High Sensitive C-reactive Protein, Magnesium and Lipid Profile Among non-diabetic, non-hypertensive Sudanese Smokers.

(A study in Khartoum State)

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

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«الآية»

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قال تعالى: (وَبِرَبِّ الْعَرْفَةِ وَخَالِدًا عَلَيْهِ الْغُفْرَانَ) وَبِحَكْمَةِ عَنْهُ

إِنَّ رَبِّي وَالَّذِينَ حَبَثُوا عَلَيْهِ الْغُفْرَانَ قَالُوا لَنْ نُعْمَلَ مَرَّةً ثُمَّ نَمُؤْنَهَا وَنَعْمَلَ وَمَا نُؤْنَهَا وَأَنْعَمْنَا عَلَى الْمُؤْمِنِينَ وَأَنْنَعَمْنَا عَلَى الْمُؤْمِنَاتِ.)

العصر

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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
Acknowledgements

I thank all the thanks first and foremost to my got Allah, who gave me the ability and success to accomplish this work and to meet all the challenge.

I also thank the scientific genius, my supervisor Dr. Ghada Abdel Rahman who served as beam that lit my way,

I would like to thank my friends and my family for their continuous support, and especially for my great brother almughirah, who have given me all giving and positive support,

Finally, sincere thanks to university of sudan, the faculty of medical laboratories, department of clinical chemistry and 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.
المستخلص
خلفية الدراسة: تدخين السجائر هو كلاسيكي وعامل خطر رئيسي في تنمية عدة أمراض مع مواد التهابية، مثل مرض الأوعية القلبية ومرض الإعاقة الرئوية المزمن، وارتفاع نسبة الدهون في الدم. هدف هذه الدراسة لتقييم مستويات البلازما للبروتين عالي الكثافة شديد الحساسية، المغنيسيوم، وملف الدهون لدى المدخنين.

المواد والطرق: هذه كانت دراسة عرضية وصفية أجريت أثناء الفترة من مايو/مايو إلى نوفمبر/تشرين الثاني 2018، ثمانون فرد سوداني تُضمن في هذه الدراسة، مستويات البلازما للبروتين عالي الكثافة شديد الحساسية كانت مقاسة باستخدام طريقة لاتكس اميون تيربدمترك، بلازما ملف الدهون والمغنيسيوم قيستا بالكشف الكيميائي باستخدام الفوتوميتر.

النتائج: أظهرت هذه الدراسة أن متوسط البلازما للبروتين عالي الكثافة شديد الحساسة مرتفع ارتفاعاً غير معنوي عند مقارنتها مع المعدلات الطبيعية مع قيمة احتمال > من (0.05)، بينما متوسطات البلازما للمغنيسيوم، الكولستيرول الكلي، الدهون الثلاثية، الدهون مرتفعة الكثافة والدهون منخفضة الكثافة كانت منخفضة بشكل ملحوظ بين مجموعة المدخنين مقابل المعدلات الطبيعية مع قيم احتمال < 0.05.

وأيضاً أظهرت الدراسة أنه على حسب الفترة الزمنية للتدخين أن هناك زيادة ذات دلالة معنوية في متوسطات كل من البروتين سي التفاعل عالي الكثافة شديد الحساسية، الكولستيرول الكلي، الدهون الثلاثية والدهون منخفضة الكثافة بينما يوجد انخفاض ذات دلالة معنوية في متوسطات كل من المغنيسيوم والدهون مرتفعة الكثافة.

الخلاصة: الخلاصة أن مستويات البلازما لدى المدخنين في كل من البروتين عالي الكثافة شديد الحساسية الكولستيرول الكلي، الدهون الثلاثية، والدهون مرتفعة الكثافة، تزيد بزيادة الفترة الزمنية للتدخين بينما كل من المغنيزيوم والدهون مرتفعة الكثافة تنخفض بزيادة الفترة الزمنية للتدخين.
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<td>TC</td>
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<td>Body mass index</td>
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<td>ATP</td>
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<td>HDL-C</td>
<td>High density lipoprotein lipase cholesterol</td>
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<td>Mg/L</td>
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<td>CDC</td>
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Chapter one

Introduction, Rationale Objectives
1. Introduction, Rationale and Objectives:

1.1 Introduction:
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).
1.2 Rationale:
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:
Conventionally, 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).

2.2.1 Structure 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 extracellular 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 Mg2+, Paracellular pathway which is a passive mechanism absorbs Mg2+ through small spaces between epithelial, The transcellular pathway involves movement of Mg2+ 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 Mg2+ is controlled largely by the kidney, which can reabsorb Mg2+ in deficiency states or readily excrete excess Mg2+ in overload states, Of the non-protein-bound Mg2+ 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 Hypermagnesia:
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 30 kg/m² is considered overweight and a person with BMI over 30 kg/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 HDL-c (mg/dl):** (Burits CA., et al)

<table>
<thead>
<tr>
<th>Total cholesterol</th>
<th>&lt; 200</th>
<th>desirable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 – 239</td>
<td>Borderline high</td>
</tr>
<tr>
<td></td>
<td>≥ 240</td>
<td>high</td>
</tr>
<tr>
<td>LDL-c</td>
<td>&lt; 100</td>
<td>Optimum</td>
</tr>
<tr>
<td></td>
<td>100 – 129</td>
<td>Near optimum</td>
</tr>
<tr>
<td></td>
<td>130 – 159</td>
<td>Borderline high</td>
</tr>
<tr>
<td></td>
<td>160 – 189</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>≥ 190</td>
<td>Very high</td>
</tr>
<tr>
<td>HDL-c</td>
<td>&lt; 40</td>
<td>low</td>
</tr>
<tr>
<td></td>
<td>≥ 60</td>
<td>high</td>
</tr>
</tbody>
</table>
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 fatty acid and glycerol, which phosphorylated in the presence of ATP and glycerol kinase to glycerol-3-phosphate that oxidized in presence of glycerol-3-phosphate 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 quinonimine 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 (m²) (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)

<table>
<thead>
<tr>
<th>Hs-crp Reference value</th>
<th>Risk to CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 mg/l</td>
<td>Low risk</td>
</tr>
<tr>
<td>1 – 3 mg/l</td>
<td>Moderate risk</td>
</tr>
<tr>
<td>&gt;3 mg/l</td>
<td>High risk</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg$^{2+}$</td>
<td>1.7 – 2.4 mg/dl</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. cholesterol</td>
<td>140 – 200 mg/dl</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>60 – 150 mg/dl</td>
</tr>
<tr>
<td>HDL-c</td>
<td>40 – 75 mg/dl</td>
</tr>
<tr>
<td>LDL-c</td>
<td>50 – 130 mg/dl</td>
</tr>
</tbody>
</table>
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/m² 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.
Table (4.1) means of study variables and parameters among study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>19</td>
<td>62</td>
<td>34.0 ± 11.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17</td>
<td>35</td>
<td>22.9 ± 3.90</td>
</tr>
<tr>
<td>Duration (year)</td>
<td>0.1</td>
<td>40.0</td>
<td>14.7 ± 11.2</td>
</tr>
<tr>
<td>HS-CRP (mg/L)</td>
<td>0.001</td>
<td>6.6</td>
<td>1.35 ± 1.80</td>
</tr>
<tr>
<td>Mg (mg/dL)</td>
<td>1.1</td>
<td>3.2</td>
<td>2.10 ± 0.40</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>98</td>
<td>386</td>
<td>164 ± 52.0</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>29</td>
<td>452</td>
<td>110 ± 81.0</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>13</td>
<td>48</td>
<td>32.2 ± 8.2</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>50</td>
<td>301</td>
<td>110 ± 47.1</td>
</tr>
<tr>
<td>Parameters</td>
<td>Frequency</td>
<td>Percentage (%)</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td><strong>HS-CRP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Risk (&lt; 1.0)</td>
<td>48</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Moderate Risk (1.0 – 3.0)</td>
<td>16</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>High Risk (&gt;3.0)</td>
<td>16</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimal (&lt;200)</td>
<td>66</td>
<td>82.0</td>
<td></td>
</tr>
<tr>
<td>Border line high (200 – 239)</td>
<td>9</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>High (≥ 240)</td>
<td>5</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td><strong>HDL-C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Risk (≥ 40)</td>
<td>16</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Moderate Risk (35 – 39)</td>
<td>19</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>High Risk (&lt; 35)</td>
<td>45</td>
<td>58.2</td>
<td></td>
</tr>
<tr>
<td><strong>LDL-C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimal (&lt; 100)</td>
<td>40</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Near Optimum (100 – 129)</td>
<td>23</td>
<td>28.8</td>
<td></td>
</tr>
<tr>
<td>Border Line High (130 – 159)</td>
<td>11</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>High (160 – 189)</td>
<td>1</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Very high (≥ 190)</td>
<td>5</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>80</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Mean ± SD</td>
<td>R.V</td>
<td>P.value</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>HS-CRP</td>
<td>1.35 ± 1.8</td>
<td>UP to 1.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Mg</td>
<td>2.13 ± 0.45</td>
<td>(1.7 – 2.4)</td>
<td>0.00</td>
</tr>
<tr>
<td>TC</td>
<td>164 ± 52.0</td>
<td>(140 – 199)</td>
<td>0.00</td>
</tr>
<tr>
<td>TG</td>
<td>110 ± 81.0</td>
<td>(60 – 150)</td>
<td>0.00</td>
</tr>
<tr>
<td>HDL-C</td>
<td>32.2 ± 8.2</td>
<td>(40 – 75)</td>
<td>0.00</td>
</tr>
<tr>
<td>LDL-C</td>
<td>110 ± 47.0</td>
<td>(50 – 130)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*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.

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

*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

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

-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

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

*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 smokers.

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.
Referencing


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)

QUESTIONNAIRE

Serial number:.....................................................................................................................................

Name:..................................................................................................................................................

Age:..................................................................................................................................................

Duration:.................................................... (years)....................................................... (months)

weight:..............................................(kg) Height:.................................................................(m)

BMI:..........................................................(kg/m²)

No. of cigarettes/day:..........................................................................................................................

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

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

Other diseases : ..........................................................................................................................

Exercise: yes (  ) NO (  )

Mobile No:.........................................................

signature..........................................................
**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 turbimetry.

**CONTENTS AND COMPOSITION**

A. Reagent: 1 x 40 ml, Glycine buffer 0.1 mol/l, sodium azide 0.01 g/l, pH 8.6
B. Reagent: 1 x 10 ml, Suspension of latex particles coated with anti-human CRP antibodies, sodium azide 0.01 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 uncontaminated by other reagents.

**AUXILIARY REAGENTS**

CRP-Na Standard: For 1 x 5 ml, BioSystems Code 31113. Human serum C-reactive protein concentration is stated on the vial label. The concentration value of the CRP-Na Standard is traceable to the Reference Material for C-reactive Protein (CRM 640/2).

Human serum used in the preparation of the standard has been tested and found to be negative for the presence of antibodies anti-β2GPI and anti-HCV, as well as for viral antigens. 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-Na Standard by 9 times saline aliquots. Multiple dilutions of the CRP-Na Standard by the corresponding factor indicated below to obtain the CRP concentration of the dilutions.

**REAGENT PREPARATION**

Working Reagent: Pour the contents of a Reagent B vial into a Reagent A bottle (Note 2). Mix thoroughly. Store for 60 days at 2-8°C.

Lesser Working Reagent volumes can be prepared by mixing 1 ml of Reagent B + 4 ml of Reagent A. Shake the Reagent B vial before pouring.

**SAMPLES**

Serum collected by standard procedures.

CRP in serum is stable for 7 days at 2-8°C.

**REFERENCE VALUES**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Men</th>
<th></th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-12 years</td>
<td>&lt; 14.5 mg/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14-17 years</td>
<td>&lt; 1.45 mg/l</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

**QUALITY CONTROL**

It is recommended to use the Protein Control Serum level I (Cat. No. 31211) and II (Cat. No. 31212) to verify the performance of the instrument.

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 analyzer. Results are similar to A15. Details on evaluation data are available on request.

- **Detection limit:** 0.15 mg/l.
- **Measurement interval:** 0.15-1.5 mg/l. For higher values dilute sample 1:5 with distilled water and repeat measurement.
- **Repeatability (within run):**

<table>
<thead>
<tr>
<th>Mean concentration</th>
<th>CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.52 mg/l</td>
<td>1.9%</td>
<td>20</td>
</tr>
<tr>
<td>4.85 mg/l</td>
<td>1.3%</td>
<td>20</td>
</tr>
</tbody>
</table>

- **Reproducibility (day to day):**

<table>
<thead>
<tr>
<th>Mean concentration</th>
<th>CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.52 mg/l</td>
<td>2.0%</td>
<td>25</td>
</tr>
<tr>
<td>4.85 mg/l</td>
<td>2.5%</td>
<td>25</td>
</tr>
</tbody>
</table>

- **Trueness:** Results obtained with this procedure do not show systematic differences when compared with a reference procedure. Details of the comparison experiments are available on request.
- **Zinc effect:** This method has no zinc effect (< 500 mg/l).
- **Interference:** Hemoglobin (10 mg/l) and bilirubin (10 mg/l) do not interfere. Lipids (100 mg/l) and heparin (3 U/ml) may interfere. Other drugs and substances may interfere.

**DIAGNOSTIC CHARACTERISTICS**

C-reactive protein (CRP), which is synthesized in the liver, is one of the first 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, stroke, 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.

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 (hs-CRP) 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 inflammatory process occurring.

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

**NOTES**

1. Share 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.

**BIBLIOGRAPHY**

Appendix (III)

PRINCIPLE OF THE METHOD
Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured spectrophotometrically.

Cholesterol ester + H₂O → Cholesterol + Fatty acid
Cholesterol + H₂O + + H₂O → Cholesterolone + H₂O
2 H₂O + 4 - Aminoantipyrine + Phenol → Quinoniminine + 4 H₂O

CONTENTS

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>COD-11805</th>
<th>COD-11505</th>
<th>COD-11606</th>
<th>COD-11506</th>
<th>COD-11507</th>
<th>COD-11508</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Standard</td>
<td>1 x 10 mL</td>
<td>1 x 10 mL</td>
<td>1 x 10 mL</td>
<td>1 x 5 mL</td>
<td>1 x 5 mL</td>
<td>1 x 5 mL</td>
</tr>
</tbody>
</table>

COMPOSITION
A. Reagent. Pipes 35 mmol/L, sodium chloride 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.

STORAGE
Store at 2-8°C.

REAGENT PREPARATION
Reagents and Standards are prepared ready for 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 labeled test tubes. (Note 1)

<table>
<thead>
<tr>
<th>Cholesterol Standard (B)</th>
<th>Sample</th>
<th>Reagent (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 μL</td>
<td>1.0 μL</td>
</tr>
<tr>
<td></td>
<td>1.0 μL</td>
<td>1.0 μL</td>
</tr>
</tbody>
</table>

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 color is stable for at least 2 hours.

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

\[ \text{Cholesterol concentration (mg/dl)} = \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times 200 \]

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:

- Up to 200 mg/dl = Normal
- 200-239 mg/dl = Borderline High
- 240 mg/dl = High

QUALITY CONTROL
It is recommended to use the Biochemistry Control Serum level I (cod. 18001, 18002 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.
- Reproducibility (within run):
  - Mean concentration: 25 mg/dl = 0.6 mmol/L
  - Reproducibility (run to run):

<table>
<thead>
<tr>
<th>Cholesterol (mg/dl)</th>
<th>CV</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>250</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

- Trueness: Results obtained with this reagent do not show significant differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Lipemia (high (over 10 g/L) does not interfere. Bilirubin (15 mg/dL) and hemoglobin (6 g/dL) may affect the results. Oth-r substances and drugs may interfere. These metabolically incompatible have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure is used.

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

Notes
1. This method 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, especially in some analysers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18001 and 18043).

BIBLIOGRAPHY
Appendix (IV)

TRIGLYCERIDES

Glycerol phosphate oxidase/peroxidase

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.

<table>
<thead>
<tr>
<th>Value</th>
<th>Normal</th>
<th>Border-line</th>
<th>High</th>
<th>Very high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 150 mg/dL</td>
<td>170-250 mg/dL</td>
<td>251-500 mg/dL</td>
<td>&gt;500 mg/dL</td>
<td></td>
</tr>
</tbody>
</table>

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005; 18009 and 19420) and II (cod. 18007; 18010; 18012 and 18014) 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 remain within the acceptable limits.

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.6 mg/dL. (-9 0.001 mg/dL.
- Linearity limit: 600 mg/dL. = 60.0 mg/dL. For higher values dilute sample 1:4 with distilled water and repeat measurement.
- Repeatability (within run): Mean Concentration CV ±

<table>
<thead>
<tr>
<th>Mean Concentration</th>
<th>CV ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/dL; 113 mg/dL</td>
<td>1.7% ± 20</td>
</tr>
<tr>
<td>245 mg/dL; 277 mg/dL</td>
<td>0.1% ± 20</td>
</tr>
</tbody>
</table>

- Reproducibility (run to run): Mean Concentration CV ±

<table>
<thead>
<tr>
<th>Mean Concentration</th>
<th>CV ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/dL; 113 mg/dL</td>
<td>2.6% ± 25</td>
</tr>
<tr>
<td>245 mg/dL; 277 mg/dL</td>
<td>1.7% ± 25</td>
</tr>
</tbody>
</table>

- Trueness: Results obtained with the 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 µL) does not interfere. Bilirubin (2.5 mg/dL) may interfere.

Other drugs and substances may interfere. These metrological characteristics have been obtained using an analytical method that may vary if a different instrument or a manual procedure is 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 lipoprotein and used by adipose tissue, muscle and other. Their primary function is to provide energy to the cell. Elevated serum triglyceride levels can be caused by liver disease, diabetes mellitus, nephrosis, hypothyroidism, alcoholism, familial hyperlipoproteinaemia IV and V, and other. 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 those cases, it is recommended to calibrate with a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

**PRINCIPLE OF THE METHOD**

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample precipitate with phosphotungstic acid 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:

\[
\text{Cholesterol ester} \rightarrow \text{Cholesterol + Fatty acid (4:4)}
\]

\[
\text{Cholesterol + } \text{H}_2\text{O} \rightarrow \text{Cholesterolone + H}_2\text{O} \quad (2:4)
\]

\[
2\text{H}_2\text{O} + 4 \rightarrow \text{Ammannitrylpyrine + Phenol (4:4)}
\]

**CONTENTS AND COMPOSITION**

A. Reagent I: 1 x 50 mL. Phosphotungstic acid 0.9 mmol/L, magnesium chloride 20 mmol/L.

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

**STORAGE**

Store at 2-8°C. Reagent and Standard should be stored until the expiry date shown on the label when stored tightly closed and if containers are protected during their use.

**ADDITIONAL REAGENTS**

These auxiliary reagents are to be used together with the Cholesterol Reagent contained in any of the BioSystems Cholesterol kits (code: 11509, 11509, 11538, 11538).

**REAGENT PREPARATION**

Reagent and Standard are provided ready to use.

**ADDITIONAL EQUIPMENT**

- Desktop centrifuge
- Thermocapillary water bath at 37°C
- Analyzer, spectrophotometer or photometer able to read at 500 ± 2.29 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**

1. Pipette into labeled centrifuge tubes (Note 1):
   - Sample
   - Reagent (5) Cholesterol-HDL kit
   - 0.2 mL
   - 0.5 mL

2. Mix thoroughly and let stand for 10 minutes at room temperature.
3. Centrifuge at a minimum of 4000 rpm for 10 minutes.
4. Carefully collect the supernatant (Note 2).
5. Chlorinometry
6. Bring the Reagent (Cholesterol-HDL kit) to room temperature.
7. Pipette into labeled test tubes (Note 3):
   - 100 mL
   - 100 mL
   - 100 mL
   - 1 mL
   - 1 mL
   - 1 mL

8. Mix thoroughly and incubate the tubes for 20 minutes at room temperature (16-20°C) or for 15 minutes at 37°C.
9. 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 formulae:

\[ \text{CHD} = \frac{\text{Sample} - \text{Blank}}{\text{Concentrate} \times \text{Sample dilution factor}} \times \text{CHD Standard} \]

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

\[ \text{CHD} = \frac{0.025 \times \text{mg/dL HDL cholesterol}}{1.36 \times \text{mmol/L HDL cholesterol}} \]

**REFERENCE VALUES**

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

- Up to 35 mg/dL: 0.91 mmol/L
- 36-49 mg/dL: 0.95-1.24 mmol/L

**QUALITY CONTROL**

It is recommended to use the Biochemistry Control Serum level I (code: 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
- Repeatability (within run):
  - Mean Concentration: 30 mg/dL = 0.78 mmol/L
  - 50 mg/dL = 1.24 mmol/L
  - 80 mg/dL = 2.06 mmol/L
  - Reproducibility (run to run):
    - Mean Concentration: 30 mg/dL = 0.78 mmol/L
    - 50 mg/dL = 1.24 mmol/L
    - 80 mg/dL = 2.06 mmol/L

**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.

**BIBLIOGRAPHY**

Appendix (VI)

Magnesium Xylydyl
Xylyl Blue, Colorimetric

Quantitative determination of magnesium
IV/D

Store at 2-8°C.

**PRINCIPLE OF THE METHOD**
Magnesium forms a coloured complex when reacts with Xylydyl carbonate in alkaline solution. The intensity of the color formed is proportional to the magnesium concentration in the sample.

**CLINICAL SIGNIFICANCE**
Magnesium is the second most abundant intracellular cation of the human body after potassium, being essential in great number of enzymatic and metabolic processes. It is a cofactor of all the enzymatic reactions that involve the ATP and comprises the membrane that maintains the electrical excitability of the muscular and nervous cells.

A low magnesium level is found in malabsorption syndrome, diabetic or renal acidosis, hyperparathyroidism or diabetics. Elevated concentration of magnesium is found in acalculous cholecystitis, chronic renal failure, gout, and malignancies. Addisons disease or intussusception therapy may also be due. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

**REAGENTS**

<table>
<thead>
<tr>
<th>R</th>
<th>Xylyl Blue</th>
<th>0.1 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thymol blue</td>
<td>0.7 mmol/L</td>
</tr>
<tr>
<td>DMSO</td>
<td>3000 mmol/L</td>
<td></td>
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**MAGNESIUM CAL**
Magnesium aqueous primary standard 2 mg/mL.

**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 chemicals in the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C protected from light and contamination prevented during their use. Do not use reagents after the expiration date.

**Signs of reagent deterioration:**
- Presence of particles, color change and turbidity.
- Blank absorbance (A) at 646 ± 10.

**ADDITIONAL EQUIPMENT**
- Spectrophotometer or colorimeter measuring at 646 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment (e.g. filter papers).

**SAMPLES**
- Serum, heparinised plasma
- Whole blood or EDTA anticoagulant
- Urine

Stability: 7 days at 4°C.

**PROCEDURE**

1. **Assay conditions:**
   - Wavelength: 646 nm
   - Cuvette: 1.0 cm light path
   - Temperature: 37°C ± 5°C

2. Adjust the instrument to zero with distilled water.
3. Dilute the sample 1:10 with distilled water and multiply the result by 10.

**CALCULATIONS**

\[
\text{mg/dL} = \frac{\text{mg/mL} \times \text{dilution factor}}{0.1}
\]

**QUALITY CONTROL**
Control sera are recommended to monitor the performance of assay procedures.

**REFERENCE VALUES**
- Serum: 1.5 to 2.5 mg/dL
- Urine: 24-24 mg/dL

The values are for orientation purposes; each laboratory should establish its own reference range.

**PERFORMANCE CHARACTERISTICS**

- **Measuring range:** From detection limit of 0.0052 mg/dL to 6 mg/dL. The results obtained were greater than 0.0052 mg/dL.
- **Sensitivity:** 1 mg/dL = 0.538 (µg/L).
- **Accuracy:** Results obtained using SPINREACT reagents (γ) did not show any significant differences when compared with other commercial reagents (µ). The results obtained were within the following:
- **Correlation coefficient (γ):** 0.9992
- **Regression equation:** y = 1.02x + 0.12
- **Precision:**
  - Intra-assay (n = 10): 1.59, 1.61, 1.68
  - Inter-assay (n = 10): 1.69, 1.71

The results of the performance characteristics depend on the analyzer used.

**INTERFERENCES**
- Heterology and anticoagulants other than heparin.
- A list of drugs and other interfering substances with magnesium determination has been reported by Young et al.

**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 thoroughly rinsed with water and dried before use.
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. It is recommended not to use a separate calibrator.
4. Use clean disposable pipettes for dispensing.

**BIBLIOGRAPHY**

**PACKAGING**
- Ref. 1001265: R: 2 x 150 mL, CAL: 1 x 5 mL
- Ref. 1001286: R: 2 x 50 mL, CAL: 1 x 2 mL