Sudan University of Science and Technology

College of Graduate Studies

Serum Total Protein, Albumin, C-reactive protein, Magnesium Levels, Aspartate Aminotransferase and Alanine Aminotransferase activity among Sudanese Male Cigarette Smokers in Khartoum State

مستويات مصل البروتين الكلي والألبومين و البروتين المتفاعل- سي والمغنيزيوم ونشاط أنزيمات ناقلة أمين الاسبارتات وناقلة أمين الألانين لدى الرجال السودانيون مدخني السجائر في ولاية الخرطوم

A dissertation submitted for partial fulfillment for the requirement of M.Sc. degree in clinical chemistry

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Dedication

... To my teachers…

... To my family**...**

…To my friends…

…. To volunteers…

..Who helps me a lot in this project and supporting me **.**

Samar Alasha Dawood

Acknowledgments

All praises is due to Almighty **Allah** the most gracious the most merciful.

I am greatly indebted to My supervisor **Dr.Ghada Abdelrahman** who gave me very clear outline to accomplish this research ,and spent much of her time in supervising and correcting my mistakes.

I would like to thanks all the volunteers who participated in this study.

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Abstract

Background and Aim: Cigarette smoking is one of the 10 greatest contributors to global death and disease. Cigarette contains harmful chemicals such as carcinogenic, cytotoxic and free radicals, which are affect many organs. The aim of this study was to evaluate the levels of serum total protein, albumin, C-reactive protein, magnesium, and aspartate aminotransferase, alanine aminotransferase activity among men smokers at Khartoum- state**.**

Method: The study was analytical cross sectional case control,100 individuals were enrolled as study group (50 cigarette smokers and 50 nonsmoker). Blood samples were drown from both groups to measure serum total proteins, albumin, magnesium levels and aspartate aminotransferase(AST), alanine aminotransferase(ALT) activity by Mindary BS-200, C-reactive protein (CRP) by i-Chroma. Data were analyzed by statistical package for social science (SPSS-16).

Result: Serum aspartate aminotransferase, alanine aminotransferase activity and C-reactive protein levels were significantly higher in cigarette smokers group versus non smokers group with (mean ±SD) for AST(25.4 ± 6.50 IU/L VS 16.9 ± 5.16 IU/L,P.V=0.00),ALT was(24.1 ± 6.99 IU/L VS 14.2 ± 5.52 IU/L ,P.V=0.00) and CRP was (4.04 ± 1.65 mg/L VS 2.85 ± 1.06 mg/L, P.V=0.00). While Serum total protein, albumin and magnesium levels were significantly lower in cigarette smokers group versus non smokers with (mean ±SD) for T.P(6.26 ± 0.45 g/dL VS 7.26 ± 0.69g/dL,P.V=0.00), albumin was (3.86± 0.59 g/dL VS 4.96 ± 0.45 g/dL, P.V=0.00), magnesium was(1.76 ± 0.27mg/dL VS 2.39 ± 0.36 mg/dL, P.V=0.00).

Conclusion: Smokers had significant increased in serum alanine aminotransferase, aspartate aminotransferase activity and C-reactive protein level ,and significant decreased in serum total protein, albumin and magnesium levels.

المستخلص

المقدمه : يعتبر التدخين من ضمن اخطر 10 اسباب عالميه تؤدي للوفاه تحتوي السيجاره على مواد كيميائيه سامه ومسرطنه تؤثر على عدة اعضاء من الجسم. يهدف الهدف من هذه الدراسه تقيم مستوى الكلي للبروتين ,الالبومين ,البروتين المتفاعل سي ,المغنيسيوم ونشاط انزيم امونيا الاسبارتات وانزيم امونيا الانين ضمن الرجال السودانيون المدخنون من ولايه الخرطوم.

الطريقه :اجريت هذه الدراسه المقطعيه بولايه الخرطوم حيث تم اختيار 100 من الاشخاص الاصحاء بطريقه عشوائيه, فقسموا لمجموعتين ,الاولى تتكون من 50 من المدخنين الاصحاء كمجموعه اختبار , والثانيه تتكون من 50 من غير المدخنين الاصحاء كمجموعه ضابطه.اخذت من كل المجموعتين عينات دم لقياس مستوى الكلي للبروتينات ,الالبومين,المغنيسيوم ونشاط انزيم امونيا الاسبارتات وانزيم امونيا الالانين باستخدام جهاز ميندري بي اس 200,والبروتين المتفاعل سي باستخدام جهاز اكروما, وتم تحليل النتيجه باستخدام حزمه احصائيه للعلوم الاجتماعيه.

النتيجه : كان مستوى مصل نشاط انزيم امونيا الاسبارتات ,انزيم امونيا الالانين و البروتين المتفاعل سي اعلى لدى المدخنين مقارنه بغير المدخنين, المتوسط $\pm$ الانحراف المعياري ل انزيم امونيا الاسبارتات(25.4 $\pm$ 6.50 الوحده الدوليه/ لتر) مقابل بغيرالمدخنين(16.90 $\pm$ 5.16 الوحده الدوليه/ لتر) القيمه الاحتماليه=0.00, انزيم امونيا الانين (24.1 $\pm$ 6.99 وحده دوليه/لتر) مقابل بغير المدخنين (14.2 $\pm$ 5.52 وحده دوليه/لتر) القيمه الاحتماليه=0.00, البروتين المتفاعل سي (4.04 $\pm$ 1.65 ملغرام / لتر) مقابل بغير المدخنين (2.85 $\pm$ 1.06 ملغرام/لتر القيمه الاحتماليه=0.00. بينما مستوى مصل البروتين الكلي,الالبيومين والمغنيسيوم اقل لدى المدخنين مقارنه بغير المدخنين , المتوسط $\pm$ الانحراف المعياري ل البروتين الكلي (6.26 $\pm$ 0.45 جرام/دسي لتر)مقارنه بغير المدخنين(0.69 $\pm$ 7.26 جرام/دسي لتر) القيمه الاحتماليه=0.00 , الالبيومين(3.86 $\pm$ 0.59 جرام/دسي لتر) مقارنه بغير المدخنين (4.96 $\pm$ 0.45 جرام/دسي لتر) القيمه الاحتماليه=0.00, المغنيسيوم(1.76 $\pm$ 0.27 مل جرام/دسي لتر) مقارنه بغير المدخنين (2.39 $\pm$ 0.36 مل جرام/دسي لتر) القيمه الاحتماليه=0.00 .

الاستنتاج : خلصت الدراسه الى ان مستويات مصل نشاط انزيم امونيا الاسبارتات ,انزيم امونيا الالانين و البروتين المتفاعل سي تزداد لدى المدخنين, بينما مستويات مصل البروتين الكلي,الالبيومين والمغنيسيوم اقل لدى المدخنين.

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List of Abbreviations

Abbreviations	Full terms
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
CRP	C-reactive protein
TP	Total protein
Mg^{+2}	Magnesium ion
PAH's	Polycyclic aromatic hydrocarbons
CO	Carbon monoxide
CPOD	Chronic obstructive pulmonary disease
TER	Trans-capillary escape rate
AMI	Acute myocardial infarction
OSA	Obstructive sleep apnea
NMDA	N-Methyl-D-aspartate
NO	Nitric oxide
NADPH	Nicotinamide adenine dinucleotide phosphate

Chapter One

Introduction

Rationale

Objectives

Chapter One

1. Introduction, Rationale and Objectives

1.1 Introduction

Cigarette smoking is a major cause of preventable morbidity and mortality.Worldwide more than3 million people currently die each year from cigarette smoking (Aurelio., 2005), many major disorders are caused and enhanced by cigarette smoking and different kind of diseases that lead to death appear all over the world every year (Funck *et al*., 2006). The risk of death in the smokers measured by the number of cigarettes smoked daily, the duration of smoking, the degree of inhalation and the age of initiation(Janson *et al*., 2001, Lubin *et al*., 2007). Cigarette smoke contains large numbers of free radicals that are capable of initiating or promoting oxidative injury, cigarette smokers are at greater risk for cardiovascular diseases, respiratory disorders, cancers, peptic ulcers gastroesophageal reflux disease, blind-ness, bone matrix loss, and hepatotoxicity comparing with non-smokers(Spiro and Silvestri., 2005) .

Smoking causes a variety of adverse effects on organs that have no direct contact with the smoke itself such as the liver (El-Zayad *et al*., 2002) cigarette smoking yields chemical substances with high cytotoxic potentials(Benowitz *et al*., 2007).These chemicals cause oxidative stress on the liver, which leads to damage to the liver cells and fibrosis (Mcbride., 1992).The chemicals that are present in cigarette smoke prevent the liver from performing its main function (Wangly *et al*., 2003). Over time the liver becomes less efficient at removing the toxins from the body, toxins which induce necroinflammation and increase the severity of hepatic lesions(fibrosis) (ChenZm *et al*., 2003).

Previous studies found that smoking increase in serum ALT and AST activities and smoker also have significantly lower total protein and albumin levels (Alsalhen., 2014), other study found that the smoking increased serum CRP level and decrease magnesium level in smokers compared with nonsmokers (Fatehuddin *et al* ., 2015).

1.2 Rationale

The number of smokers worldwide have increased every years hundreds of thousands of people around the world die from disease caused by cigarette smoking, cigarette contains over 4000 harmful compounds, 200 are toxicants and 80 are known carcinogens (CO, free radicals, nicotine, and tar). Smoke produce CO which binds to hemoglobin retard carrying of oxygen and lead to hypoxia, lung cancer, kidney cancer, heart disease and stroke, and these chemicals tend to accumulate in the liver progressively have deleterious effect on liver tissues, therefore altering some of the serum biochemical parameters related to liver efficiency.

However, according to our best knowledge there are no sufficient work at Sudan about the effect of cigarette smoking on serum total protein, albumin, CRP, magnesium Levels and AST, ALT activities, that why we attempt to do this study to assess serum total protein, albumin, CRP, magnesium levels and AST, ALT activities among Sudanese male cigarette smokers.

1.3 Objectives

1.3.1 General Objective

To assess the levels of serum total protein, albumin, magnesium, CRP levels, AST and ALT activities in Sudanese cigarette smokers in Khartoum state.

1.3.2 Specific objectives

1. To measure serum total protein, albumin, magnesium, CRP levels, ALT and AST activities in study groups.

2. To correlate between serum total protein, albumin , magnesium, CRP levels, ALT and AST activities with duration of smoking and number of cigarettes smoked per day.

Chapter Two

Literature Review

Chapter Two

2. Literature Review

2.1 Smoking

Smoking is a practice in which a substance is burned and the resulting smoke breathed in to be tasted and absorbed into the bloodstream. Most commonly the substance is the dried leaves of the tobacco plant which have been rolled into a small square of rice paper to create a small round cylinder called a "cigarette". Smoking is primarily practiced as a route of administration for recreational drug use because the combustion of the dried plant leaves vaporizes and delivers active substances into the lungs where they are rapidly absorbed into the bloodstream and reach bodily tissue. In the case of cigarette smoking these substances are contained in a mixture of aerosol particles and gasses and include the pharmacologically active alkaloid nicotine, the vaporization creates heated aerosol and gas to form that allows inhalation and deep penetration into the lungs where absorption into the blood stream of the active substances occurs (Emmanuela *et al*., 2015).

Smoking generally has negative health effects, because smoke inhalation inherently poses challenges to various physiologic processes such as respiration. Diseases related to tobacco smoking have been shown to kill approximately half of long-term smokers when compared to average mortality rates faced by non-smokers. According to a 2017 report in The Lancet, smoking has caused over five million deaths every year from 1990 to 2015(Reitsma *et al*., 2017).

Other smoking implements include pipes, cigars, bidis, hookahs ,vaporizers and bongs it has been suggested that smoking related diseases may also be contracted by non smokers .A 2007 report states that about 4.9million people worldwide each die as a result of smoking (West *et al*., 2007).

Cigars are tightly rolled of dried and fermented tobacco which are ignited so that smoke may be drawn into the smoker's mouth. they are generally not inhaled because the high alkalinity of the smoke, which can quickly become irritation to trachea and lung , the prevalence of cigar smoking varies depending on location, historical period and population surveyed. The united states is the top consuming country by far, followed by Germany and united kingdom, the US and Western Europe account for about 75% of cigar sales worldwide (Rarick., 2008).

2.1.1 Physiology of smoking

The active substance in tobacco especially cigarettes is administered by burning the leaves and inhaling the vaporized gas, inhaling the vaporized gas form of substances into the lungs is a quick and very effective way of delivering drugs into the bloodstream (as the gas diffuses directly into the pulmonary vein, then into the heart and from there to the brain) and affects the user within less than a second of the first inhalation(Gilman *et al*., 2004).

The lungs consist of several million tiny bulbs called alveoli that altogether have an area of over 70 m^2 (about the area of a tennis court). This can be used to administer useful medical as well as recreational drugs such as aerosols, consisting of tiny droplets of a medication, or as gas produced by burning plant material with a psychoactive substance or pure forms of the substance itself. Not all drugs can be smoked, for example the sulphate derivative that is most commonly inhaled through the nose, though purer free base forms of substances can, but often requires considerable skill in administering the drug properly. The method is also somewhat inefficient since not all of the smoke will be inhaled, the inhaled substances trigger chemical reactions in nerve endings in the brain due to being similar to naturally occurring substances such as endorphins and dopamine which are associated with sensations of pleasure. The result is what is usually referred to as a "high" that ranges between the mild stimulus caused by nicotine to the intense euphoria caused by heroin, cocaine and methamphetamines (Leslie., 2004).

When tobacco is smoked, most of the nicotine is pyrolyzed. However, a dose sufficient to cause mild somatic dependency and mild to strong psychological dependency remains. There is also a formation of hormone from the acetaldehyde in tobacco smoke, this seem to play an important role in nicotine addiction probably by facilitating a dopamine release in the nucleus accumbens as a receptor to nicotine stimuli .Using rate study with after repeated exposure to nicotine results in less responsiveness nucleus accumbens cells , which produce dopamiasne responsible for reinforcement (Shoaib *et al*., 2004).

2.1.1.1 Component of cigarette smoking

Carcinogen Smoke or any partially burnt organic matter, contains carcinogens (cancer-causing agents) There are over 19 known carcinogens in cigarette smoke (Jones *etal*., 2000).

The constituents of smoke are contained in either the particular phase or gas phase.

Particulate phase component include: **Tar** is defined as the nicotine- free,dry particulate mass of tobacco smoke. The nature of the chemical components in tar and their toxicity vary widely across tobacco from various sources. Tar as cilia are blocked the tars in cigarette smoke are deposited and collected on the walls of the respiratory tract and the lung, and cause them to turn block (Talhout *et al*., 2007), **Polycyclic aromatic hydrocarbons(PAH's)** are tar components produced by pyrolysis in smoldering organic matter and emitted into smoke. Several of these PAH's are already toxic in their normal form, however many of them can become more toxic in the liver. Due to the hydrophobic nature of PAH's they don't dissolve in water and are hard to expel from the body. In order to make the PAH more soluble in water, the liver creates an enzyme called Cytochrome P450 which adds an additional oxygen to the PAH, turning it into a mutagenic epoxides, which is more soluble, but also more reactive(Feng *et al*., 2006). **nicotine**: which is contained in cigarettes and other smoked tobacco products, is a stimulant and is one of the main factors leading to continued tobacco smoking. Nicotine is a highly addictive psychoactive chemical. When tobacco is smoked most of the nicotine is pyrolyzed; a dose sufficient to cause mild somatic dependency and mild to

strong psychological dependency remains. The amount of nicotine absorbed by the body from smoking depends on many factors, including the type of tobacco, whether the smoke is inhaled and whether a filter is used (Talhout *et al*., 2007).

Gas phase contains carbon monoxide(CO) (impairs oxygen transport and utilization) carbon monoxide in smoke replace the oxygen in the hemoglobin (a component of blood) adversely affecting oxygen transport and energy supply, and requiring the heart to do more work to supply the same amount of oxygen to the body. A large number of smoke constitute and particularly components of the gaseous phase of tobacco smoke, cause immunologic responses and inflammation in the cells, this causes increased stickiness of the blood, which increase the risk of clots, these processes increase the likelihood of a heart attack ,stroke or other problems with the cardiovascular system. Irritants such as nitric oxide cause hyper-secretion of mucus and substance such as a crolein, acetone and acetaldehyde cause damage to the small hair–like strands that line the airways (cilia). This damage to the cilia impairs the ability of the cilia to clear mucus causing breathing difficulties, years of smoking and daily coating of the lungs and airways in tar leads to irreversible lung damage and ultimately death from Chronic Obstructive Pulmonary Disease (CPOD) (Jonathan., 2004).

2.1.2 Health effects and regulation of smoking

Smoking is one of the leading causes of preventable death globally. In the United States about 500,000 deaths per year are attributed to smoking-related diseases and a recent study estimated that as much as 1/3 of China's male population will have significantly shortened life-spans due to smoking(Leslie., 2004).

Male and female smokers lose an average of 13.2and14.5years of life, respectively at least half of all lifelong smokers die earlier as a result of smoking (Doll *et al*., 2004). smoking most commonly leads to diseases causes may affect the heart and lungs and will most commonly affect areas such as hands or feet with first signs of smoking related health issues showing up as numbness, with smoking being a major risk factor for heart attacks, Chronic Obstructive Pulmonary Disease(COPD),

emphysema and cancer, particularly lung cancer, cancers of the mouth and pancreatic cancers(Seksik., 2009). Smoking is a risk factor in Alzheimer's disease(Cataldo *et al*., 2010), rheumatoid arthritis and systemic lupus(Majka DS., 2006)While smoking more than 15 cigarettes per day has been shown to worsen the symptoms of Crohn's disease((Lakatos *et al* ., 2007).

Cigarette smoke contains toxins and free radicals can interact with DNA and could cause genetic mutations and gene activation responsible for the development of an autoimmune disease(Costenbader., 2006, Padyukov., 2004).

2.2 The liver

The liver is the largest internal organ of the human body weighing approximately 1.2-1.5 kg in healthy adult .It is a functionally complex organ that play a critical bio chemical role in the metabolism, digestion, detoxification and elimination of substances from the body. The liver is involved in a number of excretory synthetic, and metabolic functions, all of which are essential of life. The liver is unique in the sense that it is a relatively resilient organ that can regenerate cells that have been destroyed by some short-term injury or disease. However, if the liver is damaged repeatedly over a long period of time, it may undergo irreversible changes the permanently interfere with its essential functions. (Michael *et al*., 2010).

The bile produced in the liver is collected in bile canaliculi, the bile canaliculi are small spaces between the hepatocytes that form intrahepatic duct, where excretory products of cell can drain ,the excretory system of the liver begins at the bile canaliculi. The intra-hepatic ducts (within the liver) , which drain into the right and left hepatic ducts, which merge to form the common hepatic duct. The cystic duct from the gallbladder joins with the common hepatic duct to form the common bile duct. Bile either drains directly into the duodenum via the common bile duct, or is temporarily stored in the gallbladder via the cystic duct(Michael *et al*., 2010).

The liver is an important organ that has many tasks; such as responsibility for processing drugs, alcohol and other toxins to eliminate them from the body (Pessione., 2001). The liver is unique in the sense that it is a relatively resilient organ that can regenerate cells that have been destroyed by some short term injury or disease. However if the liver is damaged repeatedly over a long period of time, it may undergo irreversible changes that permanently interfere with it is essential function (Michael *et al*., 2010) .

2.2.1 Liver disorders

There are more than a hundred kinds of liver disease. Acute and chronic Hepatitis, inflammation of the liver is caused mainly by various viruses(viral hepatitis) but also by some liver toxins (e.g. alcoholic hepatitis), autoimmunity (autoimmune hepatitis) or hereditary conditions. Cirrhosis is the formation of fibrous tissue (fibrosis) in the place of liver cells that have died due to a variety of causes, including viral hepatitis, alcohol overconsumption, and other forms of liver toxicity, cirrhosis causes chronic liver failure. Hereditary diseases that cause damage to the liver include hemochromatosis Hepatic jaundice, Gilbert's syndrome, a genetic disorder of bilirubin metabolism found in about 5% of the population, can cause mild jaundice, Crigler-najjar disease :partial or complete deficiency of UDP-glycuronyltransferase , Dubin – Johnson syndrome : defective liver cell excretion of bilirubin. Intrahepatic cholestasis : may be caused by 14 hepatocyte injury such as cirrhosis , bile duct injury such as rotor syndrome. There are also many pediatric liver diseases including: biliary atresia, and progressive familial intrahepatic cholestasis (Anna *et al*., 2002).

2.2.2 Effects of smoking on liver

The liver is an important organ that has many tasks; such as responsibility for processing drugs, alcohol and other toxins to eliminate them from the body(Pessione *et al*., 2001).Smoking yields chemical substances with cytotoxic potentials,these chemicals created by smoking induce oxidative stress associated with lipid peroxidation, which leads to activation of stellate cell and development of fibrosis(Husain *et al*., 2001) .

Smoking increases the production of pro-inflammatory cytokines(IL-1,IL-6,TNF-a) involved in liver cell injury(Moszcynki., 2001). (El-zayadi *et a*l., 2002) have reported an association between heavy smoking and liver cell injury in the form of necroinflammation, apoptosis and excess iron deposition in liver. These effects are attributed to iron overload with consequent iron deposition in hepatocyte(El-zayadi., 2002).Execess hepatic iron induces oxidative stress and lipid peroxidation(Husain., 2001).

2.3 Total protein

Serum total protein is a biochemical test for measuring the total amount of protein in serum. They serve many different functions, including transport of lipid , hormones and minerals in activity and functioning of the immune system. Other blood proteins act as enzymes, complements and protease inhibitors. Serum total protein contains albumin and globulin (DeGowin *et al*., 2004).

Serum albumin account for 55% of blood proteins and is a major contributor to maintaining oncotic pressure, and transport insoluble molecules, globulins make up 38% of blood proteins and transport ion, hormones, and lipid, assisting in immune function. Fibrinogen comprises 7% of blood proteins, conversion of fibrinogen to insoluble fibrin essential for blood clotting. The remainder of the plasma proteins(1%) are regulatory proteins, such as enzymes proenzymes and hormones. All blood proteins are synthesized in liver except for the gamma globulin. (wendy and Jean *l*., 2007).

Alteration in serum total protein concentration is used commonly in clinical practice as a non-specific indicator for underlining disease or monitor disease activity(Putignanop *et al*., 2000).Proteins are the most important compounds that are present in human serum abundantly and play a key role for the maintenance of suitable acid-alkaline balance of the body. In addition, proteins are source of energy for the tissues and muscles when adequate amount of carbohydrate is not ingested. The human serum protein level shows a significant decrease in smokers (Alsalhen *et al*., 2014).

2.4 Albumin

The single most important protein is Albumin and present in highest concentration in the plasma , albumin is synthesized in the liver from 585 amino acids at the rate of 9-12 grams per day, with no reserve or storage (Michael *et al*., 2010) .

Albumin exists in the interstitial space, Albumin leaves the circulation at rate 4%-5% of total intravascular albumin per hour , this rate movement is known trans-capillary escape rate(TER), which measures systemic capillary efflux of albumin . albumin forms a large proportion of all plasma protein, It normally constitutes about 50% of human plasma protein. Serum albumins are important in regulating blood volume by maintaining of the colloid osmotic pressure , albumin buffers PH , negative acute-phase reactant protein (Michael *et al*., 2010).

Albumins serve as carriers for molecules of low water solubility including thyroid hormones , fat soluble hormones , ions and fatty acids, and some drugs like warfarin phenobutazone and phenytoin. For this reason it is sometimes referred as a molecular "taxi", Specific types include { human serum albumin – bovine serum albumin (BSA), often used in medical and molecular biology labs (Farrugia., 2010).

Deceased concentrations of serum albumin caused by: An inadequate source of amino acids occurs in malnutrition and malabsorption , Liver disease resulting decreased synthesis by hepatocytes, protein losing enteropathy and disease of intestinal tract as diarrhea, kidney loss to urine in renal disease , skin loss such as burns, and acute disease, and high albumin levels caused by: dehydration or excessive albumin infusion (Michael *et al*., 2010).

Albumin is protein made in liver in acute inflammation the liver switches from making albumin to making more urgent proteins ,lowe serum albumin is related to current smoking(J.clin., 2004), Chemicals in cigarette directly alters the binding properties of albumin and results in its degradation in the liver and loss of albumin by the kidney (Ramamurthy *et al*., 2012)

Albumin one of the well-known acute phase protein, has antioxidant properties and it can be considered as a marker of inflammation (Pongpaew *et al*., 2001).

Cigarette smoke increases the production of pro-inflammatory cytokines (IL-1, IL-6 and IL-13 and TNF- alpha) (Yang *et al* ., 2006).Cigarette smoke induce oxidative stress by stimulating Nicotinamide adenine dinucleotide phosphate(NADPH) oxidase and decreasing antioxidant defenses (Agarwal ., 2005) .

2.4.1 Relationship between Albumin and cigarette smoking

Albumin as one of the extracellular antioxidants, which acts as sacrificial antioxidant by inhibiting the generation of free radicals through an immediate attacks of albumin molecule itself so the radical reaction ontinues on albumin surface and causes damage to albumin molecule(Jwan and Saad., 2014).

2.5 Liver enzymes

2.5.1 Aspartate Aminotransferase(AST)

AST is an enzyme belonging toclass of transferase. commonly referred to as a transaminase(AST catalyzes the reversible transfer of an amino group between aspartate and a-keto acid with formation of glutamate and oxaloacetate), the older terminology Serum glutamic-oxaloacetic transaminase (SGOT-GOT), AST widely distributed in human tissue, the highest concentration are found in cardiac tissue, liver and skeletal muscle, smaller amount found in the kidney , pancreas and erythrocyte (Michael.*et al*., 2010).

2.5.1.1 Diagnostic Significant of AST

The clinical use of AST is limited mainly to the evaluation of hepatocellular disorder and skeletal muscle involvement. In myocardial infarction AST level begin to rise within 6 to 8 hours, peak at 24 hours, and return to normal within 5days. AST may be elevated also in diseases affecting other organs, such as pulmonary embolism. AST level are highest in acute hepatocellular disorders. AST exists as two isoenzyme fraction located in the cell cytoplasm and mitochondria , the intracelllular concentration

of AST may be 7000 times higher than the extracellular concentration, hemolysis should be avoided because it can increase serum AST concentration (Michael *et al*., 2010).

2.5.2 Alanine Aminotransferase(ALT)

ALT is a transferase with enzymatic activity similar to AST. ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. pyridoxal phosphate (PLP) function as coenzyme. The older terminology was Serum glutamic-pyruvic transaminase(SGPT-GPT).ALT is high concentration in liver, it is considered the more liver specific enzyme of transferase(*Michael et al.,* 2010).

2.5.2.1 Diagnostic significance of ALT

Clinical applications of ALT is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. Higher elevations are found in hepatocellular disorder than in extra-hepaticor intra-hepatic obstructive disorder. In acute inflammatory conditions of liver ALT elevations are frequently higher than AST and tend to remain elevated longer (half life of ALT 16-24 hours) (Michael *et al*., 2010).

ALT in conjunction with an elevated AST, is used to assess liver involvement with diagnosis of Acute myocardial infarction(AMI). ALT dose not exhibit a significant increase in muscular dystrophy, and it is not affected in case of pulmonary emboli or acute pancreatitis (Anna *et al*., 2002).

2.5.2.2 Relationship between AST- ALT and cigarette smoking

Cigarette smoke propagates the lipid peroxidation, which damage the biological cell membrane of the liver and serum aminotransferases are enzymes that act as sensitive

indicators of hepatocellular damage ,The enzymes are leaked out into blood and increased the level of AST and ALT in smokers (Rochling., 2001).

2.6 C-reactive protein (CRP)

2.6.1 Definition

C-reactive protein is synthesized in the liver and in one of the first acute-phase proteins to rise in response to inflammatory disease. CRP received its name because it precipitate with somatic C carbohydrate antigen of capsule of pneumococci (Michael *et al*., 2010).

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

It rises within two hours of the onset of inflammation, up to a 50,000-fold, and peaks at 48 hours. Its half-life of 18 hours is constant, and therefore its level is determined by the rate of production and hence the severity of the precipitating cause. CRP is thus a marker for inflammation that can be used to screen for inflammation(Enocsson *et al*., 2009).

2.6 .2 Clinical significance of CRP

CRP is used mainly as a marker of inflammation, apart from liver failure, there are few known factors that interfere with CRP production (Pepys and Hirschfield; 2003). Interferon alpha inhibits CRP production from liver cells which may explain the relatively low levels of CRP found during viral infections compared to bacterial infections (Enocsson *et al*., 2009).It is more sensitive and accurate reflection of the acute phase response, the utility of CRP in differentiating inflammatory diseases (including

inflammatory bowel disease, intestinal lymphoma and intestinal tuberculosis(Liu *et al*., 2013).

2.6.2.1 Cardiovascular Disease

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

2.6.2.2 Fibrosis and inflammation

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

2.6.2.3 Relationship between CRP and cigarette smoking

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

2.7 Magnesium (Mg^{+2})

Magnesium is a mineral found in the earth , sea , plants, animal and humans.

About 60% of the magnesium in your body is found in bone. While the rest is in muscles, soft tissues and fluids, including blood (Schmidt and kisters ., 2015).

Magnesium is an essential element in biological system ,occur typically as the Mg^{2+} it is an essential mineral nutrient for life (Johan and sons., 2006), magnesium main roles is acting as a cofactor in the biochemical reactions continuously performed by enzymes

.involved more than 600 reactions in your body including: Energy creation: helps convert food into energy, Protein formation: helps create new protein from amino acids, Gene maintenance: helps create and repair DNA and RNA, Nervous system regulation: helps regulate neurotransmitters, which send messages throughout your brain and nervous system(Baajj *et al*., 2015).

Magnesium dependent enzymes appear in virtually every metabolic pathway: specific binding of Mg^{+2} to biological membrane, used as signaling molecule and including all reactions that require release of energy from ATP(Romani., 2002).

Inadequate magnesium intake causes muscle spasms and has been associated with cardiovascular disease, anxiety disorders, osteoporosis and cerebral infarction (Romani., 2013) .The incidence of major depression the neuroses and the neuroiritability are higher in adult smokers than in non-smokers (Parrott., 2012). In patients with major depression the plasma level of magnesium is decreased compared to normal adults(Eby., 2010, Nechifor., 2009) .The anxiety major depression and panic disorders are associated with tobacco use. The vulnerability to addiction is increased by stress, the tobacco smoking is higher in stressed adults, the stress favors the loss of magnesium and a low magnesium concentration, a magnesium deficit is involved in some clinical symptoms of drug dependencies, this fact could be involved in the development of tobacco addiction (Nechifor ., 2012).

The key point for addiction is the increase of dopaminergic and glutamatergic activity in the reward system, drug self-administration is regulated by nucleus accumbens dopamine levels (Veeneman., 2012), the N-Methyl-D-Aspartate(NMDA) receptors and nicotinic cholinergic receptors have a cooperative of contribution in the control of pre synaptic dopamine release, the presynaptic nicotinic receptors stimulation increases the release of dopamine and norepinephrine. An increasing magnesium concentration reduces the nicotine stimulated norepinephrine release. the stimulation of pre-synaptic nicotine receptors into the brain induces an increase in the calcium entry into neurons

And also increases glutamate release ,the glutamate one of the most important neurotransmitters involved in all dependences including nicotine dependence stimulate different receptors in the brain (Nechifor ., 2012).

The NMDA receptors stimulation increases the dopamine release .all addictive drugs increase the dopamine level in nucleus accumbens. this is an essential step in development of addiction(Di Chiara and Bassareo., 2007) .

Another way by which magnesium can impair the nicotine addiction is the inhibition of nitric oxide(NO) synthesis. NO is involved in the mechanism of nicotine addiction. The mechanism of reinforcing properties of addictive substances(including nicotine)involves brain nitric oxide (Di Matteo., 2010). The experimental pretreatment with inhibitors of NO synthase blocked the nicotine induced increase of dopamine synthesis and dopamine neurons activity by this way can reduce the nicotine dependence and the tobacco use(DiMatteo., 2010). Magnesium association could by a good way for increasing the eicacy of this treatment, by increasing the magnesium concentration we can moderately improve the stimulation of the reward system and can reduce the needs of stimulation by nicotine(Nechifor ., 2012).

2.7.1 Relationship between magnesium and cigarette smoking

The cigarette smoking causes decreased supply of magnesium caused due to lesser appetite and reduced absorption due to digestive system disturbances (Winiarczyk., 2008).

Chapter Three

Materials and Methods

Chapter Three

3.Materials and Methods

3.1 Study design

This was cross sectional case control study .

3.2 Study area and period

The study was conducted in Khartoum state from Jun to August 2018.

3.3 Study population

There were Fifty cigarette smokers enrolled as test group and 50 normal healthy non-smokers(age matched with test group) were enrolled in this study as a control group.

3.3.1 Inclusion criteria

Cigarettes male smokers in Khartoum state and non cigarettes smokers as a control group.

3. 3.2 Exclusion criteria

Cigarettes male smokers who have alcoholic abuse, inflammation, chronic diseases Liver drug users, burn and any recent injury and liver disease were excluded.

3. 4 Ethical consideration

The study was approved by scientific committee of Sudan clinical chemistry, and the aim of this study were explained to participants and an informed consent was obtained from each participants.

3.5 Data collection

A questionnaire was used to obtain the clinical data for each participants in this study

(appendix I) from each participant.

3.6 Blood samples collection

five ml of venous blood was collected from each subject. The blood was collected under aseptic conditions allowed to clot in a plain tube for 20 minute at room temperature. The serum was separated by centrifugation at 3000 rpm for 5 minutes, then each subject serum was stored in plain tubes frozen at -20 C until used.

3.7 Estimation of serum total Protein (appendix II)

3.7.1 Principle of method:

Protein + Cu^{++} -------^{Alkali}--------→ Colored Complex

In the assay reactions , protein in serum form a violet complex when reacted with cupric ions in an alkaline solution. The intensity of the violet color is proportional to the amount of protein present when compared to a solution with known protein concentration . (Henry *etal*., 1974)

3.7.2 Procedure: (appendix II).

3.7.3 Calculation: The Mindary BS-200 analyzer calculates the concentration of each sample automatically (appendix II).

3.8 Estimation of serum albumin (appendix III)

3.8.1 Principle of method:

Albumin is bound by the BCG dye to procedure an increase in the blue-green color measured at 630 nm. The color increase is proportional to the concentration of albumin in sample (Tietz, N., 1976).

3.8.2 Procedure: (appendix III)

3.8.3 Calculation: The Mindary BS-200 analyzer calculates the concentration of each sample automatically (appendix III).

3.9 Estimation of serum magnesium (appendix IV)

3.9.1 Principle of method:

Serum magnesium ions react with Xylidyl Blue in alkaline medium to produce a red complex that is measured spectrophotometrically. The intensity of color produced is directly proportional to magnesium concentration.Calcium interference is virtually eliminated by use of EGTA and a surfactant system is included to remove protein interference. (Basinski., 1965) .

3.9.2 Procedure: (appendix IV).

3.9.3 Calculation: the Mindary BS-200 analyzer calculates the concentration of each sample automatically (appendix IV).

3.10 Estimation of serum aspartate aminotransferase(AST) :(appendix V)

3.10.1 Principle of method:

L-Aspartate + α-Ketoglutarate $\xrightarrow{AST}$ Oxalacetate + L-Glutamate

Oxalacetate + NADH + H^+ $\xrightarrow{MDH}$ L-Malate + NAD^+ + H_2O

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α-Ketoglutarate to yield oxalacetate and L-glutamate.The oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum (Kaplan., 1989).

3.10.2 Procedure: (appendix V).

3.10.3 Calculation: The Mindary BS-200 analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process Conversion factor of traditional unit (IU/L) into SI- units (n Kat/L).(appendix V)

3.11 Estimation of serum alanin aminotransferase : (appendix VI)

3.11.1 Principle of method:

L-Alanine + α -Ketoglutarate ----^{ALT}--------→ Pyruvate + L-Glutamate

Pyruvate + NADH + H^+ ----- ^{LDH} -----→ L-Lactate + NAD^+ +H_2O

ALT catalyzes the transfer of the amino group from L-alanine to α -ketoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH to NAD. This change in absorbance is directly propotional to the activity of ALT in the sample . (Scand., 1974)

3.11.2 Procedure: (appendix VI).

3.11.3 Calculation: The Mindary BS-200 analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process Conversion factor of traditional unit (IU/L) into SI- units (n Kat/L).(appendix VI)

3.12 Estimation of serum CRP(appendix VII)

3.12.1 Principle of the method

The test uses a sandwish immunodetection method , the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigen in sample forms the more antigen-antibody complex and leads to sronger intensity of fluorescence signal on detector antibody , which is processed by

instrument for ichroma tests to show RF igM conentration in sample.(Pepys and Hirschfield., 2003)

3.12.2 Procedure(appendix VII).

3.12.3 Calculation: The instrument ichroma tests calculates the tests result automatically and display CRP concentration of the test sample in term mg/l.

3.13 Quality control

The Mindary BS-200 analyzer was calibrated at first step, before running of sample, control sera used to be sure that results obtained were accurate and precise.

3.14 Statistical analysis

Data was analyzed to obtain means and standard deviation and correlation of the sampling using Statistical package for social science (SPSS, version 16), t-test was used for comparison and correlation.

Chapter Four

Results

Chapter Four

4.Results

4.1 Results

Hundred Sudanese males (50 smokers and 50 non smokers) were enrolled in this study to study the effect of smoking on The levels of biochemical parameter of Serum AST and ALT activities, total protein (TP), albumin, CRP and magnesium levels.

Table(4.1):Shows a comparison between means of serum total protein ,albumin magnesium and CRP levels, AST and ALT activities in Sudanese males smokers and control group. There were significantly decrease in Serum total protein , albumin and magnesium levels of smokers compared to non- smokers TP (mean ±SD: 6.26 ± 0.45 g/dL vs 7.26 ± 0.69 g/dL p.value=0.00) , albumin (mean ±SD: 3.86 ± 0.59 g/dL vs 4.96 ± 0.45 g/dL p.value=0.00) , Mg^{+2} (mean ±SD: 1.76 ± 0.27 mg/dL vs 2.39 ± 0.36 mg/dL p.value=0.00), and significantly increase in AST, ALT activities and CRP level of cigarette smokers compared to the non-smokers AST (mean ±SD: 25.4 ± 6.50 IU/L vs 16.9 ±5.16 IU/L p.value=0.00), ALT (mean ±SD: 24.1 ± 6.99 IU/L vs 14.2 ± 5.52 IU/L p.value=0.00), CRP (mean ±SD: 4.04 ± 1.65 mg/L vs 2.85 ± 1.06 mg/L p.value=0.00) all of these differences were statistically significant (P< 0.05).

Figure (4.1a): a scatter plot shows a significant negative correlation (P=0.01, r = -0.34) between total protein level and duration of smoking.

Figure (4.1b) :a scatter plot shows a significant negative correlation (P=0.00., r = -0.43) between total protein level and number of cigarette.

Figure (4.2a): a scatter plot shows a significant negative correlation (P=0.01, r=-0.35) between albumin level and number of cigarette per day among Sudanese smokers.

Figure (4.2b): a scatter plot shows a significant negative correlation (P=0.00, r = -0.42) between albumin level and duration of smoking among Sudanese smokers.

Figure (4.3a): a scatter plot shows a significant positive correlation (P=0.00, r=0.64) between AST activity and number of cigarette among Sudanese smokers.

Figure (4.3b): a scatter plot shows a significant positive correlation (P=0.00, r =0.58) between AST activity and duration of smoking among Sudanese smokers.

Figure (4.4a): a scatter plot shows a significant positive correlation (P=0.00, r= 0.66) between ALT activity and number of cigarette among Sudanese smokers.

Figure (4.4b): a scatter plot shows a significant positive correlation (P=0.00, r=0.58) between ALT activity and duration of smoking among Sudanese smokers.

Figure (4.5a): a scatter plot shows a significant positive correlation (P=0.00, r =0.72) between CRP level and number of cigarette among Sudanese smokers.

Figure (4.5b): a scatter plot shows a significant positive correlation (P=0.00, r=0.59) between CRP level and duration of smoking among Sudanese smokers.

Figure (4.6a): a scatter plot shows a significant negative correlation (P=0.00, r =-0.54) between Mg^{+2} level and number of cigarette among Sudanese smokers.

Figure (4.6b): a scatter plot shows a significant negative correlation (P =0.00, r =-0.49) between Mg^{+2} level and duration of smoking among Sudanese smokers.

Table (4.1) Comparison between means of serum total protein, albumin, AST, ALT activities, CRP and magnesium levels in Sudanese smokers and non-smokers

variable	**Smokers N=(50) Mean ±SD**	**Non-smokers N=(50) mean±SD**	**P.value**
Total Protein (g/dL)	6.26 ± 0.45	7.26 ± 0.69	0.00*
Albumin (g/dL)	3.86 ± 0.59	4.96 ± 0.45	0.00*
AST (IU/L)	25.4 ± 6.50	16.9 ± 5.16	0.00*
ALT(IU/L)	24.1 ± 6.99	14.2 ± 5.52	0.00*
CRP (mg/L)	4.04 ± 1.65	2.85 ± 1.06	0.00*
Magnesium(mg/dL)	1.76 ± 0.27	2.39 ± 0.36	0.00*

Independent T test was used to compare between two means

Results given mean ± SD,

P.value ≤ 0.05, was considered significant.

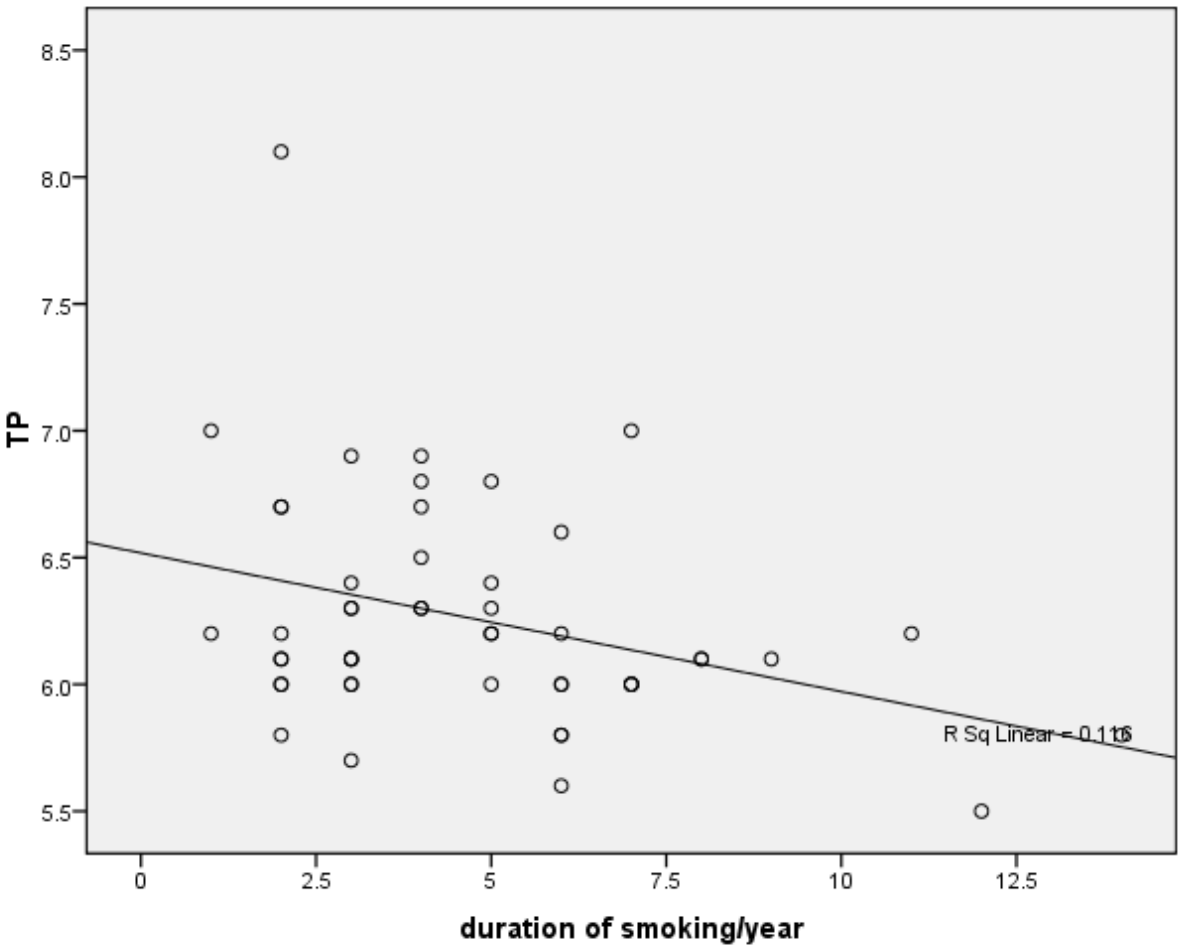


Figure (4.1a): a scatter plot shows a significant negative correlation between Total protein level and duration of smoking among Sudanese smokers.

(P=0.01, r = -0. 34)

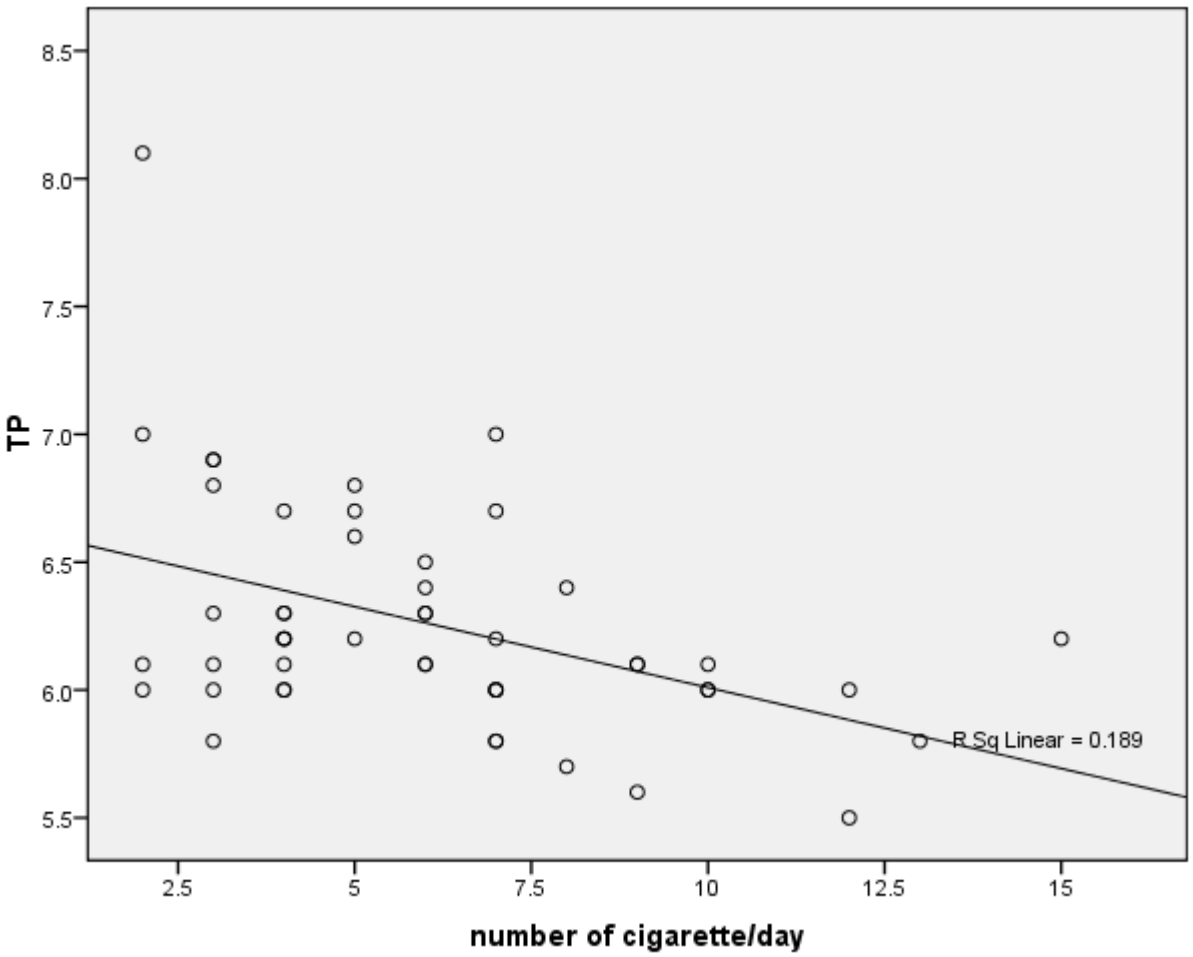


Figure (4.1b): a scatter plot shows a significant negative correlation between Total protein level and number of cigarette per day among Sudanese smokers (P=0.00, r = -0.43).

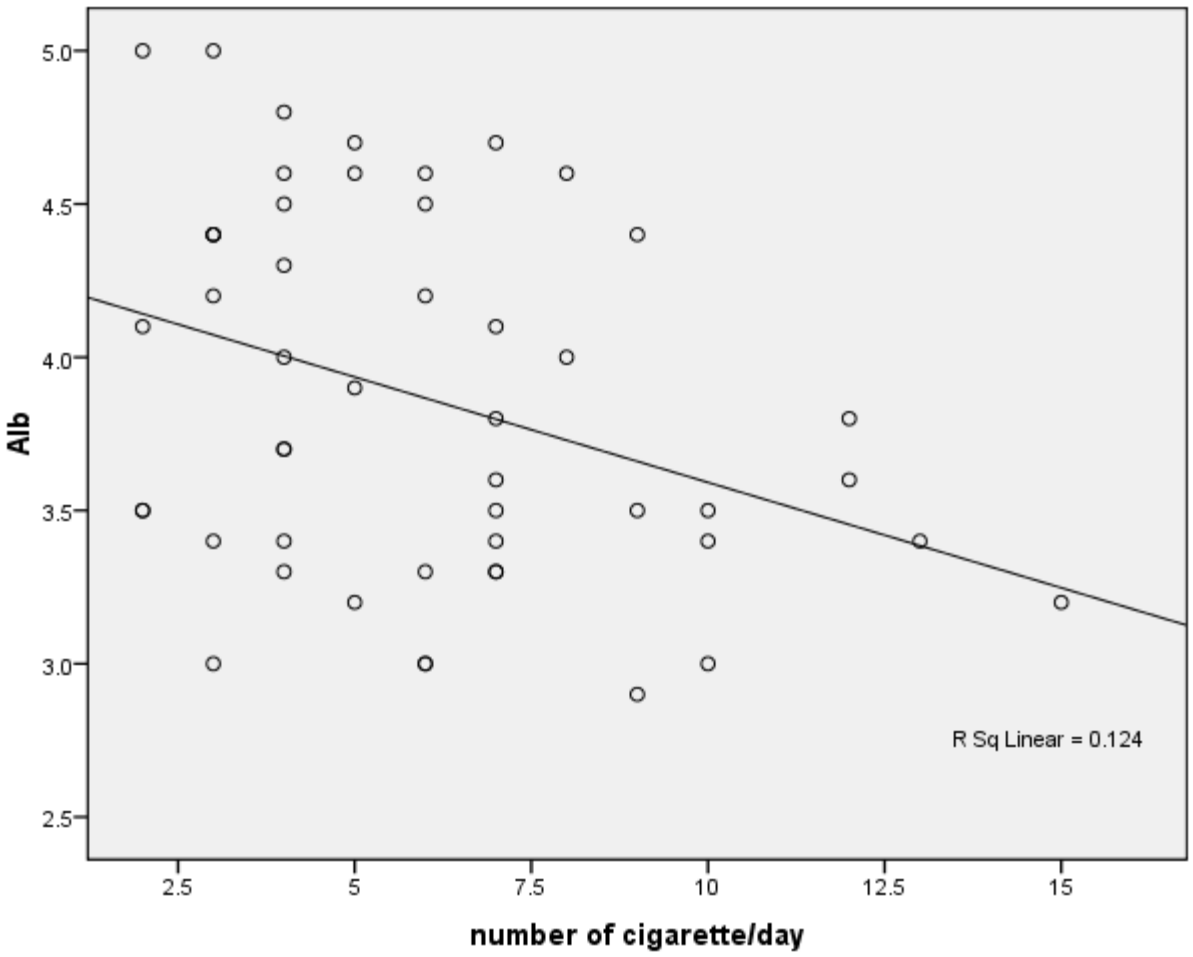
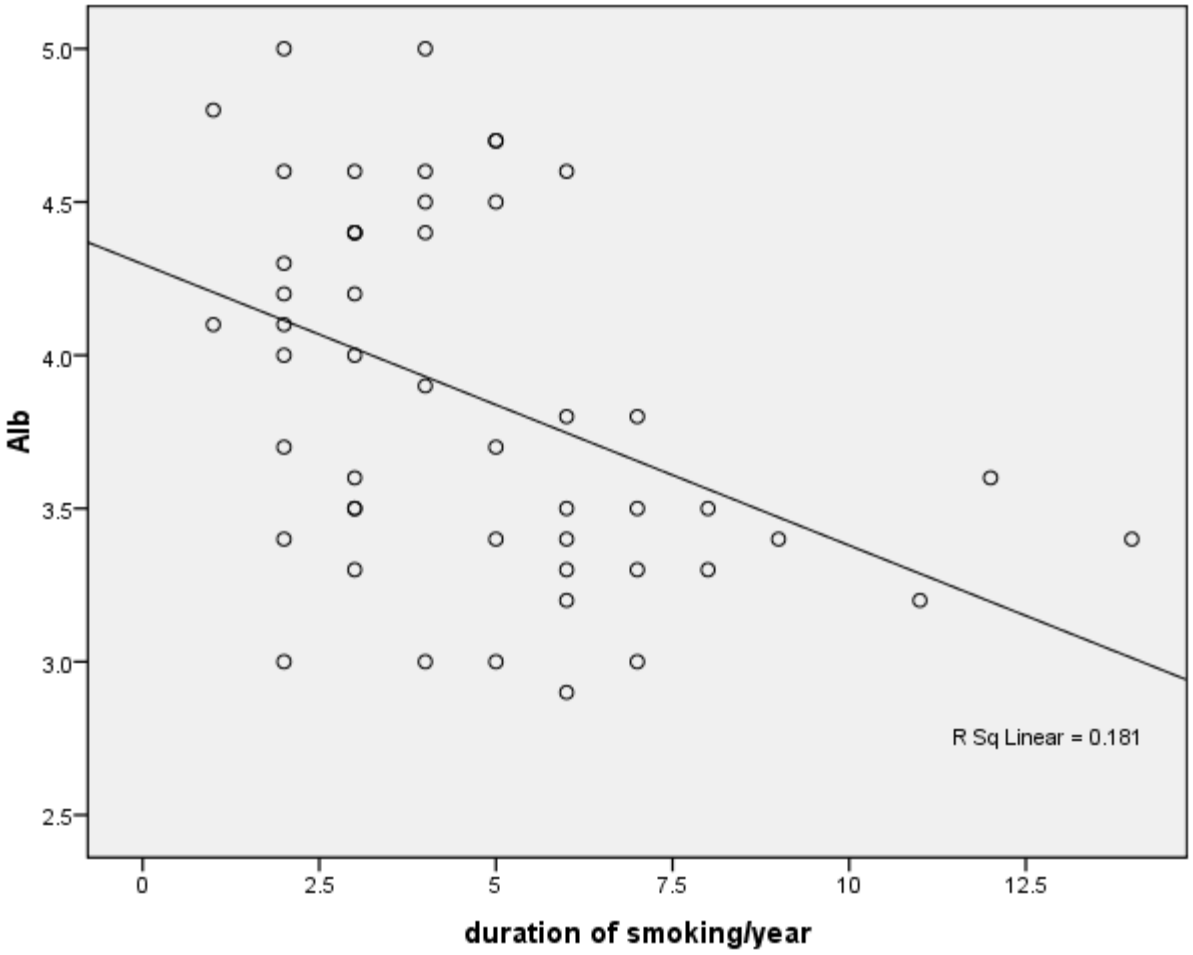


Figure (4.2a): a scatter plot shows a significant negative correlation between Albumin level and number of cigarette per day among Sudanese smokers.(P=0.01, r = -0.35)



Figure(4.2b): a scatter plot shows a significant negative correlation between Albumin level and duration of smoking among Sudanese smokers.

(P=0.00, r = -0. 42)

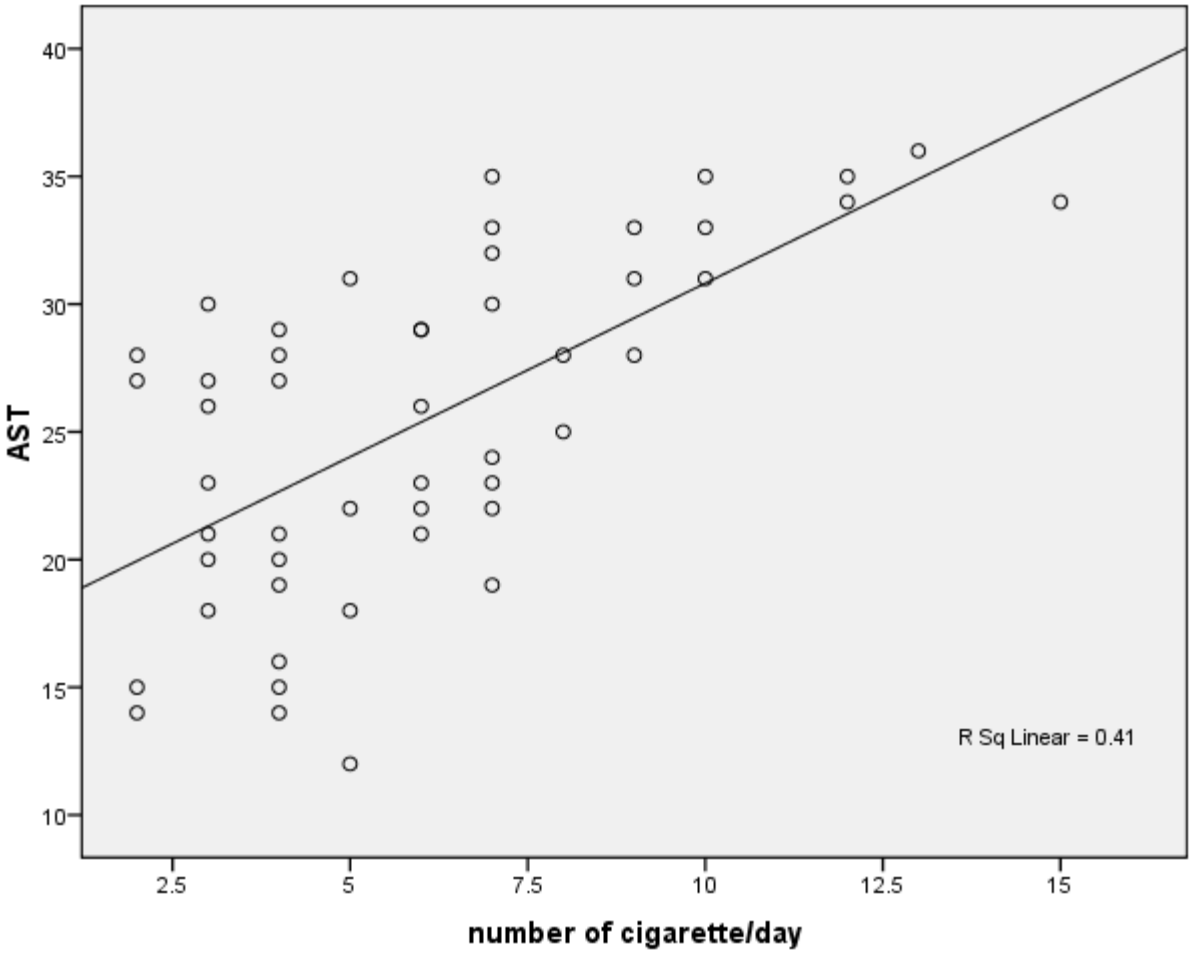


Figure (4.3a): a scatter plot shows a significant positive correlation between AST activity and number of cigarette per day among Sudanese smokers.

(P=0.00, r =0.64)

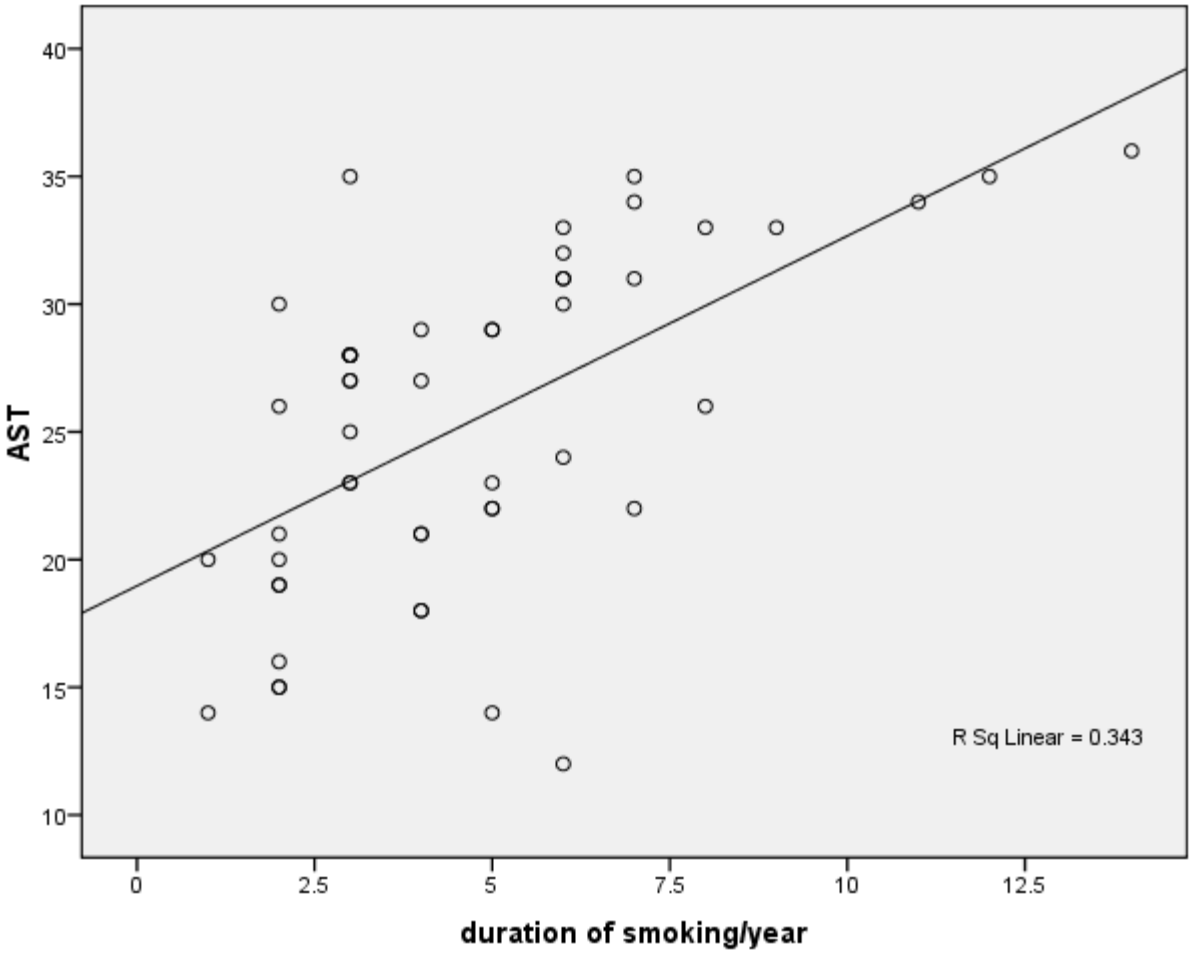


Figure (4.3b): a scatter plot shows a significant positive correlation between AST activity and duration of smoking among Sudanese smokers.

(P=0.00, r =0.58)

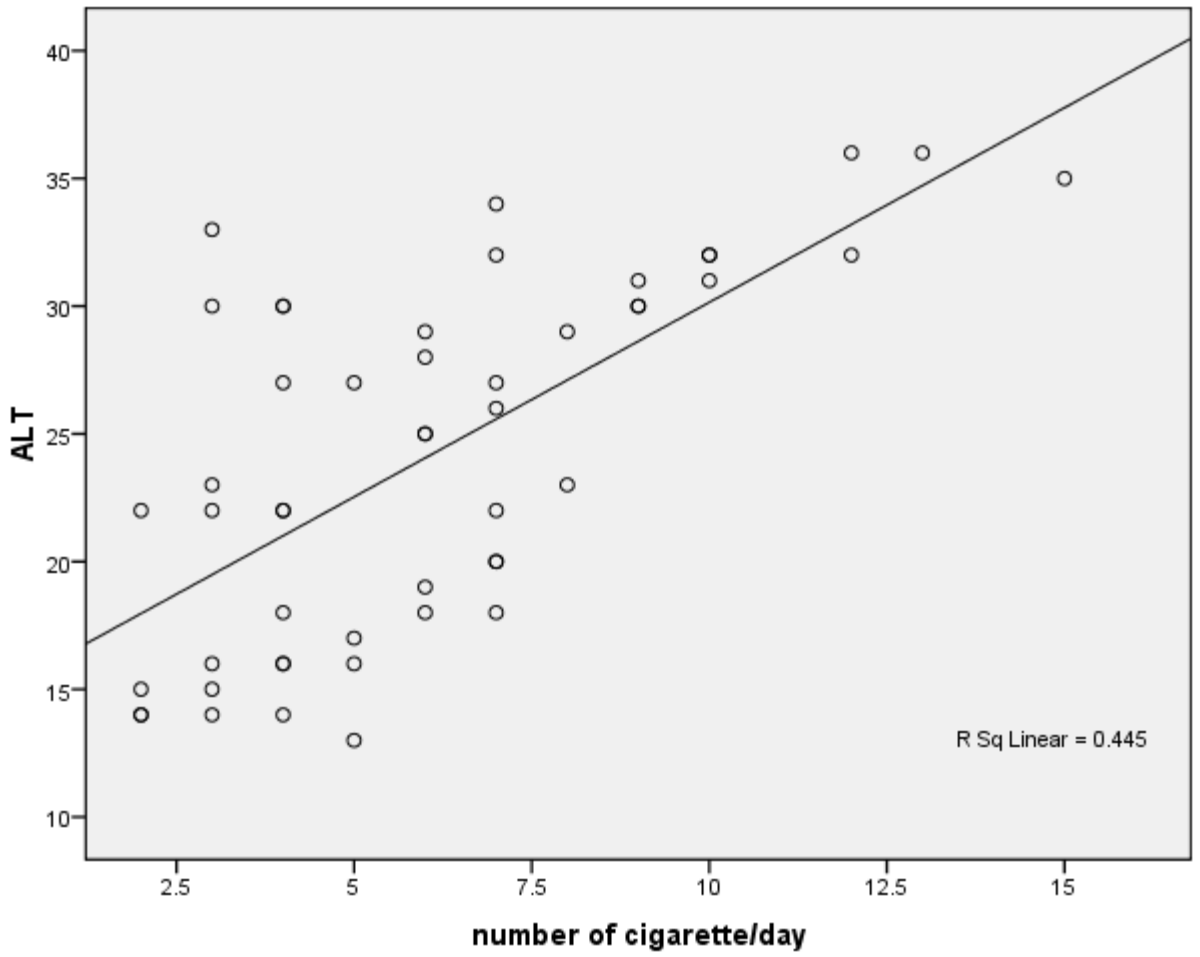


Figure (4.4a): a scatter plot shows a significant positive correlation between ALT activity and number of cigarette per day among Sudanese smokers.

(P=0.00, r = 0.66)

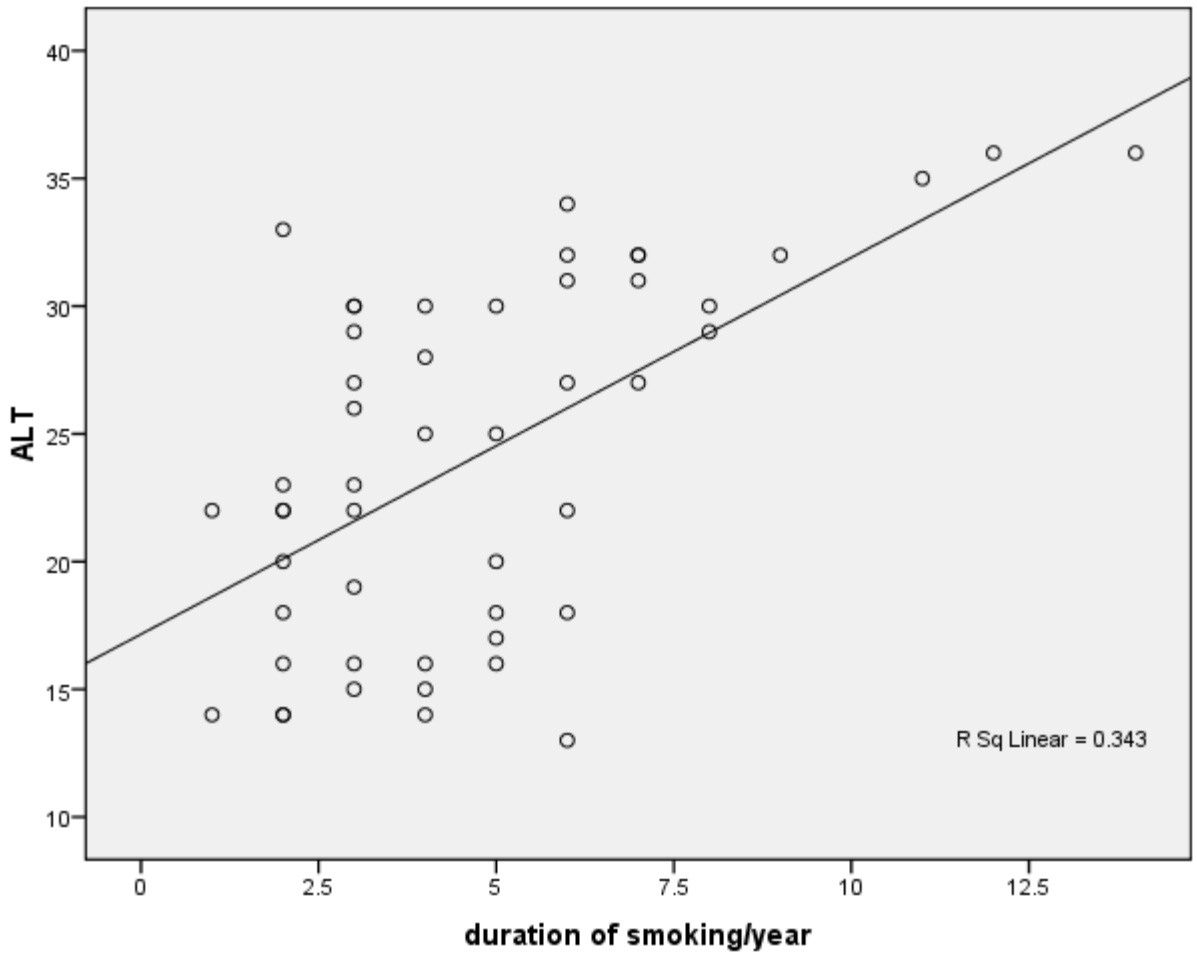


Figure (4.4b): a scatter plot shows a significant positive correlation between ALT activity and duration of smoking among Sudanese smokers.

(P=0.00, r =0.58)

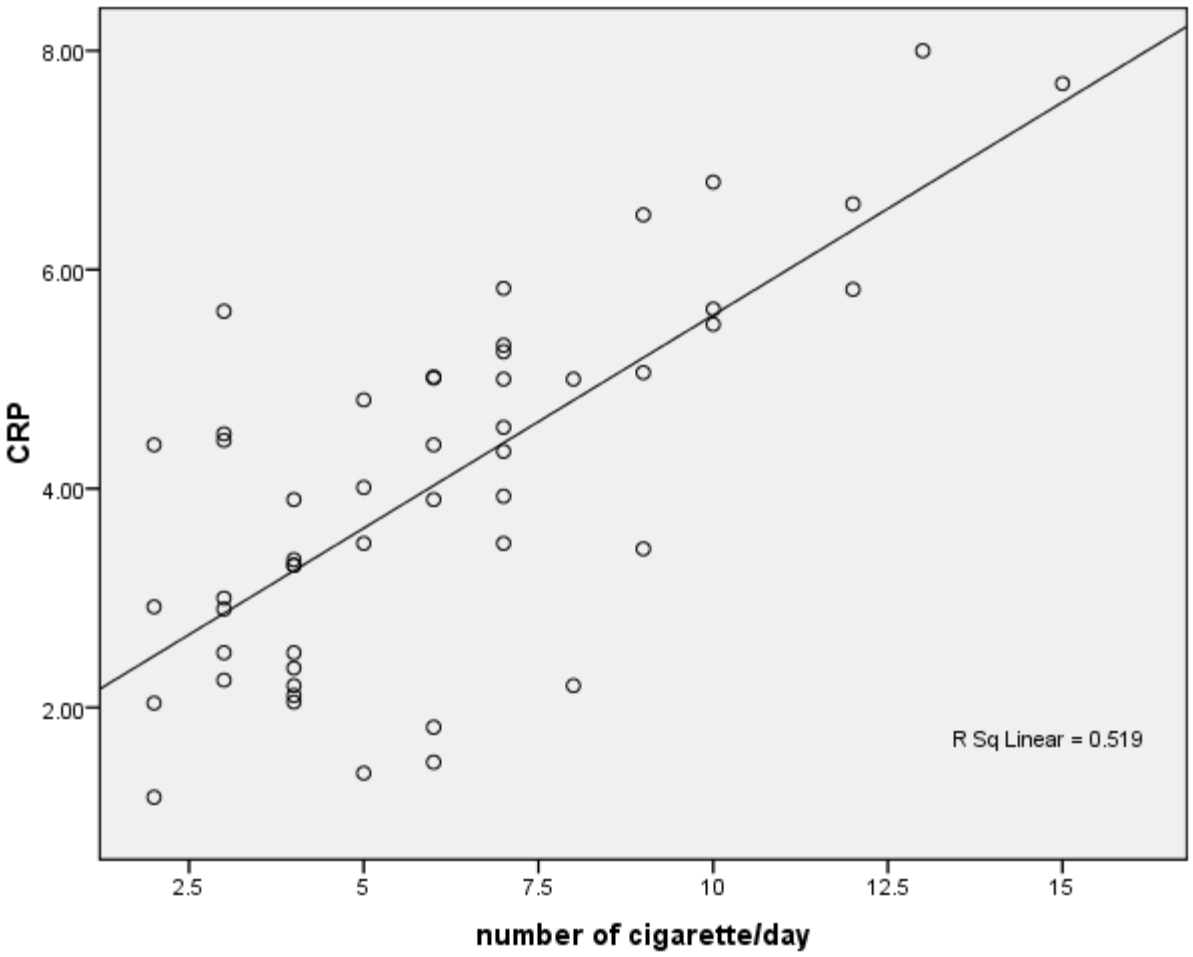


Figure (4.5a): a scatter plot shows a significant positive correlation between CRP level and number of cigarette per day among Sudanese smokers.

(P=0.00, r = 0.72)

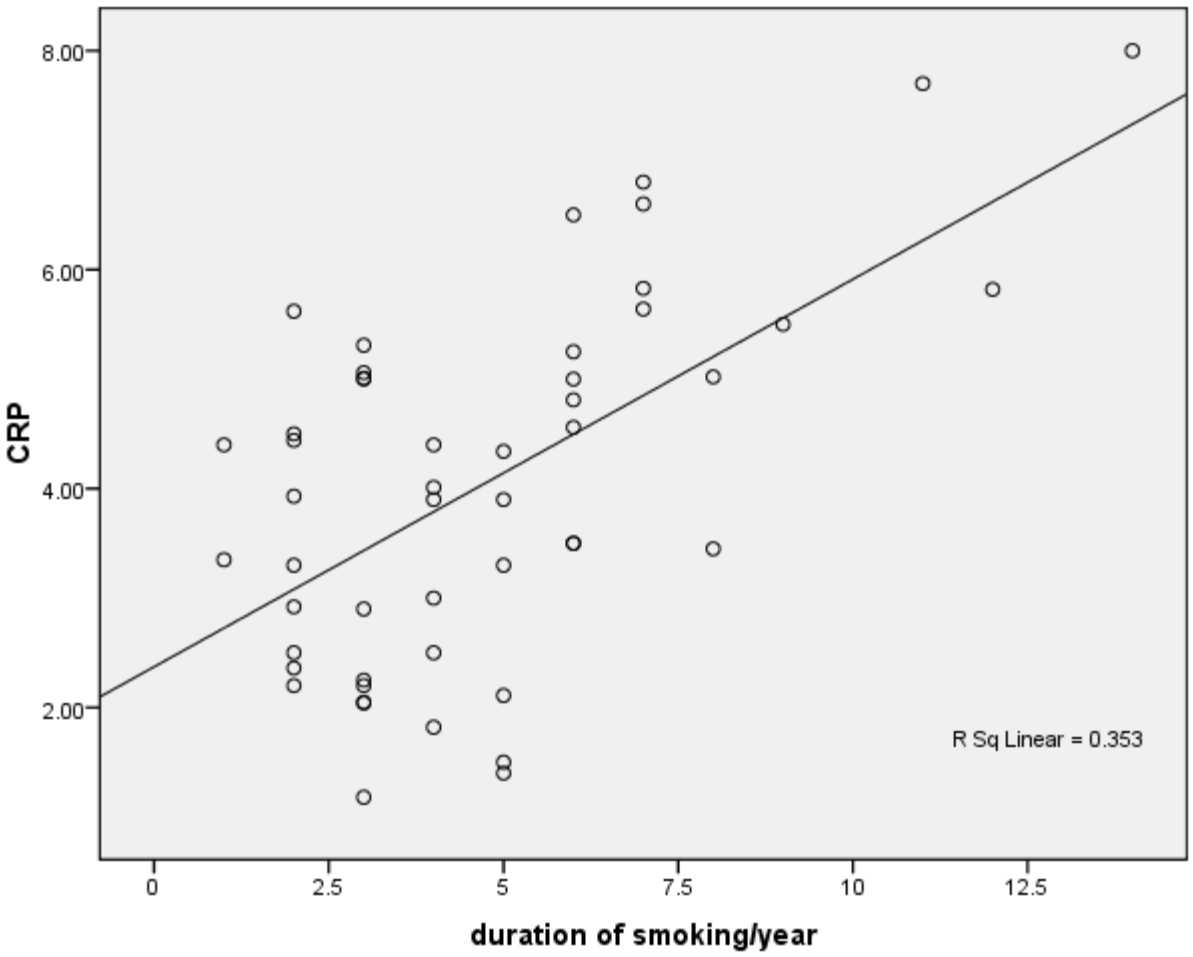


Figure (4.5b): a scatter plot shows a significant positive correlation between CRP level and duration of smoking among Sudanese smokers.

(P=0.00, r =0. 59)

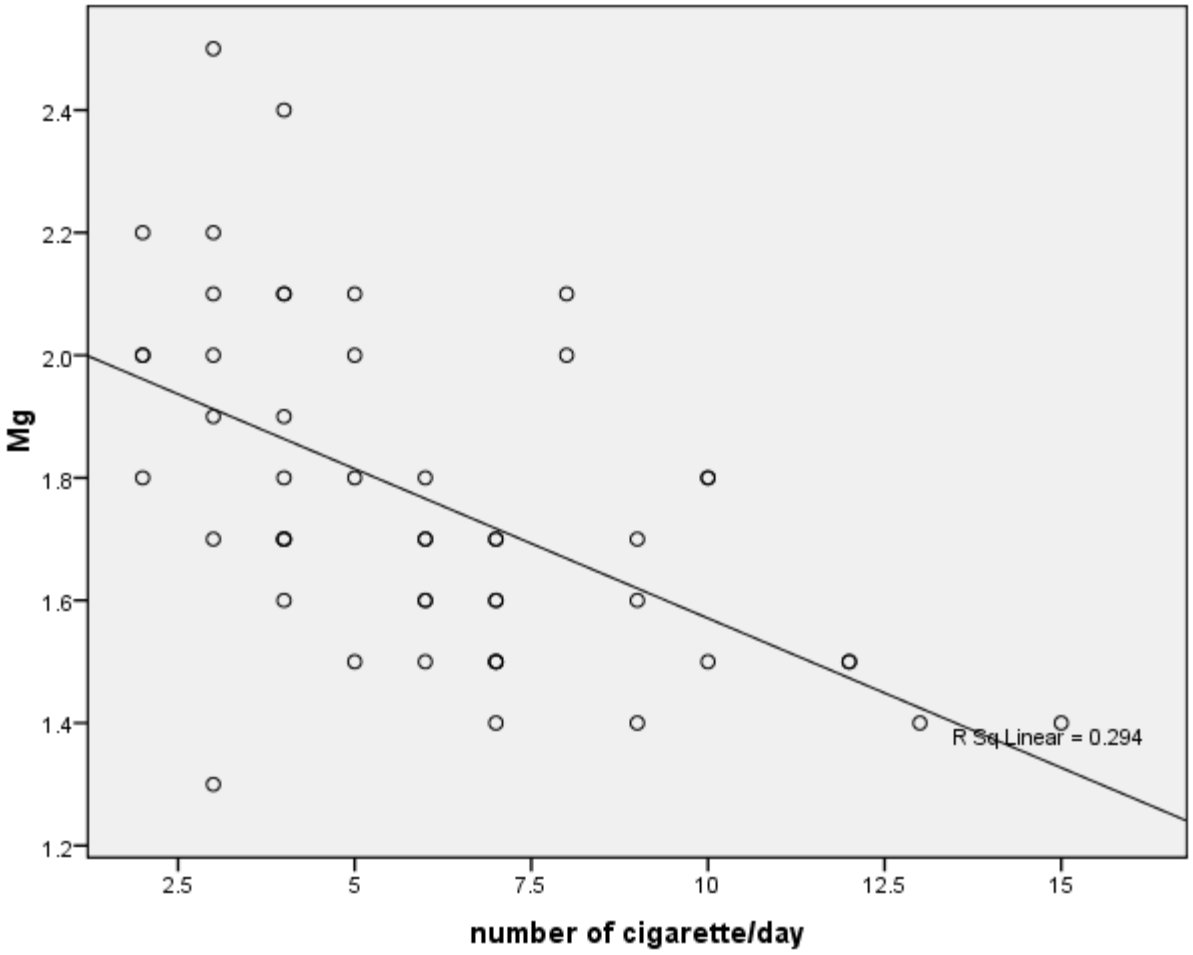


Figure (4.6a): a scatter plot shows a significant negative correlation between Mg level and number of cigarette per day among Sudanese smokers.

(P=0.00, r = -0. 54)

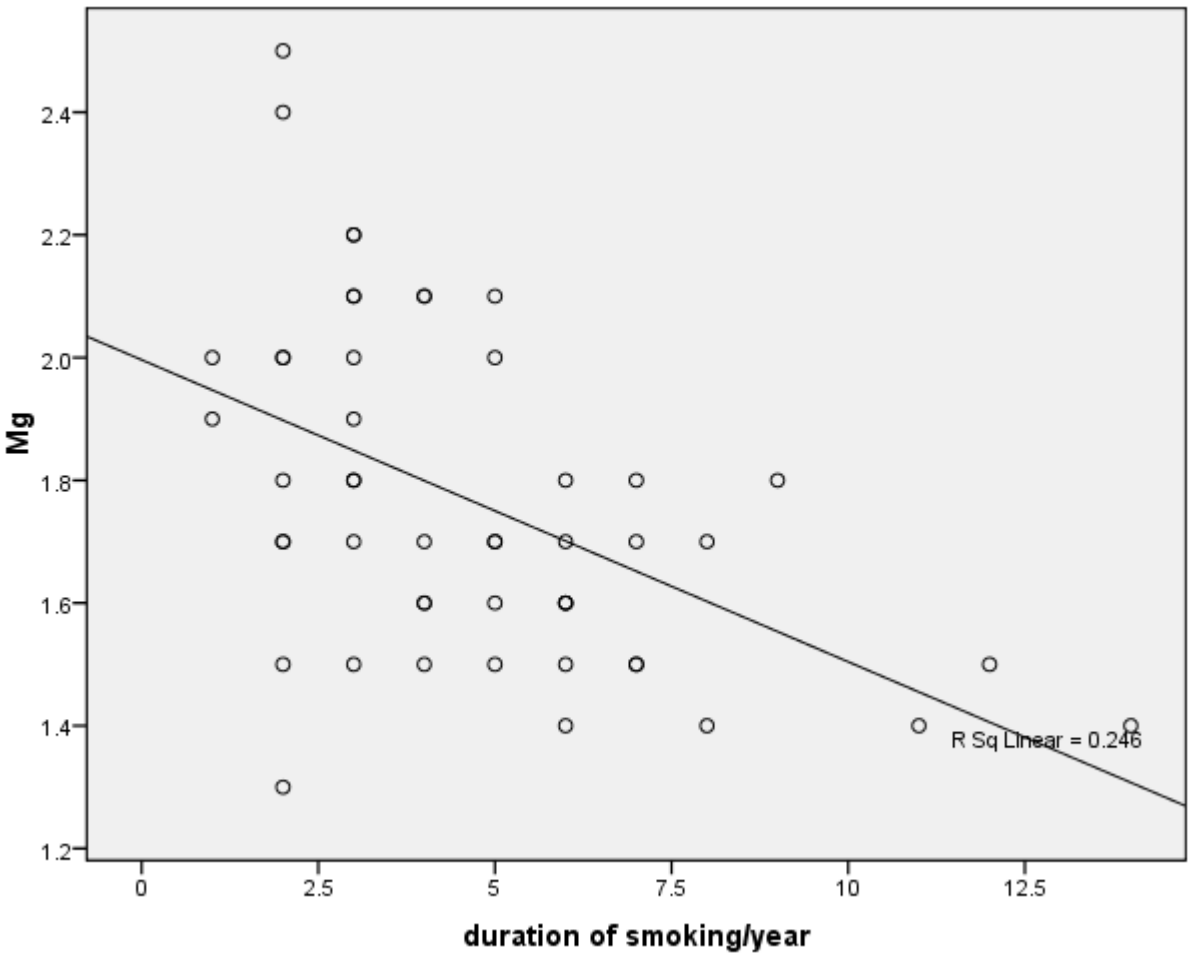


Figure (4.6b): a scatter plot shows a significant negative correlation between Mg level and duration of smoking among Sudanese smokers.

(P =0.00, r = -0.49)

Chapter Five

Discussion, Conclusion and Recommendations

Chapter Five

5. Discussion, Conclusion and Recommendations

5.1 Discussion

Smoking has been considered as one of the 10 greatest contributors to global health problems and it is considered as harmful agent causing disease with multiple organ dysfunction and deaths (Leone., 2005), nicotine is the major component of cigarette smoke plays an important role in the development of many diseases (Czernin and waldherr., 2003). Cigarette smoke contains over 4000 different chemicals, 400 of which are proven to be carcinogenic; it also contains various oxidants such as oxygen free radicals which are probably the major causes of damage to biomolecules (Yeh *et al*., 2008).

The current study showed a significant decrease levels of total protein and albumin in smoker than non smoker (P.value =0.000) this result agree with another result carried by (Jang *et al*., 2012) whom found lowered serum total protein and albumin levels in smoker than non smoker, this result can be explained due to dietary effect on albumin synthesis since smoker may have consumed low protein(lesser appetite)in diet than non smoker (Nathwani *etal*., 2005).

The study show a significant increase in activity of AST and ALT in smoker than non smoker(P.value =0.00) this result agree with another result carried by(Alsalhen., 2014) which showed that the smoker had significantly higher than non smokers in AST and ALT activities .This difference may be due to cigarette smoke propagates the lipid per oxidation, which damage the cell membrane of the liver and serum AST,ALT are enzymes that act as sensitive indicators of hepatocellular damage (Rochling., 2001)

The result obtained from this study indicated that there was significantly lower of the mean serum magnesium level in smokers compared to non –smokers(P.value =0.000) this could be due to smokers may get less magnesium because they tend to eat less and reduced absorption due to digestive system disturbances (Winiarczyk., 2008), also showed CRP level was significantly higher in smokers compared to non –smokers (P.value =0.000), the cause can be smoking triggers inflammation and CRP synthesis increases dramatically in the liver in response to cytokines released by adipocytes and macrophages(Lau D.C *et al*., 2005),which agree with another result done by (Fatehuddin and Shumaila., 2015) ($p<0.001$).

The study showed there were a significant negative correlation between serum total protein, albumin with duration of smoking per years and number of cigarettes per day, result was in agreement with result carried by(Jang *et al*) they found there was significant negative correlation between serum total protein ,albumin with duration of smoking per years and number of cigarettes per day (Jang *et al*., 2012).The result due to dietary effect since smoker may have consumed low protein(lesser appetite)in diet.

The study revealed that significant positive correlation between AST,ALT activities with the number of cigarettes per day and duration of smoking per years, result was in agreement with result carried by(Jang *et a*l), who found there was significant positive correlation between AST,ALT activities with the number of cigarette smoked per day and duration of smoking per years(Jang *et a*l., 2012). Cigarette smoke propagates the lipid peroxidation which damage hepatocytes and serum aminotransferases are enzymes that act as sensitive indicators of hepatocellular , the enzymes are leaked out into blood.

This study showed that, there was a significant positive correlation between CRP level and number of cigarette smoked per day, the result was agree with result carried by (Lowe *et al*), who found there was significant positive correlation between CRP level and the number of cigarette smoked per day(Lowe *et al*., 2001). The cause smoking triggers inflammation and CRP synthesis increases dramatically in the liver.

The study showed that there was a significant positive correlation between CRP level and duration of smoking per years, which agree with result carried by(Shamima) the study showed that there was significant positive correlation between CRP level and number of cigarette smoked per day(Shamima *et al*., 2015) .

The study showed that there was a significant negative correlation between serum magnesium with the number of cigarette smoked per day and duration of smoking per years, which agree with result carried by (Sulafa *et al*) which found that significant negative correlation between serum magnesium and the number of cigarette smoked per day and duration of smoking per years(Sulafa *et al*., 2013).

5.2. Conclusion

The study concludes that Smokers had significant increased in serum ALT, AST activites and CRP level, and significant decreased in serum total protein, albumin and magnesium levels, Smoker also had Serum total proteins, albumin and magnesium were significantly negative correlation with duration of smoking and number of cigarettes smoked per day, where there were a significant positive correlation between serum AST,ALT and CRP with duration of smoking and number of cigarettes smoked per day.

5.3 Recommendations

1. Educational programs is necessary to increase people awareness about serious health effects of smoking and the importance of proteins to the body.
2. Evaluation of liver function test profile helpful in early diagnosis of liver disease among smokers, especially liver enzymes activity to have an early alarm about the oxidative damage to liver .
3. Serum magnesium should be monitored in smokers in addition magnesium supplementation should be given when necessary.

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Appendices

Appendix I

Sudan University of Science and Technology

College of Graduate Studies

Serum Total Protein, Albumin, C-reactive Protein, Magnesium Levels and Aspartate Aminotransferase and Alanine Aminotransferase Activity among Sudanese Male cigarette Smokers in Khartoum State

Questionnaire

Name: ..... No of sample ( ).

Age: ..... Tel.....

Duration of smoking/ years: .....

Number of cigarettes / day: .....

History of other diseases: .....

Results:

Total protein: ……..g /dL

Albumin: …… g /dL

CRP: …… mg/L

Magnesium: …… mg/dL

AST Activity : …….. IU/L

ALT Activity : …….. IU/L

Appendix II

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Total Protein (Biuret) Reagent Set

Intended Use
For the quantitative determination of total protein concentration in serum using the Mindray BS-200 analyzer.

Method History
The color reaction of protein molecules with cupric ions, known as the Biuret color reaction, has been known since 1878. Since the Riegler[1] publications of 1914, several attempts have been made to stabilize the cupric ions in the alkaline reagent. Kingsley,[2,3] modified the procedure in 1939 and 1942 to include the use of sodium potassium tartrate as a complexing agent. This procedure was later modified by Weichselbaum[4] and Gomall.[5] The present method is based on these modifications.

Principle
Protein + Cu^{++} ---(Alkali)---➢ Colored Complex

Protein in serum forms a violet colored complex when reacted with cupric ions in an alkaline solution. The intensity of the violet color is proportional to the amount of protein present when compared to a solution with known protein concentration.

Reagent Content
Sodium Hydroxide 600mM, Copper Sulfate 12mM, Sodium Potassium Tartrate 32mM, Potassium Iodide 30mM, Non-reactive ingredients.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion. DO NOT PIPETTE BY MOUTH. In case of ingestion drink large amounts of water and seek medical attention quickly.
3. Avoid contact with skin and eyes. The reagent contains sodium hydroxide which is corrosive. In case of contact with skin, flush with water. For eyes, seek medical attention.

Reagent Preparation
Reagent comes in a ready to use form.

Reagent Storage
Store reagent at room temperature(15-30˚C). The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration
The reagent should be a clear, pale blue solution. Turbidity or the presence of a black precipitate indicates reagent deterioration and should not be used.

Interferences
Young, et al.[7] has reviewed a number of drugs and substances that may affect protein concentrations.

Materials Provided
Total Protein reagent

Materials Required but not Provided
1. Mindray BS-200 Analyzer
2. BS-200 operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number 12-C7592-50

BS-200 Test Parameters

Test :	TPRO	R1:	250
No.:	030	R2:	0
Full Name:	Total Protein	Sample Volume:	5
Standard No.:		R1 Blank:	
Reaction Type:	End-point	Mixed Rgt. Blank:	
Pri. Wave:	546 nm	Linearity Range:	1.0 – 15.0
Sec. Wave:	670nm	Linearity Limit:	
Direction:	Increase	Substrate Limit:	
Reac. Time:	0 / 11	Factor:	
Incuba. Time:	0	□ Prozone check	
Unit:	g/dl	q1: q2: q3:	q4:
Precision:	0.1	PC: Abs:	

Calibration Parameters

Rule:	Two-point linear	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	Chem Cal
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Correlation Coefficient:			

Specimen Collection and Storage

1. Unhemolyzed serum is the specimen of choice.
2. Gross hemolysis will cause elevated results because of the released hemoglobin as well as the increase in background color.
3. Lipemic sera cause elevated results. A serum Blank should be performed.
4. Samples with bromosulfophthalein (BSP) will result in falsely elevated results.[8]
5. Protein in serum is stable for one week at room temperature (18-25°C) and for at least one month refrigerated (2-8°C) when guarded against evaporation.[6]

Calculation (Example)

Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of standard} = \text{Total Protein (g/dl)}$$

Example: Abs. of Unknown = 0.350, Abs. of Standard = 0.400
Concentration of Standard = 8 g/dl

Then: $\frac{0.350}{0.400} \times 8 = 7.00$ g/dl

Limitations

1. Samples with values above 15.0 g/dl should be diluted 1:1 with 0.9% saline, re-run and result multiplied by two.
2. The Biuret procedure is not sensitive at low ranges (<1 g/dl). Do not use for urine or spinal fluid.

Calibration

Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Quality Control

1. Use control sera with known total protein concentrations to monitor the integrity of the reaction.
2. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values[8]

6.2 – 8.5 g/dl

1. The effect of posture, when blood is drawn, varies with the individual but recumbent values are usually lower than ambulatory. Differences may be as much as 1.2 g/dl.
2. It is strongly recommended that each laboratory establish its own range.

Performance

1. Linearity: 1.0 – 15.0 g/dl
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer and method, resulting in a correlation coefficient of 0.996 with a regression equation of y=0.919 x + 0.36.
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[9]

Within Run			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
4.77	0.13	2.7	4.78	0.07	1.5
7.46	0.16	2.1	7.63	0.14	1.8

Appendix III

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Albumin Reagent Set

Intended Use
For the quantitative determination of Albumin in serum using the Mindray BS-200 analyzer.

Method History
Determination of serum albumin is usually made using an ultra centrifugation, salt fractionation, electrophoretic or dye binding method. Dye binding procedures are the simplest to perform, and lend themselves to high volume testing and automation. They are also the procedures most widely used in combination with total protein determinations to yield an A/G ratio.[1,2] In 1953, the use of methyl orange[3] for direct determination was described. This method suffered from non-specific binding characteristics.[4,5] The use of a HABA[6] dye was introduced in 1954. This method was specific for albumin but displayed poor sensitivity, poor correlation with electrophoresis methods and significant interference from bilirubin, lipids, salicylates, penicillin and sulfonamides.[7]

A bromocresol green (BCG) dye-binding procedure was first proposed in 1964.[8] This procedure exhibited greater sensitivity and much lower

Specimen Collection and Storage [14]
1. Serum is the specimen of choice.
2. Avoid excessive hemolysis since every 100 mg/dl of hemoglobin corresponds to about 100 mg/dl of albumin.
3. Albumin in serum is reported stable for one week at room temperature (18-30°C) and approximately one month when stored in the refrigerator (2-8°C) and protected against evaporation.

Interferences
1. See Young et al[15] for a list of interfering substances.
2. Ampicillin has been found to seriously interfere with BCG methods.[16]

Materials Provided
Albumin reagent.

Materials Required but not Provided
1. Mindray BS-200 Analyzer.
2. BS-200 Operation manual.
3. Chemistry Calibrator, catalog number C7506-50

Reagent Storage
Store the reagent at room temperature (15-30˚C). The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration
The reagent should be clear, yellow-green solution. Turbidity or precipitation makes the reagent unsatisfactory and it should be discarded.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion.
3. Avoid contact. Reagent is an acid solution. Flush with water when contact occurs.
4. Reagent contains Sodium Azide as a preservative. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

Calibration Parameters

Rule:	Two-point linear	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	Chem Cal
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Correlation Coefficient:			

Limitations

1. The dye-binding properties of albumin, other than human, differ among species.[17]
2. Samples with values above 8.0 g/dl should be diluted with 0.9% saline 1:1, re-run, and results multiplied by 2. Samples with results below 0.5 g/dl should be done electrophoretically.
3. Severely lipemic serums should have a serum blank.
    A. Add 0.01 ml (10ul) sample to 1.0 ml deionized water and read absorbance against deionized water at 630 nm.
    B. Subtract the serum blank absorbance from the test absorbance and use the corrected absorbance in the calculations.

Calibration

Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Calculation (Example)

Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of Std.} = \text{Albumin (g/dl)}$$

Quality Control

The validity of the reaction should be monitored by use of normal and abnormal control sera with known albumin concentrations. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values[1]

3.5 – 5.3 g/dl

It is strongly recommended that each laboratory establish its own normal range.

Performance

1. Linearity: 0.5 – 8.0 g/dl
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer and method, resulted in a correlation coefficient of 0.952 with a regression equation of y = 1.076x-0.30 (n=29).
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[18]

Within Run			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
2.92	0.10	3.3	3.15	0.06	1.9
4.44	0.08	1.8	4.84	0.11	2.3

Appendix IV

POINTE SCIENTIFIC, INC.
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Magnesium - XB Reagent Set

Intended Use
For the quantitative determination of magnesium in serum using the Mindray BS-200 analyzer. For *in vitro* diagnostic use only.

Clinical Significance
Magnesium in the body is found primarily in bone with some in soft tissue, blood cells, and serum. Decreased levels have been observed in cases of diabetes, alcoholism, diuretics, hyperthyroidism, hypothyroidism, malabsorption, hyperalimenation, myocardial infarction, congestive heart failure and liver cirrhosis. Increased serum magnesium levels have been found in renal failure, diabetic acidosis, Addison's disease, and vitamin D intoxication.

Precautions
This reagent is for *in vitro* diagnostic use only.
Reagents are Poison/Caustic, Avoid All Contact.
All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd ed., 1988, HHS Publication No. (CDC) 88-8395.

Specimen Collection and Storage
1. Use fresh, unhemolyzed serum or heparinized plasma.
2. Red cells contain twice the magnesium concentration as serum. A hemolyzed sample would falsely elevate results.[10]
3. Grossly icteric or lipemic specimens should not be used in this method.

Principle
Serum magnesium ions react with Xylidyl Blue in alkaline medium to produce a red complex that is measured spectrophotometrically. The intensity of color produced is directly proportional to magnesium concentration. Calcium interference is virtually eliminated by use of EGTA and a surfactant system is included to remove protein interference.

Reagent Composition
When combined the reagent contains: xylidyl blue 0.1mM, EGTA 0.13mM, DMSO 1.4M, Buffer, surfactant, non-reactive stabilizers including potassium cyanide at 0.02% w/v. Caution: Poison/Caustic, Avoid All Contact.

Reagent Preparation
The reagents are ready to use.

Mindray BS-200 Test Parameters

Test :	MG	R1:	200
No.:	026	R2:	200
Full Name:	Magnesium	Sample Volume:	3
Standard No.:		R1 Blank:	
Reac.Type:	Endpoint	Mixed Rgt. Blank:	
Pri. Wave:	546nm	Linearity Range:	0.05 – 4.86
Sec. Wave:	670nm	Linearity Limit:	
Direction:	Increase	Substrate Limit:	
Reac. Time:	0 / 10	Factor:	
Incuba. Time:	3	□ Prozone check	
Unit:	mg/dl	q1: q2: q3: q4:	
Precision:	0.1	PC: Abs:	

Calibration

Use an NIST-traceable serum based calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

Quality Control

The validity of the reaction should be monitored by use of control sera with known normal and abnormal magnesium values. These controls should be run at least with every working shift in which magnesium assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Calculation (Ratiometric Calculation) (Example)

Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of Standard} = \text{Value mg/dl}$$

Example: Abs. of Unknown = .140
Abs. of Standard = .120
Conc. of Standard = 2.4 mg/dl

Then: $\frac{.140}{.120} \times 2.4 \text{ mg/dl} = 2.8 \text{ mg/dl}$

NOTE: "mg/dl" may be converted to "mEq/L" by dividing the result by 1.21525.

Expected Values

Newborns	1.8 - 2.8 mg/dl
Children	1.7 – 2.3 mg/dl
Adults	1.6 – 3.0 mg/dl

The expected values were taken from literature.[13] Each laboratory should establish its own normal range.

Performance

Linearity: 0-05 - 4.86 mg/dl

Comparison: A study was performed between the Mindray BS-200 and a similar analyzer using this method, resulting in a correlation coefficient of correlation of 0.983 with a regression equation of y=0.945x + 0.05. (N=36).

Appendix V

POINTE SCIENTIFIC, INC.
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Liquid AST (SGOT) Reagent Set

Intended Use
For the quantitative determination of Aspartate Aminotransferase (AST) in human serum using the Mindray BS-200 analyzer.

Clinical Significance
AST is widely distributed in tissues with the highest concentrations found in the liver, heart, skeletal muscle and kidneys. Diseases involving any of these tissues can lead to elevated levels of AST in serum. Following myocardial infarction, AST levels are elevated and reach a peak after 48 to 60 hours.

Hepatobiliary diseases such as cirrhosis, metastatic carcinoma and viral hepatitis can show increased levels of AST. Other disorders which can lead to an elevated level of AST are muscular dystrophy, dermatomyositis, acute pancreatitis and infectious mononucleosis.[1]

Method History
Karmen[2] developed a kinetic assay procedure in 1955 which was based upon the use of malate dehydrogenase and NADH. Optimized procedures were presented by Henry[3] in 1960 and Amador and Wacker[4] in 1962. These modifications increased accuracy and lowered the effect of interfering substances. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology[5] published a recommended method based on optimized modifications in 1974. In 1976, the Expert Panel on Enzymes of the International Federation of Clinical Chemistry (IFCC)[6] proposed the addition of pyridoxal-5-phosphate to the reaction mixture to ensure maximum activity. The IFCC[7] published a recommended method that included P-5-P in 1978. The present method is based on IFCC recommendations but does not contain P-5-P since most specimens contain adequate amounts of this cofactor for full recovery of AST activity.[8,9,10]

Principle
$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate} + \text{L-Glutamate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^+ + \text{H}_2\text{O}$$

Reagent Deterioration
Do not use reagent if:
1. The initial absorbance at 340nm is below 0.800.
2. The reagent fails to meet stated parameters of performance.

Precautions
1. This reagent set is for *in vitro* diagnostic use only.
2. The reagent contains sodium azide (0.28%) as a preservative. Do not in gest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

Specimen Collection and Storage[11]
1. Non-hemolyzed serum is recommended. Red cells contain AST which can give falsely elevated results.
2. AST in serum is reported stable for ten days when refrigerated (2-8°C), two weeks when frozen (-20°C), and four days when stored at room temperature (15-30°C).

Interferences
1. A number of drugs and substances affect AST activity. See Young, et al.[12]
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.[13]
3. Bilirubin to at least 18 mg/dl, and hemoglobin to at least 300 mg/dl, have been found to have a negligible effect on this procedure.

Materials Provided
AST (SGOT) Reagents R1 and R2

Materials Required but not Provided
1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry control, catalog number 12-C7592-50

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α-Ketoglutarate to yield oxalacetate and L-glutamate. The oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

Reagents
After combining R1 and R2, the reagent contains: L-aspartic acid 200mM, α-ketoglutaric acid 11mM, LDH (microbial) > 1000U/L, MDH (microbial) ≥800U/L, NADH >0.18mM, buffer, sodium azide 0.28%, stabilizers.

Reagent Preparation
The reagents are ready to use.

Reagent Storage
Store the reagents at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed.

BS-200 Test Parameters

Test::	AST	R1:	200
No.:	005	R2:	50
Full Name:	AST	Sample Volume:	10
Standard No.:		R1 Blank:	
Reaction Type:	Kinetic	Mixed Rgt. Blank:	
Pri. Wave:	340nm	Linearity Range:	0 - 500
Sec. Wave:	405nm	Linearity Limit:	0.2
Direction:	Decrease	Substrate Limit	0.15
Reac. Time:	3 / 11	Factor:	4200
Incuba. Time:	3	□ Prozone check	
Units	U/L	q1: q2: q3: q4:	
Precision:	Integer	PC: Abs:	

Liquid AST (SGOT) Reagent Set

Calibration Parameters

Rule:		Calibrator 1:	
Sensitivity:		Calibrator 2:	
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Correlation Coefficient:			

Limitations
1. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.[13]

Calibration
The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

Calculation (Example)
One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

Expected Values[13]
8 to 22 IU/L (30°C)
5 to 34 IU/L (37°C)
Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Performance
1. Linearity: 0-500 IU/L.
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer using this method, resulting in a correlation coefficient of 0.996 and a regression equation of y=1.069 x + 0.6. (n=50).
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[14]

Within Run			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
39.8	1.7	4.2	50.3	1.4	2.78
182.6	3.2	1.8	194.5	3.8	1.95

4. Sensitivity: The sensitivity for this reagent was investigated by reading the change in absorbance at 340nm for a saline sample and samples with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, the AST (SGOT) reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L AST activity gives a ΔAbs/Min. of 0.0004.

$$\text{AST (IU/L)} = \frac{\Delta\text{Abs./Min.} \times 1.10 \times 1000}{6.22 \times 0.10 \times 1.0} = \Delta\text{Abs./min.} \times 1768$$

Where ΔAbs./Min. = Average absorbance change per minute
1000 = Conversion of IU/ml to IU/L
1.10 = Total reaction volume (ml)
6.22 = Millimolar absorptivity of NADH
0.10 = Sample Volume (ml)
1.0 = Light path in cm

Example: If the average absorbance change per minute = 0.12 then 0.12 x 1768 = 212 IU/L

NOTE: If test parameters are altered the factor has to be recalculated using the above formula.

SI Units: To convert to SI Units (nkat/L) multiply IU/L by 16.67.

Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal AST (SGOT) values. These controls should be run at least with every shift in which AST (SGOT) assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Appendix VI

POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

Liquid ALT (SGPT) Reagent Set

Intended Use
For the quantitative determination of Alanine Aminotransferase (ALT) in serum using the Mindray BS-200 analyzer.

Clinical Significance
ALT is widely distributed in tissues with the highest concentrations found in the liver and kidneys. Even so, ALT is considered more liver-specific than AST. Elevated levels of ALT are often only observed in liver diseases such as cirrhosis, hepatitis, or metastatic carcinoma. However, there can be elevated levels of ALT with infectious mononucleosis, muscular dystrophy, and dermatomyositis.[1]

Specimen Collection and Storage
1. Hemolyzed samples cannot be used as red cells contain ALT.[7]
2. ALT in serum is stable for three days at room temperature (15-30°C), seven days refrigerated (2-8°C), and thirty days frozen (-20°C).[7]

Interferences
1. A number of drugs and substances affect ALT activity. See Young, et al.[8]
2. Bilirubin to at least 30 mg/dl, and hemoglobin to at least 400 mg/dl, have been found to have a negligible effect on this procedure.

Materials Provided
ALT (SGPT) Reagents R1 and R2

Method History
UV methods for ALT determination were described by Henley[2] in 1955 and Wroblewski and La Due[3] in 1956. The procedure was improved and optimized by Henry et al[4] in 1960. In 1974, the Scandinavian Society for Clinical Chemistry[5] recommended optimized reaction conditions. The International Federation of Clinical Chemistry (IFCC)[6] published a proposed recommended method in 1980 utilizing the LDH-NADH coupled assay. The procedure described herein is based on that method.

Materials Required but not Provided
1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry control, catalog number 12-C7592-50

Principle
$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+ + H_2O$$

ALT catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH to NAD. The resulting rate of decrease in absorbance is directly proportional to ALT activity.

Reagents
After combining R1 and R2, the reagent contains: L-alanine >450mM, α-ketoglutaric acid >14mM, LDH(microbial) >2000IU/L, NADH >0.18mM, buffer, sodium azide 0.28%, Stabilizers.

BS-200 Test Parameters

Test :	ALT	R1:	200
No.:	003	R2:	50
Full Name:	ALT	Sample Volume:	10
Standard No.:		R1 Blank:	
Reac. Type:	Kinetic	Mixed Rgt. Blank:	
Pri. Wave:	340nm	Linearity Range:	0 - 500
Sec. Wave:	405nm	Linearity Limit:	0.2
Direction:	Decrease	Substrate Limit:	
Reac. Time:	3 / 11	Factor:	4700
Incuba. Time:	3	□ Prozone check	
Unit:	U/L	q1: q2: q3: q4:	
Precision:	Integer	PC: Abs:	

buffer, sodium azide 0.28%, stabilizers.

Reagent Preparation
The reagents are ready to use.

Reagent Storage
Store the reagents at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration
Do not use reagent if:
1. The initial absorbance at 340nm is below 0.800.
2. The reagent fails to meet stated parameters of performance.

Precautions
1. This reagent set is for *in vitro* diagnostic use only.
2. The reagent contains sodium azide (0.28%) as a preservative. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

Calibration Parameters

Rule:		Calibrator 1:	
Sensitivity:		Calibrator 2:	
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Correlation Coefficient:			

Liquid ALT (SGPT) Reagent Set

Limitations
1. Turbid or highly icteric samples may give readings whose initial absorbance exceeds the capabilities of the spectrophotometer. More accurate results may be obtained by using 0.05ml (50ul) of sample and multiplying the final answer by two.
2. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Calibration
The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

Calculation (Example)
One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$\text{ALT (IU/L)} = \frac{\Delta\text{Abs./Min.} \times 1.10 \times 1000}{6.22 \times 0.10 \times 1.0} = \Delta\text{Abs./min.} \times 1768$$

Where ΔAbs./Min. = Average absorbance change per minute
1000 = Conversion of IU/ml to IU/L
1.10 = Total reaction volume (ml)
6.22 = Millimolar absorptivity of NADH
0.10 = Sample Volume (ml)
1.0 = Light path in cm

Example: If the average absorbance change per minute = 0.12 then 0.12 x 1768 = 212 IU/L

3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

Within Run (n=20)			Day to Day (n=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
35.4	1.3	3.6	41.8	1.5	3.6
89.5	1.1	1.3	111.9	2.6	2.3

4. Sensitivity: The sensitivity for this reagent was investigated by reading the change in absorbance at 340nm for a saline sample and serums with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, the ALT (SGPT) reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L ALT activity gives a ΔAbs/Min. of 0.0004.

NOTE: If test parameters are altered the factor has to be recalculated using the above formula.

SI Units: To convert to SI Units (nkat/L) multiply IU/L by 16.67.

Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal ALT (SGPT) values. These controls should be run at least with every shift in which ALT (SGPT) assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values[9]

4 to 24 IU/L (30°C)
4 to 36 IU/L (37°C)
Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Performance

1. Linearity: 0-500 IU/L.
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer using this method, resulting in a correlation coefficient of 0.999 and a regression equation of y=0.94x + 5.8. (n=33).

Appendix VII

Infection

ichroma™ CRP

INTENDED USE

ichroma™ CRP is a fluorescence Immunoassay (FIA) for the quantitative determination of CRP in human whole blood / serum / plasma. It is useful as an aid in management and monitoring of autoimmune diseases and infectious processes, such as rheumatoid arthritis.[1,2]

For *in vitro* diagnostic use only.

INTRODUCTION

The C-Reactive Protein (CRP) is synthesized by the liver in response to interleukin-6 and well known as one of the classical acute-phase reactants and as a marker of inflammation. CRP is the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage. The acute-phase response comprises the nonspecific physiological and biochemical responses of endothermic animals to most forms of tissue damage, infection, inflammation, and malignant neoplasia. The serum CRP level may rise from a normal level of <5 mg/L to 500 mg/L during the body's general, non-specific response to infectious and other acute inflammatory events. For some time, the measurement of CRP concentration has been used as a clinical tool for monitoring autoimmune diseases and infectious processes, such as rheumatoid arthritis.[1,2]

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show CRP concentration in sample.

COMPONENTS

ichroma™ CRP consists of 'Cartridges', 'Detection Buffer Tubes', 'Sample Collectors' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human CRP at the test line, while rabbit IgG at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human CRP-fluorescence conjugate, anti rabbit IgG-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is pre-dispensed in a tube. 25 detection buffer tubes are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.

- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Do not keep the sample in a freezer, which could affect the test value of CRP. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ CRP** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ CRP** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ CRP** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, heparin sodium, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4 - 30°C.
- The detection buffer pre-dispensed in a tube is stable for 20 months if stored at 2 - 8°C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF i-CHROMA CRP-25

Components of **ichroma™ CRP**

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
  - Sample Collectors 25
- Box containing Detection Buffer tubes
  - Detection Buffer Tubes 25

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ CRP**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ D** REF 13303
- **ichroma™ Printer** REF FPRR007
- **ichroma™ CRP Control** REF CFPO-2

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ CRP** is human whole blood / serum / plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood. If longer storage is required, e.g. if the test could not be performed within 24 hours, serum or plasma should be immediately frozen below -20 °C. The freezing storage of sample up to 3 months does not affect the quality of results.
- The whole blood sample should not be kept in a freezer in any case.
- Once the sample was frozen, it should be thawed one time and only for test, because repeated freezing and thawing can result in the change of test values.
- Fingertip blood sample should be collected as follows:
  - Position the hand with the palm facing upwards. Blood should be normally drawn from the middle or ring finger of the non-dominant hand. Apply intermittent pressure towards its tip.
  - Wipe the fingertip clean with an alcohol pad.
  - Allow the finger to dry completely because blood will not form a drop if the puncture site is moist and because the residual alcohol at the fingertip may dilute the blood sample and affect the test result.
  - Hold the finger and puncture the fingertip by firmly pressing a new sterile lancet against it at an off-center position.
  - Wipe away the first drop of blood with a sterile gauze pad or cotton ball.
  - Massage the finger towards its tip to form a new drop of blood. Blood will flow easily if the finger is held lower than the elbow.
  - Hold the handle of a capillary tube and touch the mouth of the capillary to the drop of blood.
  - Let the blood fill the capillary tube completely.
  - It may be sometimes necessary to massage the finger again for an additional drop of blood for filling the capillary tube.

TEST SETUP

- Check the contents of **ichroma™ CRP**: Sealed Cartridge, Detection Buffer Tubes, Sample Collector and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the detection buffer.
- Keep the sealed cartridge (if stored in refrigerator) and the detection buffer tube at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests.
- Press the 'Select' button on the instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

1) Make a puncture on the top of the detection buffer tube by inserting an empty sample collector.
2) Draw 10 µL (Human whole blood / serum / plasma / control) of sample with a sample collector.
3) Assemble the sample collector and the tube into one.
4) Shake the 10 times or more until the sample out of the sample collector by inversion. The mixture of buffer and the sample has to be used within 30 seconds.
5) Remove the cap off the top of assembled tube. Discard two drops of reagent onto the paper towel before applying to the cartridge
6) Load only two drops of the mixture onto the sample well of the cartridge.
7) Leave the Cartridge at room temperature for 3 min before inserting the device into the holder.
8) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
9) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
10) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
11) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays CRP concentration of the test sample in terms of mg/L.
- The cut-off (reference value) : 10 mg/L
- Working range : 2.5~300 mg/L.
- Effect of Hematocrit
  The CRP Whole Blood of ichroma™ Reader is calibrated to read the CRP serum concentration of a blood sample with a hematocrit of 40%. If the actual hematocrit value deviates from 40%, the result should be corrected by multiplying with the respective factor in the table: deviates from 40%, the result should be corrected by multiplying with the respective factor in the table:

Hct %	Factor	Hct %	Factor
20-29	0.8	56-58	1.4
30-36	0.9	59-61	1.5
37-42	1.0	62-63	1.6
43-47	1.1	64-65	1.7
48-51	1.2	66-67	1.8
52-55	1.3	68-69	1.9

Reference range, HCT:

- Woman: 35 – 44 %
- Men: 39 – 48 %

QUALITY CONTROL

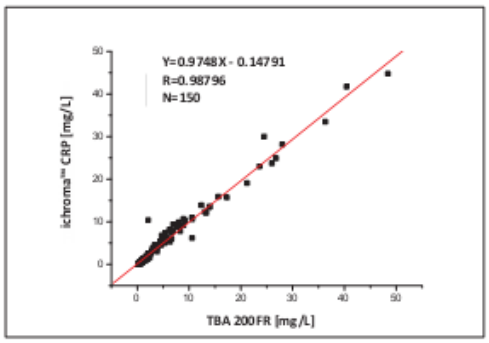
- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ CRP**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Specificity**: There, in test samples, are biomolecules such as hemoglobin, CEA, AFP, ALT, Troponin I, CK-MB, Albumin, and serum amyloid P component in higher concentration than their normal physiological levels. But this doesn't interfere with the **ichroma™ CRP** test measurements, nor occurs any significant cross-reactivity.
- **Precision**: The intra-assay precision was calculated by one evaluator, who tested different concentration of control standard twenty times each with three different lots of **ichroma™ CRP.** The inter-assay precision was confirmed by 3 different evaluators with 3 different lots, testing ten times each different concentration.

CRP Concentration (mg/L)	Intra assay		Inter assay	
	Mean value (mg/L)	CV (%)	Mean value (mg/L)	CV (%)
5	4.9	4.2	4.6	7.2
100	100	2.3	101.6	5.9
250	251.3	3.9	251.2	4.6

- **Comparability:** CRP concentrations of 150 serum samples were quantified independently with ichroma™ CRP and TBA 200FR as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=0.9748X - 0.14791 and R = 0.98796 respectively.



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Note: Please refer to the table below to identify various symbols

	Read instructions for use
	Use by
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance; please contact:
Boditech Med Inc.'s Technical Services
Tel: +82 33 243-1400
E-mail: sales@boditech.co.kr

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www.boditech.co.kr

EC REP **Obelis s.a**
Bd. Général Wahis 53,
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E-Mail: mail@obelis.net

CE

Appendix VII

Infection

ichroma™ CRP

INTENDED USE

ichroma™ CRP is a fluorescence Immunoassay (FIA) for the quantitative determination of CRP in human whole blood / serum / plasma. It is useful as an aid in management and monitoring of autoimmune diseases and infectious processes, such as rheumatoid arthritis.[1,2]

For *in vitro* diagnostic use only.

INTRODUCTION

The C-Reactive Protein (CRP) is synthesized by the liver in response to interleukin-6 and well known as one of the classical acute-phase reactants and as a marker of inflammation. CRP is the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage. The acute-phase response comprises the nonspecific physiological and biochemical responses of endothermic animals to most forms of tissue damage, infection, inflammation, and malignant neoplasia. The serum CRP level may rise from a normal level of <5 mg/L to 500 mg/L during the body's general, non-specific response to infectious and other acute inflammatory events. For some time, the measurement of CRP concentration has been used as a clinical tool for monitoring autoimmune diseases and infectious processes, such as rheumatoid arthritis.[1,2]

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show CRP concentration in sample.

COMPONENTS

ichroma™ CRP consists of 'Cartridges', 'Detection Buffer Tubes', 'Sample Collectors' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human CRP at the test line, while rabbit IgG at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human CRP-fluorescence conjugate, anti rabbit IgG-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is pre-dispensed in a tube. 25 detection buffer tubes are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Do not keep the sample in a freezer, which could affect the test value of CRP. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ CRP** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ CRP** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ CRP** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, heparin sodium, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4 - 30°C.
- The detection buffer pre-dispensed in a tube is stable for 20 months if stored at 2 - 8°C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.