بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Graduate Studies

Evaluation of High Sensitive C reactive Protein among Adult Smokers in Khartoum State.

تقويم عالي الحسّاسية بروتين (سي) التفاعلي لدي المدخنيين البالغين في ولاية الخرطوم

A dissertation Submitted in partial Fulfillment for requirements of MSc Degree in Medical Laboratory Science (Clinical Chemistry)

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قال تعالي:

﴿وَيُحِلُّ لَهُمُ الطَّيِّبَاتِ وَيُحَرِّمُ عَلَيْهِمُ الْخَبَائِثَ﴾

صدق الله العظيم

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Dedication

To..

My life beats, for whom I work to make their dreams, my parents **Ekhlas ,** and soul of my father **Hamza** …

To ..

My life brightness, without whom I could not continue smile, my brothers **Muaiz , Montser** ..

To ..

My life flavor, my lovely sister **Mozen**..

To ..

My life **friends** , they are filling all my days ..

To ..

The absolutely necessary person..the gentle **reader** ..

Mohammed

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Allah enabled me to conduct this study by his blessing therefore thanks for my god **Allah** firstly and lastly..

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Great thanks for **my family**, always encourage me for thebetter..

Thanks for **my friends**, who helped me and made my work wonderful ..

Last thanks for **everyone** helped me in my research ..

Abstract

Cigarette smoking is a classical and a major risk factor in the development of several diseases such as cardiovascular diseases.

A cross sectional study conducted at the February and March 2015 aim to evaluate the effect of cigarette smoking on High Sensitive C Reactive Protein level in Sudanese smokers. In this study 175 subjects were chosen for determination of plasma High Sensitive C Reactive Protein. Fifty were heavy smokers, fifty were light smokers, Twenty five were ex-smokers the other fifty were apparently healthy and not smoker subjects servers as control group for the comparison. All the tests and control group are fall within the middle age (29 years). Plasma High Sensitive C Reactive Protein was estimated by Cobas C 311 automated chemistry analyzer and data analysis done by using t-test and Pearson correlation, by using the computer program Statistical Package for Social Sciences. Analysis showed that there is a significant increases in the mean value of High Sensitive C Reactive Protein in smokers when compared with control group with (*P*-value 0.013) and the mean values of High Sensitive C Reactive Protein in heavy and ex-smokers was significant high when compared to control with (*P*-value 0.000) (*P*-value 0.025) respectively. While showed no statistical significances difference in High Sensitive C Reactive Protein level in light smokers when compared to control with (*P*-value 0.402).

There was no statistical significant difference in High Sensitive C Reactive Protein level in current smokers with ex-smokers with (*P*-value 0.995).

However, There was a positive correlation between High Sensitive C Reactive Protein and smoking duration (r= 0.281, p =0.006).

The study concluded that plasma High Sensitive C Reactive Protein is elevated in cigarette smokers individual and elevation of hs-CRP is depending on smoking type and duration.

مستخلص الدراسة

تدخين السجائر هو عامل خطر و رئيسي في تطورالعديد من الأمراض مثل أمراض القلب والأوعية الدموية.

أجريت دراسة مستعرضة وصفية في فبراير ومايو 2015 هذه الدراسةِ أجريت في الخرطوم لتقويمتأثيرَ تدخين السجائر علي عالي الحساسية بروتين سي التفاعلي لدي المدخنين السودانين.

وتم اختيار مئة وخمس وسبعين فردا لدراسة عالي الحساسية بروتين سي التفاعلي في البلازما خمسون منهم كَانوا مدخّنونبإسرافَ ،وخمسونكَانوا قليلي التدخين،خمسة وعشرون كَانوامدخنين سابقينَ ،خمسون كَانوا أصحّأ على ما يبدو ولَيسوا مدخّنين أخضعُوا كمجموعة قياسيةللمقارنةِ كُلّ المدخنين والمجموعة القياسية يَقِعانِ ضمن العُمرِ المتوسّطِ (29 عاما).

تم قياس عالي الحساسية بروتين سي التفاعلفي بلازما الدم بواسطة محلّل الكيمياءِ الآليِ كوباس سي 311 وتحليل البيانات تم باستخدام اختبار (ت) وارتباط بيرسون باستخدام برنامج الكومبيوتر الحزمة الإحصائية للعلوم الاجتماعية.

أظهرت النتائج ان هناك زيادة كبيرة في القيمةِ المتوسطةِ لمستوى عالي الحساسية بروتين سي التفاعلي في المدخنين مقارنة بالمجموعة القياسيةِمَع (قيمة بي0.013).

والقِيَم المتوسطة لمستوي عالي الحساسية بروتين سي التفاعلي لدي المدخنين الثقيلينالوسابقينِ كَانَ عالي عندما قورن بالمجموعة القياسيةمَع (قيمة بي 0.000) (قيمة بي 0.025) على التوالي.

بينما لاتوجد أهمية أحصائية للقيم المتوسطة لمستوي عالي الحساسية بروتين سي التفاعلي لدي قليلي التدخين عندما قَورنوا بالمجموعة القياسية علي التوالي مع (قيمة بي 0.402).

بينما لاتوجد دلالات فرق أحصائية للقيم المتوسطة لمستوي عالي الحساسية بروتين سي التفاعلي لدي المدخّنِين الحاليِن مَع المدخّنِين السابقِين مَع (قيمة بي 0.995).

ومع ذلك هنالك ارتباط أيجابي مباشر بين مستوي عالي الحساسية بروتين سي التفاعلي و مدة التدخين وقيمة بيرسون ساوت (0.281) وقيمة بي ساوت (0.006).

في هذه الدراسة تم التوصل الي ان مستوي عالي الحساسية بروتين سي التفاعلي مرتفع في مدخنين السجائر مقارنة بغير المدخنين ويعتمد علي نوع التدخين ومدة التدخين.

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List of Abbreviations

Abbreviation	Full Name
AD	Alzheimer's Disease
COPD	Chronic Obstructive Pulmonary Disease
CRP	C Reactive Protein
CVD	Cardiovascular Disease
Da	Dalton
dL	Milligrams per deciliter
ELISA	Enzyme Linked Immunosorbant Assay
ESR	Erythrocyte Sedimentation Rate
G	Gram
H	Hour
hs-CRP	High sensitivity C Reactive Protein
IBD	Inflammatory Bowel Disease
IL	Inter Leukin
Mg	Milligram
Mg/dl	Milligrams per deciliter
Ml	Milliliter
OSA	Obstructive Sleep Apena
PRR	Pattern Recognition Receptor
rpm	Round Per Minute
SAP	Serum Amyloid protein
SLE	Systemic Lupus Erythematosus
SPSS	Statistical Package of Social Science

TNF	Tumor Necrosis Factor
WBC	White Blood Cell
WHO	World Health Organization

Chapter One

Introduction and Literature Review

1. Introduction and Literature Review

1.1 Introduction

Cigarette smoking is a classical and a major risk factor in the development of several diseases with an inflammatory component including cardiovascular disease and chronic obstructive pulmonary disease. Improvements in assays for protein markers of inflammation have led to many studies on these factors and their roles in disease. The links between smoking and increased morbidity and mortality have been long established and current trends indicate that of the one billion smokers worldwide, 500 million will die prematurely from smoking-related diseases. Smoking has been shown to have harmful effects on numerous organs of the body and the list of diseases where smoking has been recognized as a contributory factor is extensive. It has long been accepted that cigarette smoking is a classical and major risk factor in the development of cardiovascular disease (CVD) and atherosclerosis. More recently it has been recognized that CVD contains a component of inflammation and has even been referred to as an inflammatory disease. In addition a link has been established between several other chronic inflammatory diseases and smoking including chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, systemic lupus erythematosus (SLE) and Crohn's disease. Although the mechanisms linking smoking to these diseases are not well understood interest in the relationship between inflammatory markers and smoking has been gathering pace in an attempt to provide explanations for smoking-mediated morbidity and mortality. It has been proven that an elevated CRP level, with a cut-off point of approximately 3 mg/l, is associated with an increased risk of occlusive arterial disease, especially acute coronary syndromes (Tonstad *et al*, 2009). As well as the major increases in expression of C- Reactive protein (CRP) in response to infection or tissue injury, minor elevation in CRP levels has been recognized as a possible marker of disease in systemic conditions

This has been aided by the development of assays to measure CRP levels with far greater sensitivity than previous methods (described as high-sensitivity or hs-CRP assays) and has led to a flood of literature investigating CRP levels in healthy and diseased individuals (Ridker, 2001).

1.2. Objective

1.2.1 General objective

To evaluate the effect of cigarette smoking on high sensitive C reactive protein among a Sudanese cigarette smokers in Khartoum state during February to May 2015.

1.2.2 Specific objectives

1. To estimate high sensitivity C reactive protein level in cigarette smokers.

2. To compare means concentration of high sensitivity C reactive protein in cigarette smokers and control groups.

3. To compare means concentration of high sensitivity C reactive protein in heavy, light, Ex-smokers and control groups.

4. To correlate between high sensitivity C reactive protein and cigarette smoking duration.

1.3 Rationale

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

In Sudan smoking is become a common condition with increasing rate in both sex's males and females and occurs in different age groups, it can cause many organ damages and dysfunctions; no previous study was done in Sudan.

This study was conducted to verify the effect of cigarette smoking on C reactive protein using hs-C reactive protein as a sensitive marker of inflammatory condition.

1.4.1 Tobacco smoking

1.4.1.1 Definition

Tobacco smoking is the practice where tobacco is burned and the vapor either tasted or inhaled (Gately *et al*, 2001). Smoking is the most common method of consuming tobacco, and tobacco is the most common substance smoked. The agricultural product is often mixed with other additive and then pyrolyzed (Pyror *et al*, 1993). The resulting vapourare often inhaled and the active substance absorbed through the alveoli in the lung. The active substances trigger chemical reactions in nerve ending, which heightens heart rate, memory, alertness, and reaction time (Parrot *et al*, 1989). Dopamine and later endorphins are released, which are often associated with pleasure (Gilman *et al*, 2004). As of 2000 smoking is practiced by some 1.22 billion people. Men are more likely to smoke than women, though the gender gap decline with younger age (WHO, 2001). To help you to understand the magnitude of smoking-related deaths, we can compare them with other sources of premature death. For example, the number of people who die from using tobacco is greater than the combined total number of people who die from murder, suicide, car accidents, fire, AIDS, and using alcohol, cocaine, and heroin. Tobacco use also accounts for one-third of all cancers. Smokers die from cancer at a rate that is twice as high as nonsmokers, and heavy smokers die at a rate that is four times higher than nonsmokers. Lung cancer is the number one cancer killer of both men and women, and smoking is associated with nearly 90 percent of lung cancer cases. In addition smoking is a leading cause of cancers of the mouth, tongue, throat, larynx (voice box), esophagus, stomach, pancreas, cervix, kidney, ureter, and bladder (Gilman *et al*, 2004).

Cancer is not the only disease caused by smoking. Smoking also causes chronic bronchitis and emphysema, and worsens asthma (Gilman *et al*, 2004).

Cigarette smoking substantially increases the risk of coronary heart disease, including stroke, heart attack, aneurysm and vascular disease. It also contributes to peptic ulcers, varicose veins, osteoporosis, periodontal disease, Alzheimer's disease, atherosclerosis, high blood pressure, allergies, and impotence. The bottom line is that continuing to smoke puts you at risk of contracting a serious, life-threatening illness. If you contract any one of the diseases listed above, your quality of life will deteriorate and you will shorten your life span (Gilman *et al*, 2004).

1.4.1.2 History of tobacco smoking

The history of tobacco smoking dates back to as early as 5000 BC in shamanistic rituals. Many ancient civilizations, such as the Babylonians, Indians and Chinese, burnt incense as a part of religious rituals, as did the Israelites and the later Catholic and Orthodox Christian churches. Smoking in the Americas probably had its origins in the incense-burning ceremonies of shamans but was later adopted for pleasure, or as a social tool. The smoking of tobacco, as well as various hallucinogenic drugs was used to achieve trances and to come into contact with the spirit world (Gately *et al*, 2001).

1.4.1.3 Physical and biochemical properties of smoking

Convent ally, cigarette smoke is divided into two phases: a tar phase and a gas phase. The tar or particulate phase is defined as material that trapped when the smoke stream is passed through the Cambridge glass fiber filter that retains 99.9% of all particulate material with a size>0.1µm (Tylor *et al*, 1992).

The gas phase is the material that passes through the filter. The particulate (tar) phase of cigarette smoke contain> 10^{17} free radicals/g, and the gas phase contain>10^{15}free radicals/puff. The radical associated with the tar phase are long-lived (hours to months), where as the radicals associated with gas phase have a shorter life span (seconds) (Tylor *et al*, 1992).

Cigarette smoke that is drawn through the tobacco into an active smoker's mouth is known as mainstream smoke. Side stream cigarette smoke emitted from the burning end of cigarette. Mainstream cigarette smoke comprises 8% of tar and 92%of gaseous component (Tylor *et al*, 1992).

Environmental tobacco smoke result from the combination of side stream smoke (85%) and small fraction of exhaled mainstream smoke(15%)from smokers (Glantz , 1991).

Side stream cigarette smoke contain a relatively higher concentration of the toxic gaseous component than main stream cigarette smoke of the entire known constituent, nicotine, a component of the tar phase, is the addictive substance of cigarette smoke (Powell,1998).

1.4.1.4 Epidemiology

According to the World Health Organization (WHO), smoking is currently responsible for approximately 3.5 million deaths worldwide each year. Smoking is the leading preventable cause of death in the United States, and it kills more than 400,000 U.S. citizens each year. The World Health Organization predicts that by 2020, the worldwide death toll from smoking will reach 10 million each year, causing nearly 18 percent of all deaths in the developed world (WHO, 2014).

1.4.1.5 Reasons for smoking

Many people smoke because of it is calming effect on the nervous system, but it should be discouraged of it is negative effect on the body. It damages the lung, blood vessels, and other organs such as heart, but also harms that of others. There are many reasons why people take-up smoking. Many learn smoking from parents or their friends. Many people who spend their time with friends seem to catch the habit. Advertising plays a considerable role in encouraging individual to start smoking. People also look up to their elders such as teachers, doctors, and family leaders. They think that it is a prestigious to smoke (Bhrag, 1976).

1.4.1.6 Cigarette smoke affects all the organs of the body

The effect of cigarette in the body is related to: the age of person, how long they have smoked, and how much smoke per day. Tobacco produce gases and many chemical compounds including pesticide residues artificial flavors, burning agents and poisonous substances such as nicotine, carbon dioxide, tar, acetone, ammonia methyl chloride, arsenic, nickel and others. There are many poisonous chemical in cigarette smoke, and some of these have been shown to be carcinogenic. The most poisonous of these are nicotine, carbondioxide, and tar (Bhrag, 1976). Nicotine is the substance that cause addiction, swallowing one drop of nicotine can kill person, is smaller and more frequent dose, it can cause addiction among smoker. It also stimulates the release of epinephrine and other substance in the body. These increase heart and blood pressure and narrow the blood vessels. Carbon monoxide is a poisonous gas produced by the incomplete burning of cigarette. In the lungs it combines with the hemoglobin from carrying adequate amount of oxygen through the circulatory system (Bhrag, 1976).

The tar in cigarette smoke in cancer- producing substance that contain number of carcinogens (Bhrag, 1976).

The effect of cigarette are considerable: the heart rate goes up, the blood pressure increases and the skin temperature drops smoking lead to shortness of breath and coughing; it reduce fitness causes yellow teeth and fingers decrease the sense of taste and smell it lead to impotence severe period pain irregular period underweight birth, premature babies, lung cancer, stomach cancer, and many other un pleasant and potentially deadly thing (Bhrag, 1976).

Within a second of a person smoking a cigarette, the heart rate become faster and the blood pressure increase. In a short time, nicotine reaches the brain and stimulates the central nervous system. The effect of smoking is dependent on the amount of tobacco smoked (Bhrag, 1976).

Compared with a non smokers smoking is estimated to increase the risk of coronary heart disease by 2 to 4 times stroke by 2 to 4 time men developing lung cancer by 23 times women developing lung cancer by 13 times and dying from chronic obstructive lung disease (such as chronic bronchitis and emphysema) by 12 to 13 times (Ramage *et al*, 2004)

1.4.1.6.1 Smoking and respiratory disease

Smoking causes lung cancer, lung disease (e.g emphysema bronchitis, chronic air way obstruction) by damaging the air ways and alveoli of the lung (Ramage *et al*, 2004).

1.4.1.6.2 Smoking and cancer

Smoking cause the following cancer: acute myeloid leukemia, bladder cancer, cancer of the larynx (voice box) , lung cancer, cancer of the oral cavity (mouth),

cancer of the pharynx (throat), stomach cancer, and cancer of the uterus (Ramage *et al*, 2004).

1.4.1.6.3 Smoking and other health effect

Smoking has many adverse reproduction and early childhood effects, including increase risk for: infertility, preterm birth, still birth, low birth weight, and sudden infant death syndrome (Ramage *et al*, 2004).

.1.5.2 C-reactive protein (CRP)

1.5.2.1 Definition

Is an annular ring-shaped, pentameric protein found in the blood plasma, the levels of which rise in response to inflammation (i.e., C-reactive protein is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion from macrophages and T cells) (Thompson *et al*, 1999).

Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex (Thompson *et al*, 1999).

1.5.2.2 Synthesis

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

Native CRP is synthesized in a soluble form by hepatocytes and then secreted into the circulation (Ganter *et al*, 1989;Yap *et al*,1991).

The production of CRP is induced by proinflammatory cytokines IL-1, IL-6 and IL-17 in the liver, although extrahepatic production can contribute to systemic concentrations (Eklund, 2009).

However, reports of extrahepatic CRP synthesis by some other cell types have not always been reproducible. Several investigators reported the presence of CRP in lung epithelial cells (Ramage *et al*, 2004), renal cortical tubular epithelial cells (Jabs *et al*, 2005), human coronary artery smooth muscle cells (Calabro *et al*, 2003), atherosclerotic plaques(Jabs *et al*, 2005; Yasojima *et al*, 2001) and gingival tissue (Lu, 2010).

Although possibly relevant to potential local effects of CRP, the contribution to plasma concentrations is probably negligible with respect to that from the liver (Yasojima *et al*, 2001).

demonstrated that CRP can be produced in pyramidal neurons (K *et al*, 2000), at least in patients with Alzheimer's disease (AD), and there is also evidence of intrathecal synthesis of SAP and CRP in AD patients (Mulder *et al*, 2010).CRP is found in the cerebrospinal fluid of normal subjects and is raised in that of subjects with acute bacterial meningitis, but not in those with viral meningitis (Stearman, 1994).

Cytokines exert their biological effects on CRP by signaling through their receptors on hepatic cells and activating different kinases and phosphatases, leading to the translocation of various transcription factors on the CRP gene promoter and the production of CRP (Eklund, 2009).

CRP is apparently cleared from the plasma and catabolized exclusively by hepatocytes, and the plasma half-life in humans of approximately 19 h is the same in all individuals, regardless of the presence of disease or the circulating

concentration of CRP (Pepys *et al*, 2003).The sole determinant of the plasma concentration is therefore the synthesis rate (Pepys *et al*, 2003; Gabay *et al*, 1999). The rise in blood CRP after tissue insult or injury is rapid and robust, the concentration doubles every 8 h and peaks at 36–50 hr (Young *et al*, 1991).

Although that depends on the stimulus and its severity (Kushner *et al*, 1978).In response to an inflammatory insult, CRP concentration can increase above 500 mg/l that is as much as a 1000-fold or more concentration change (Young *et al*, 1991).

1.5.2.3 The acute phase response

Was 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. In particular, the synthesis of a number of proteins is rapidly up regulated, principally in hepatocytes, under the control of cytokines originating at the site of pathology. Other acute-phase proteins include proteinase inhibitors and coagulation, complement, and transport proteins, but the only molecule that displays sensitivity, response speed, and dynamic range comparable to those of CRP is serum amyloid a protein (SAA) (Pepys *et al*, 1983).

1.5.2.4 History and nomenclature

CRP was so named because it was first identified as a substance in the serum of patients with acute inflammation that reacted with the C-polysaccharide of Pneumococcus (Pepys *et al*, 2003).

Discovered by Tillett and Francis in 1930 it was initially thought that CRP might be a pathogenic secretion since it was elevated in a variety of illnesses, including cancer (Pepys *et al*, 2003).

The later discovery of hepatic synthesis demonstrated that it is a native protein (Kennelly *et al*, 2009; Pincus *et al*, 2007; Ratey *et al*, 2007).

1.5.2.5 Genetics and structure

The CRP gene is located on the first chromosome (1q21-q23). It is a member of the small pentraxins family. It has 224 amino acids, has a monomer molecular mass of 25106 Da, and has an annular pentameric discoid shape (Pepys *et al*, 2003).

1.5.2.6 Function

CRP binds 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 so-called acute phase response occurs as a result of a rise in the concentration of IL-6, which is produced by macrophages (Pepys *et al*, 2003)

As well as adipocytesn 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.CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin-mediated

phagocytosis), which express a receptor for CRP. It plays a role in innate immunity as an early defense system against infections. CRP rises within two hours of the onset of inflammation, up to a 50,000-fold, and peaks at 48 hours. Its half-life of 48 hours is constant, and therefore its level is determined by the rate of production and hence the severity of the precipitating causes (Pepys *et al*, 2003).

CRP is thus a screen for inflammation (Dhillon *et al*, 2005).

1.5.2.7 Diagnostic use and measurement

CRP is used mainly as a marker of inflammation. Apart from liver failure, there are few known factors that interfere with CRP production (Pepys *et al*, 2003) Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments. ELISA, immunoturbidimetry, rapid immunodiffusion, and visual agglutination are all methods used to measure CRP.A high-sensitivity CRP (hs-CRP) test measures low levels of CRP using laser nephelometry. The test gives results in 25 minutes with sensitivity down to 0.04 mg/L. The risk of developing cardiovascular disease is quantified as follows:

Low: hs-CRP level under 1.0 mg/L

Average: between 1.0 and 3.0 mg/L

High: above 3.0 mg/L (Pepys *et al*, 2003).

Normal concentration in healthy human serum is usually lower than 10 mg/L, slightly increasing with aging. Higher levels are found in late pregnant women, mild inflammation and viral infections (10–40 mg/L), active inflammation, bacterial infection (40–200 mg/L), severe bacterial infections and burns (>200 mg/L) (Glantz *et al*, 1991).

CRP is a more sensitive and accurate reflection of the acute phase response than the ESR (Erythrocyte Sedimentation Rate). ESR may be normal and CRP elevated. CRP returns to normal more quickly than ESR in response to therapy several studies investigated differential diagnostic values of CRP in a series of inflammatory disease (including inflammatory bowel disease, Intestinal Lymphoma, Intestinal Tuberculosis and Behcet's Syndrome), and compared CRP to other inflammatory biomarkers, such as ESR and WBC (Liu et al, 2013).

1.5.2.8 Cancer

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

In a 2004 prospective cohort study on colon cancer risk associated with CRP levels, people with colon cancer had higher average CRP concentrations than people without colon cancer (Thompson *et al*, 1999).

It can be noted that the average CRP levels in both groups were well within the range of CRP levels usually found in healthy people. However, these findings may suggest that low inflammation level can be associated with a lower risk of colon cancer, concurring with previous studies that indicate anti-inflammatory drugs could lower colon cancer risk (Baron *et al*, 2003).

1.5.2.9 Cardiovascular disease

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

A study of over 700 nurses showed that those in the highest quartile of trans fat consumption had blood levels of CRP that were 73% higher than those in the lowest quartile(Lopez Garcia *et al*, 2005).

Although one group of researchers indicated that CRP may be only a moderate risk factor for cardiovascular disease (Danesh *et al*, 2004).

This study (known as the Reykjavik Study) was found to have some problems for this type of analysis related to the characteristics of the population studied, and there was an extremely long follow-up time, which may have attenuated the association between CRP and future outcomes (Koenig *et al*, 2006).

Others have shown that CRP can exacerbate ischemic necrosis in a complement-dependent fashion and that CRP inhibition can be a safe and effective therapy for myocardial and cerebral infarcts, so far, this has been demonstrated in animal models only (Pepys *et al*, 2006).

It has been hypothesized that patients with high CRP levels might benefit from use of statins, This is based on the JUPITER trial that found that elevated CRP levels without hyperlipidemia benefited. Statins were selected because they have been proven to reduce levels of CRP (Pepys *et al*, 2003; Ridker *et al*, 2008).Studies comparing effect of various statins in hs-CRP revealed similar effects of different statins (Sindhu *et al*, 2011; Jialal *et al*, 2001).

A subsequent trial however failed to find that CRP was useful for determining statin benefit (Emberson J et al, 2011). In a meta-analysis of 20 studies involving 1,466 patients with coronary artery disease, CRP levels were found to be reduced after exercise interventions. Among those studies, higher CRP concentrations or poorer lipid profiles before beginning exercise were associated with greater reductions in CRP (Swardfager *et al*, 2012).

To clarify whether CRP is a bystander or active participant in atherogenesis, a 2008 study compared people with various genetic CRP variants. Those with a high CRP due to genetic variation had no increased risk of cardiovascular disease compared to those with a normal or low CRP (Zacho *et al*, 2008). A study published in 2011 shows that CRP is associated with lipid responses to low-fat and high-polyunsaturated fat diets (Onge et al, 2009).

1.5.2.10 Fibrosis and inflammation

Scleroderma, polymyositis, and dermatomyositis elicit little or no CRP response. CRP levels also tend not to be elevated in SLE unless serositis or synovitis is present. Elevations of CRP in the absence of clinically significant inflammation can occur in renal failure. CRP level is an independent risk factor for atherosclerotic disease. 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*, 2006).

1.5.2.11 Obstructive sleep apnea

C-reactive protein (CRP), a marker of systemic inflammation, is also increased in obstructive sleep apnea (OSA). CRP and interleukin-6 (IL-6) levels were significantly higher in patients with OSA compared to obese control subjects. Patients with OSA have higher plasma CRP concentrations that increased corresponding to the severity of their apnea-hypopnea index score. Treatment of OSA with CPAP (continuous positive airway pressure) significantly alleviated the effect of OSA on CRP and IL-6 levels (Latina *et al*, 2013).

1.5.2.12Coronary heart disease risk

Arterial damage results from white blood cell invasion and inflammation within the wall. CRP is a general marker for inflammation and infection, so it can be used as a very rough proxy for heart disease risk. Since many things can cause elevated CRP, this is not a very specific prognostic indicator (Lloyd-Jones *et al*, 2006).

Nevertheless, a level above 2.4 mg/L has been associated with a doubled risk of a coronary event compared to levels below 1 mg/L (Pepys *et al*, 2003).

However the study group in this case consisted of patients who had been diagnosed with unstable angina pectoris, whether elevated CRP has any predictive value of acute coronary events in the general population of all age ranges remains unclear. Currently, C-reactive protein is not recommended as a cardiovascular disease screening test for average-risk adults without symptoms (Lloyd-Jones *et al*, 2006).

The American Heart Association and U.S. Centers for Disease Control and Prevention have defined risk groups as follows:

- Low Risk: less than 1.0 mg/L
- Average risk: 1.0 to 3.0 mg/L
- High risk: above 3.0 mg/L (Pepys *et al*, 2003).

But hs-CRP is not to be used alone and should be combined with elevated levels of cholesterol, LDL-C, triglycerides, and glucose level. Smoking, hypertension and diabetes also increase the risk level of cardiovascular disease (Pepys *et al*, 2003).

1.5.2.13 CRP a target for therapy in human disease

We have long speculated that CRP may have significant proinflammatory effects, and that, by binding to ligands exposed on cells or other autologous structures as a result of infection, inflammation, ischemia, and other pathologies, and triggering complement activation, it may exacerbate tissue damage, leading to more severe disease (Pepys *et al*, 2003).

The rat myocardial infarction model provided the first direct evidence of these processes in vivo but they are not necessarily confined to cardiovascular disease. The excellent correlation of circulating CRP concentrations with the severity extent and progression of much different pathology and the prognostic significance of these associations are consistent with CRP not just being a marker of disease but also contributing to pathogenesis. A definitive way to test this concept will be the use of novel drugs that specifically block CRP binding and its proinflammatory effects in vivo (Pepys, 1999).If these compounds are effective they may find very broad applicability. Such drugs would be a powerful tool for determining whether increased CRP production merely reflects atherosclerosis or does indeed participate in its pathogenesis and complications and they could also have cardio protective effects in acute myocardial infarction. Knowledge of the structure and function of CRP including its three-dimensional structure alone and complexed with ligands (Thompson *et al*, 1999).

Coupled with experience in developing an inhibitor of the related protein SAP establishes an excellent platform for drug design (Pepys *et al*, 2002).

1.5.2.14 CRP levels vary according to smoking status

In recent years there has been a large volume of studies some of which are conflicting, in which serum CRP concentrations have been measured in parallel to smoking status because of the possible link between smoking and the induction of inflammatory pathways (Yanbaeva *et al*, 2007).

Smokers have increased numbers of white blood cells mainly because of a particular increase in polymorphonuclear neutrophils which are released from the bone marrow and recruited to inflamed tissue (Van Eeden *et al*, 2000).

IL-β and IL-6 which are increased in response to lung inflammation and are implicated in the induction of CRP gene expression may mediate the stimulation of bone marrow cells (Van Eeden *et al*, 2005). In one study, levels of inflammatory markers were measured in the bloodstream of intermittent smokers 24 h after they had two cigarettes following 9 days of abstinence. TNF-α, IL-10 and IL-1β did not change, but levels of IL-8 increased after 3 hr (Van der Vaart *et al*, 2005).

In one of the earlier studies of CRP levels in smokers, and before the advent of assays with higher sensitivities, CRP was found to be significantly higher in male and female smokers compared with non-smokers (median values of 1.0 mg/l and 11.2 mg/l for male non-smokers and smokers, respectively, and for females 2.0 mg/l and 11.6 mg/l, respectively) (Das, 1985). Such strikingly different values have not been observed in more recent studies. Furthermore, more than half of the smoking cohort from this initial study had CRP values > 10 mg/l, which could be considered as reflecting an inflammatory episode (Lowe and Pepys, 2006).

The complexity of cytokine-mediated inflammation is highlighted by a study showing that although smoking status did correlate with a significant elevation in levels of IL-6 and serum amyloid protein A, another acute phase protein, the increase in CRP levels observed in smokers was not found to be statistically significant (Helmersson *et al*, 2005). Another larger study found that mean CRP levels were significantly lower in never-smokers ($p < 0.0001$) than in current smokers (Wannamethee *et al*, 2005). A dose-dependent correlation between CRP and smoking habits was demonstrated in the 'Speedwell' survey of British men. CRP levels were increased from 1.13 mg/l in never-smokers to 1.87, 2.32 and 2.05 mg/l in those who smoked 1–14, 15–24 and > 25 cigarettes, respectively per day (Lowe *et al*, 2005). However another study conducted in people of Japanese ethnicity (the Iwate-Kenpoku Cohort study) failed to identify any significant relationship between serum CRP concentration and the number of cigarettes smoked per day (Ohsawa *et al*, 2005).

1.5.2.14 CRP levels following smoking cessation

Most studies that have examined CRP status in former smokers suggest that levels fail to fall immediately upon cessation, which reflects the fact that the underlying tissue damage caused by smoking takes some time to recover (Lowe *et al*, 2007).

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Study approach

A quantitative method was used to measure high sensitivity C reactive protein in Sudanese cigarette smoking in Khartoum state, during a period from February to May 2015.

2.2 Study Design and time

Descriptive cross-sectional study, conducted during the period of February to May 2015.

2.3 Study Area

This study was carried out in Khartoum state.

2.4 Study Population

Sudanese smokers.

2.5 Inclusion criteria

Specimens were collected from cigarette smoker's people, plasma specimens collected from these smokers.

2.6 Exclusion criteria

Persons with chronic infection, coronary heart disease, surgery, neoplastic proliferation and SLE.

2.7 Sample size

175 samples (100 samples from cigarette smokers, 50 samples from non smokers, 25 samples from ex-smokers).

2.8 Method of data collection and tools

Data were collected using structural interviewing questionnaire, which was designed to collect and maintain all information concerning each case examined.

2.9 Collection of Samples

Samples were collected by using dry, plastic syringes, tourniquet was used to make the veins more prominent, blood samples (5ml) was collected in containers from each volunteer under septic condition, then they were centrifuged at 4000 rpm to obtain the plasma samples, and stored in -20° until the analyzed.

2.10 Ethical Considerations

A Study was approved from ethical committee of the Sudan University of Science and Technology, verbal informed consent was obtained and all patients were informed by aims of the study.

2.11 Method

2.11.1 Estimation of hs-CRP

According to the procedure provided, serum levels of Hs-CRP were measured using the Particle enhanced immunoturbidimetric assay method (Cobas C 311 automated chemistry analyzer). Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined

turbidimetrically, samples dispensed and all processes done automatically and concentration obtained for each sample.

2.11.2 Instruments

Cobas C 311 automated chemistry analyzer. With following information:

Model =c 311

Serial NO =0812 – 02.

2.11.3 Statistical Analysis

The Student's t-test was employed to compare differences between the means of continuous variables. P-values less than 0.05 were considered statistically significant. Correlations between hs-CRP and smoking duration were assessed using bivariate correlations. $P < 0.05$ was considered statistically significant. Data were analyzed by SPSS statistical package of social science (version 17.0, SPSS Inc.).

Chapter Three

Results

3. Results

In this study 175 subjects were chosen for determination of plasma hs-CRP. 50 of them were heavy smokers, 50 were light smokers, 25 were ex-smokers the other 50 were apparently healthy and not smoker subjects represent the control group, during the period February to march 2015. The results obtained were statistically analyzed, using SPSS T.test. The level of significance was expressed as P value < 0.05 for significant.

Table 3.1 show the mean values of hs-CRP in smokers was significant high when compared to control (1.737±2.105) (0.926 ± 1.211) mg/L respectively with P=0.013.

Table 3.1 show the mean values of hs-CRP in heavy smokers was significant high when compared to control (2.289±2.095) (0.926 ± 1.211) mg/L respectively with P=0.000.

Table 3.1 show the mean values of hs-CRP in light smokers was insignificant when compared to control (1.213 ±2.075) (0.926 ± 1.211) mg/L respectively with P=0.402.

Table 3.1 show the mean values of hs-CRP in ex-smokers was significant high when compared to control (1.762±1.945) (0.926 ± 1.211) mg/L respectively with P=0.025.

Table 3.1 show the mean values of hs-CRP in current smokers was insignificant low when compared to ex-smokers (1.737±2.105) (1.762 ± 1.954) mg/L respectively with P=0.955.

Table 3.2 show the mean values of hs-CRP of smokers for more than 20 years is significant high when compared to hs-CRP of smoking duration for (less than 10 years) (3.189 ± 2.893) (1.374±1.735) mg/L respectively with P=0.006.

Table 3.2 show the mean values of hs-CRP of smokers for (less than 10 years) is insignificant low when compared to hs-CRP of smoking duration for (10 to 20 years) (1.374±1.735) (2.384 ± 3.364) mg/L respectively with P=0.329.

Table 3.2 show the mean values of hs-CRP of smokers for (10 to 20 years) is insignificant low when compared to hs-CRP of smoking duration for more than 20 years (2.384±3.364) (3.189 ± 2.893) mg/L respectively with P=0.553.

As shown in table 3.3 there is a direct correlation between smoking duration and hs-CRP (r= 0.281, p =0.006).

Table 3.1 Descriptive summary of the the mean and Standard Divisions (mean± SD) of plasma hs-CRP between smokers, heavy smokers, light smokers, current smokers, Ex-smokers and control group.

		Mean ± SD	P.value
hs-CRP	Smokers	(1.737±2.105)	0.013
	Control	(0.926±1.211)	
	Heavy smokers	(2.289±2.095)	0.000
	Control	(0.926±1.211)	
	Light smokers	(1.213±2.075)	0.402
	Control	(0.926±1.211)	
	Ex-smoker	(1.762±1.945)	0.025
	Control	(0.926±1.211)	
	Current smoker	(1.737±2.105)	0.955
	Ex-smoker	(1.762±1.945)	

Independent sample T .Test. $P < 0.05$ was considered statistically significant

Table 3.2 shows a descriptive summary of the mean and Standard Divisions (mean± SD) of plasma hs-CRP between Smoking duration.

		Mean ± SD	P.value
hs-CRP	More than20 years	(3.189±2.893)	0.006
	Less than10 years	(1.374±1.735)	
	10 to20years	(2.384±3.364)	0.329
	Less than10years	(1.374±1.735)	
	More than 20 years	(3.189±2.893)	0.553
	10 to 20 years	(2.384±3.364)	

Independent sample T .Test. $P < 0.05$ was considered statistically significant

Table 3.3 Shows Pearson correlation analysis showed the correlation between hs-CRP and smoking duration.

	Statistic	
	Person correlation	P.value
Smoking duration	0.281	0.006

Correlation test. $P < 0.05$ was considered statistically significant

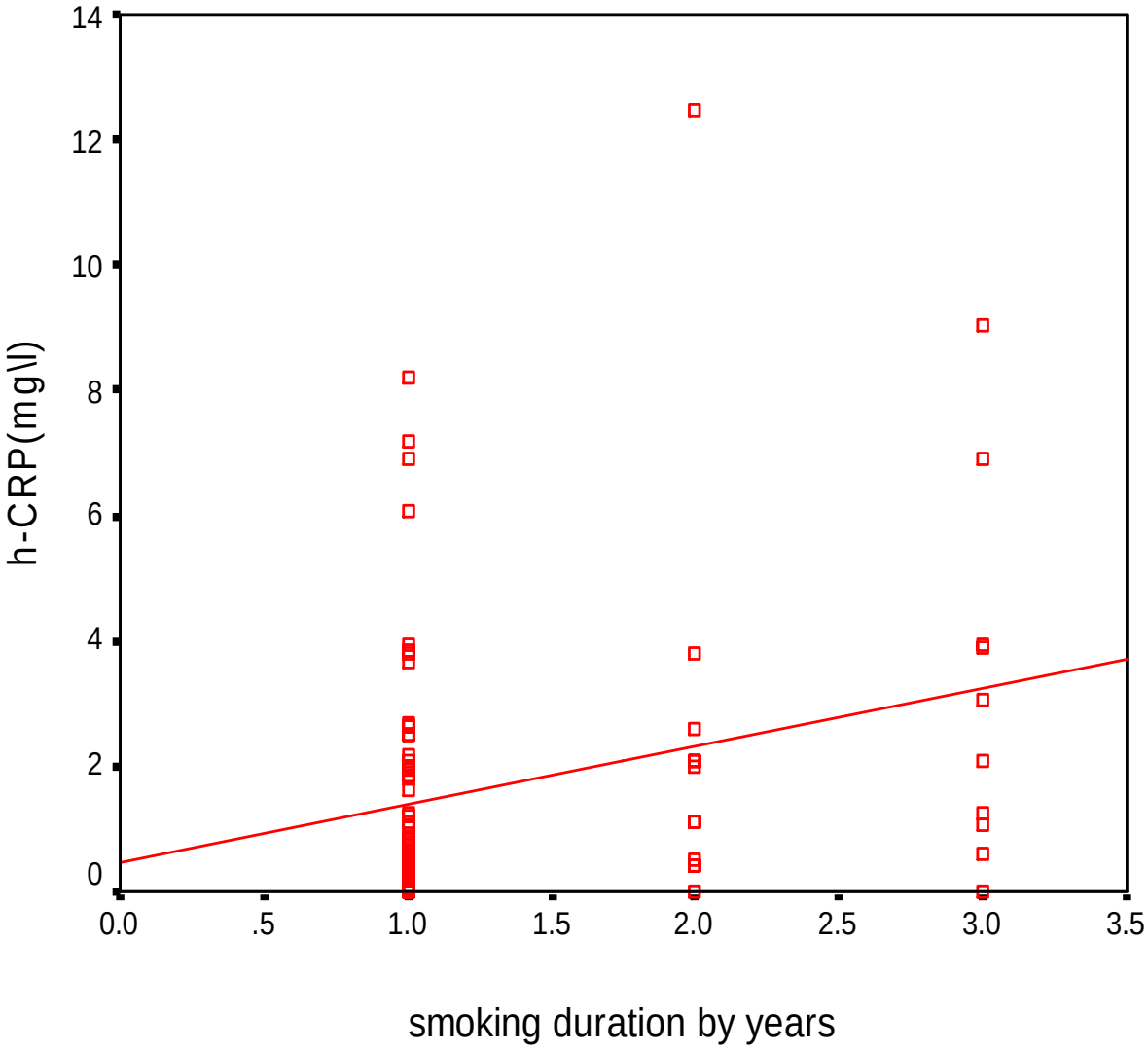

Figure 3.1correlation between duration of smoking and hs-CRP.

Chapter Four

Discussion, Conclusion and Recommendations

4. Discussion

CRP is an acute-phase reactant produced mainly by the hepatocytes in response to inflammatory stimuli. It has been shown to be a sensitive nonspecific biomarker of systematic inflammation (Pepys, *et al*, 2003).

The circulating value of CRP reflects ongoing inflammation and/or tissue damage and is associated with cardiovascular disease, type 2 diabetes, smoking, and a sedentary lifestyle (Calle *et al*, 2010).

Previous studies reported that smoking is a part of inflammation process, therefor observed elevated levels of CRP in smoking individuals. Accordingly the results of present study showed significant increase in hs-CRP of case in comparison with control group with (*p*-value 0.013) Our findings were agreed with the study done by (Kleber *et al*, 2015 and Lowe *et al*, 2006), reported that hs-CRP were significantly higher in smokers than in control. Also the study agreed with study done by (Loughlin *et al*, 1999) which reported that hs-CRP were significantly higher in smokers than in control.

The results of independent t-test showed mean hs-CRP level was significantly higher in heavy smoker than control group with (*P*-value 0.000)it agreed with study done by (Zhao *et al*, 2014),he found that hs-CRP significantly greater in heavy smokers than those of controls.

In contrast the results of hs-CRP showed insignificant lower level in light smoker than control group with (*P*-value 0.797).

In addition the results of independent t-test showed that mean hs-CRP level was significantly higher in Ex-smokers than control group with (*P*-value 0.025).which agreed with study done by (Lowe *et al*, 2007).

While showed no statistical significances in hs-CRP level in current smoker with ex-smoker with (*P*-value 0.995).

The results of independent t-test showed mean hs-CRP level was significantly higher in smokers for more than 20 years than smokers for 10 to 20 years with (*P*-value 0.006).

There is a direct correlation between hs-CRP and duration of smoking (r= 0.281, p =0.006).

4.2 Conclusion

The study concluded that, hs-CRP is elevated in cigarette smokers individual and elevation of hs-CRP is depending on smoking status and duration, and it can be used as predictive marker for cardiovascular disease in heavy smokers.

4.3 Recommendations

- Further study with estimation of other parameter to link between hs-CRP and cardiovascular disease.
- More studies compare the results with more data collected from patients such as gender, body mass index, and increase sample size.
- Further exploration of the effect of smoking on other parameter.

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Appendices

Sudan University of Science and Technology

Faculty of Medical Laboratory Science

Questionnaire:

Date: ____\2015

No: ____

Area: _____

Name: _____

Telephone No:_____

Gender: Male ( ) Female ( )

Age: _____

Type of smoking: _____

No of cigarette per day:_____

Smoking Duration: _____

Time of cessation from smoking(for Ex-smokers):_____

Type of disease: _____

hs-CRP: **mg/l**

Cardiac C-Reactive Protein (Latex) High Sensitive

Catalog Number: 04628918190

SYSTEM INFORMATION

For cobas c 311/501 analyzers:

CRPHS: ACN 217

For cobas c 502 analyzer:

CRPHS: ACN 8217

INTENDED USE

In vitro test for the quantitative determination of C-reactive protein (CRP) in human serum and plasma on Roche/Hitachi cobas c systems. Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. Highly sensitive measurement of CRP may also be used as an aid in the assessment of the risk of future coronary heart disease. When used as an adjunct to other laboratory evaluation methods of acute coronary syndromes, it may also be an additional independent indicator of recurrent event prognosis in patients with stable coronary disease or acute coronary syndrome.

STORAGE AND STABILITY

CRPHS

Shelf life at 2-8 °C:	See expiration date on cobas c pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on cobas c pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

APPLICATION FOR SERUM AND PLASMA

cobas c 311 test definition

Assay type	Rate A	
Reaction time / Assay points	10 / 7-57	
Wavelength (sub/main)	– / 546 nm	
Reaction direction	Increase	
Units	mg/L (nmol/L, mg/dL)	
Reagent pipetting		Diluent (H2O)
R1	82 µL	42 µL

R2	28 µL	20 µL	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	6 µL	–	–
Decreased	6 µL	10 µL	140 µL
Increased	6 µL	–	–
cobas c 501 test definition			
Assay type	Rate A		
Reaction time / Assay points	10 / 12-70		
Wavelength (sub/main)	– / 546 nm		
Reaction direction	Increase		
Units	mg/L (nmol/L, mg/dL)		
Reagent pipetting		Diluent (H2O)	
R1	82 µL	42 µL	
R2	28 µL	20 µL	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	6 µL	–	–
Decreased	6 µL	10 µL	140 µL
Increased	6 µL	–	–
cobas c 502 test definition			
Assay type	Rate A		
Reaction time / Assay points	10 / 12-70		
Wavelength (sub/main)	– / 546 nm		
Reaction direction	Increase		
Units	mg/L (nmol/L, mg/dL)		
Reagent pipetting		Diluent (H2O)	
R1	82 µL	42 µL	
R2	28 µL	20 µL	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	6 µL	–	–
Decreased	6 µL	10 µL	140 µL
Increased	12 µL	–	–

CALIBRATION

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).25

Calibrators	S1: H2OS2: C.f.a.s. Proteins	
	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the 6-point calibration curve:	
	S2: 0.0125	S5: 0.100
	S3: 0.0250	S6: 0.200
	S4: 0.0500	
Calibration mode	Line Graph	
Calibration frequency	Full calibrationafter reagent lot change as required following quality control procedures	

LIMITS AND RANGES

Measuring range

0.15-20.0 mg/L (1.43-190 nmol/L, 0.015-2.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:15 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 15.

Lower limits of measurement

Lower detection limit of the test

0.15 mg/L (1.43 nmol/L, 0.015 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Functional sensitivity

0.3 mg/L (2.96 nmol/L, 0.03 mg/dL)

The functional sensitivity is the lowest CRP concentration that can be reproducibly measured with an inter-assay coefficient of variation of < 10 %.

SPECIFIC PERFORMANCE DATA

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

PRECISION

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Meanmg/L (nmol/L, mg/dL)	SDmg/L (nmol/L, mg/dL)	CV%
Precinorm Protein	9.00 (85.7, 0.900)	0.10 (1.0, 0.010)	1.2
CRP T Control N	4.34 (41.3, 0.434)	0.04 (0.4, 0.004)	1.0
Human serum 1	15.9 (151, 1.59)	0.1 (1, 0.01)	0.4
Human serum 2	0.54 (5.14, 0.054)	0.01 (0.10, 0.001)	1.6

Intermediate precision	Meanmg/L (nmol/L, mg/dL)	SDmg/L (nmol/L, mg/dL)	CV%
Precinorm Protein	9.06 (86.3, 0.906)	0.11 (1.1, 0.011)	1.3
CRP T Control N	4.28 (40.8, 0.428)	0.11 (1.1, 0.011)	2.6
Human serum 3	13.3 (126, 1.33)	0.3 (3, 0.03)	2.1
Human serum 4	0.53 (5.05, 0.053)	0.05 (0.48, 0.005)	8.4

METHOD COMPARISON

CRP values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared to those determined with the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 192

Passing/Bablok33	Linear regression
y = 0.992x + 0.254 mg/L	y = 0.946x + 0.514 mg/L
t = 0.944	r = 0.996

The sample concentrations were between 0.500 and 19.7 mg/L (4.76 and 188 nmol/L, 0.050 and 1.97 mg/dL).