, 11539).

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Desktop centrifuge.
- Thermostatic water bath at 37°C.
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum or plasma collected by standard procedures.

HDL cholesterol in serum or plasma is stable for 7 days at 2-8°C. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

Precipitation

1. Pipette into labelled centrifuge tubes (Note 1):

Sample	0.2 mL
Reagent (A) (Cholesterol HDL kit)	0.5 mL

2. Mix thoroughly and let stand for 10 minutes at room temperature.
3. Centrifuge at a minimum of 4000 r.p.m. for 10 minutes.
4. Carefully collect the supernatant (Note 2).

Colorimetry

5. Bring the Reagent (Cholesterol kit) to room temperature.
6. Pipette into labelled test tubes: (Note 3)

	Blank	Standard	Sample
Distilled water	100 µL	—	—
HDL Cholesterol Standard (S)	—	100 µL	—
Sample supernatant	—	—	100 µL
Reagent (A) (Cholesterol kit)	1.0 mL	1.0 mL	1.0 mL

7. Mix thoroughly and incubate the tubes for 30 minutes at room temperature (16-25°C) or for 10 minutes at 37°C.
8. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

The HDL cholesterol concentration in the sample is calculated using the following general formula:

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} \times \text{Sample dilution factor} = C_{\text{Sample}}$$

If the HDL Cholesterol Standard provided has been used to calibrate (Note 4):

$\frac{A_{\text{Sample}}}{A_{\text{Standard}}}$	x 52.5 = mg/dL HDL cholesterol x 1.36 = mmol/L HDL cholesterol

REFERENCE VALUES

HDL cholesterol concentrations vary considerably with age and sex. The following cut-off point has been recommended for identifiying individuals at high risk of coronary artery disease[3].

Up to 35 mg/dL = 0.91 mmol/L	High
> 60 mg/dL = > 1.56 mmol/L	Low

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005 and 18009) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 3.0 mg/dL = 0.078 mmol/L.
- Linearity limit: 150 mg/dL = 3.9 mmol/L.
- Repeatibility (within run):

Mean Concentration	CV	n
30 mg/dL = 0.78 mmol/L	3.3 %	20
55 mg/dL = 1.42 mmol/L	2.0 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
30 mg/dL = 0.78 mmol/L	4.2 %	20
55 mg/dL = 1.42 mmol/L	3.2 %	20

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 4). Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides 10 g/L) does not interfere. Bilirubin (10 mg/dL) and hemoglobin (5 g/L) may intefere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

HDL play an important part in the removal of cholesterol from tissues and its transportation to the liver for removal as bile acids.

Decreased plasma HDL-cholesterol concentrations are positively correlated with the incidence of atherosclerosic diseases, basis of myocardial infarction and cerebrovascular accidents[5,6].

There are several disease states or environmental influences associated with reduced levels of HDL: acute or chronic hepatocellular diseases, intravenous hyperalimentation, severe malnutrition, diabetes, chronic anemia, myeloproliferative disorders, Tangier disease, analphalipopro-teinemia, acute stress, some drugs and smoking[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Sample and Reagent A volumes may be varied as long as the same ratio is maintained.
2. Supernatant must be clear. When supernatant is turbid or the pellet floats, add again 0.5 mL of Reagent A, mix thoroughly and centrifuge. Multiply the obtained concentration by 1.7 (dilution).
3. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
4. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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Appendix (VI)

CE MAGNESIUM

Magnesium Xylidyl

Xylidyl Blue. Colorimetric

Quantitative determination of magnesium
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

Magnesium forms a coloured complex when reacts with Magon sulfonate in alkaline solution.
The intensity of the color formed is proportional to the magnesium concentration in the sample[1].

CLINICAL SIGNIFICANCE

Magnesium is the second more abundant intracellular cation of the human body after potassium, being essential in great number of enzymatic and metabolic processes.
Is a cofactor of all the enzymatic reactions that involve the ATP and comprises of the membrane that maintains the electrical excitability of the muscular and nervous cells.
A low magnesium level is found in malabsortion syndrome, diuretic or aminoglucoside therapy; hyperparathyroidism or diabetic acidosis.
Elevated concentration of magnesium is found in uremia, chronic renal failure, glomerulonephritis, Addisons's disease or intensive anti acid therapy[1,4,5].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R	Xylidyl Blue	0,1 mmol/L
	Thioglycolic acid	0,7 mmol/L
	DMSO	3000 mmol/L
MAGNESIUM CAL	Magnesium aqueous primary standard 2 mg/dL	

PRECAUTIONS

R: H314-Causes severe skin burns and eye damage.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

The reagent and standard are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles, color change and turbidity.
- Blank absorbance (A) at 546 ≥ 1,8.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 546 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment (Note 2).

SAMPLES

- Serum, heparinised plasma[1]:
  Free of hemolysis and separated from cells as rapidly as possible.
  Do not use oxalates or EDTA as anticoagulant.
  Stability: 7 days at 2-8ºC.
- Urine[1]:
  Should be acidified to pH 1 with HCl.
  If urine is cloudy; warm the specimen to 60ºC for 10 min. to dissolve precipitates.
  Dilute the sample 1/10 with distilled water and multiply the result by 10.
  Stability: 3 days at 2-8ºC

PROCEDURE

1. Assay conditions:
   Wavelength: ..... 546 nm
   Cuvette: ..... 1 cm light path
   Temperature: ..... 37ºC / 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette (Note 6):

	Blank	Standard	Sample
R (mL)	1,0	1,0	1,0
Standard (Note 1,3) (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 5 min at room temperature or 3 min a 37ºC.
5. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

$\frac{(A)Sample-(A)Blank}{(A)Standard-(A)Blank}$ x 2 (Standard conc.) = mg/dL magnesium de in the sample

Conversion factors:
mg/dL x 0,412 = mmol/L
0,5 mmol/L = 1,0 mEq/L = 1,22 mg/dL = 12,2 mg/L[1].

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma:
1,6 – 2,5 mg/dL ≅ 0,66 – 1,03 mmol/L
Urine:
24 – 244 mg/24 h ≅ 2 – 21 mEq/L/24 h
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,0052 mg/dL to *linearity limit* of 6 mg/dL. If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	1,99	3,55	1,98	3,41
SD	0,03	0,04	0,09	0,15
CV (%)	1,68	1,14	4,55	4,42

Sensitivity: 1 mg/dL = 0,5536 (A).
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained were the following:
Correlation coefficient (r)[2]: 0,92276
Regression equation: y=1,027x + 0,102
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Haemolysis and anticoagulants other than heparin[1].
A list of drugs and other interfering substances with magnesium determination has been reported by Young et. al[2].

NOTES

1. MAGNESIUM CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
2. It is recommended use disposable material to avoid magnesium contamination. If glassware is used the material should be scrupulously clean with H_2SO_4- $K_2Cr_2O_7$ and then thoroughly rinsed with distilled water and dried before use.
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. It is recommended to use a serum Calibrator.
4. Use clean disposable pipette tips for its dispensation.
5. **SPINREACT has instruction sheets for several automatic analyzers.**

BIBLIOGRAPHY

1. Farrell E C. Magnesium. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1065-1069.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
5. Tietz NW et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref: 1001285	Cont.	R: 2 x 150 mL, CAL: 1 x 5 mL
Ref: 1001286		R: 2 x 50 mL, CAL: 1 x 2 mL

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