SUDAN UNIVERSITY OF SCIENCE
AND TECHNOLOGY
COLLEGE OF GRADUATE STUDIES

Evaluation of High Sensitive C Reactive Protein Level in Type II Diabetic Sudanese Patients in Khartoum State

تقويم مستوى بروتين سي التفاعلي للسودانيين المصابين بمرض السكري
من النوع الثاني في ولاية الخرطوم

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

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Dedication

ThisResearch is lovingly dedicated to my respective parents who have been my constant source of inspiration. They have given me the drive and discipline to tackle any task with enthusiasm and determination. Without their love and support this project would not have been madepossible. Also Idedicatedto mybrothers, sistersand friends.

Nada

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I deeply thank my families and everyone who helped us to conduct this study.

Abbreviations

Abbreviation	
ADA	American diabetes association
BMI	Body mass index
CRP	C reactive protein
DM	Diabetes mellitus
DN	Diabetic nephropathy
ESRD	End stage renal disease
HA1c	Glycated hemoglobin
Hs-CRP	High sensitive c reactive protein
IDDM	Insulin dependent diabetes mellitus
MAU	Microalbuminuria
mg/dl	Milligrams per deciliter
mg/l	Milligrams per liter
µl	Micro liter
mmol/mol	Milimol/mol
NIDDM	Non insulin dependent diabetes mellitus
NSAID	Non steroid anti inflammatory drugs
SD	Standard deviation
UAE	Urinary albumin excretion
WHO	World health organization

Abstract

Background: Inflammatory markers such as CRP has been related to inflammatory disease, previous studies have been established to link between CRP and Cardiovascular disease specially in DM patients. This study aims to evaluate hs-CRP level as a predictor marker for cardiovascular diseases among uncontrolled type II diabetic patients in Khartoum state.

Materials and Methods: One hundred and twenty individual were enrolled in this study, classified into 60 type II DM patients and 60 apparently health as control group. Serum hs-CRP and whole blood glycated hemoglobin were measured using immunoturbidymetric method and florescence sandwich immune-detection method, respectively.

Results: The results showed hs- CRP level is higher in type II DM with *P*-value 0.000 specially uncontrolled DM patients with *P*-value= 0.006, also positive correlation between hs- CRP and duration, HbA1C with *P*-value = 0.002 , *P*-value =0.000 respectively. No correlation between hs-CRP when associated with BMI and age with *P*-value =0.59, *P*-value =0.15 respectively.

Conclusion: The study concluded that, hs-CRP is higher in type II DM especially uncontrolled DM patients, in fact that, hs-CRP used as a marker for cardiovascular diseases.

المستخلص

خلفية الدراسة: علامات الالتهاب كبروتين سي المتفاعل ترتبط بالامراض الالتهابية.اجريت دراسات سابقة لايجاد علاقة بين بروتين سي المتفاعل وامراض الاوعية القلبية خاصة في مرضى السكري من النوع الثاني.هذة الدراسة تهدف الى تقييم بروتين سي المتفاعل كعلامة تنبئية لامراض الاوعية القلبية بين مرضى السكري الغير متحكمين في ولاية الخرطوم.

طرق ومواد الدراسة: مائة وعشرون شخص شاركوا في هذة الدراسة.تم تصنيفهم الى ستين مريضا بمرض السكري من النوع الثاني وستين شخصا اصحاء كمجموعة تحكم.بروتين سي في المصل والهيموقلوبين السكري في الدم الكامل تم تقييمهم باستخدام طريقة التعكر المناعية والطريقة المناعية للكشف المتألق ، تتابعيا .

نتيجة الدراسة: اظهرت النتائج ان بروتين سي المتفاعل اظهر ارتفاعا بدلالة معنوية لدى مرضى السكري من النوع الثاني (P-value 0.000) خاصة عند مرضى السكري الغير متحكمين (P-value 0.006) ، ايضا هناك ارتباطا ايجابيا بين بروتين سي المتفاعل ومدة المرض والهيموقلوبين السكري (P-value = P-value 0.000, 0.002)على التوالي.

لا يوجد ارتباط بين بروتين سي المتفاعل و معامل كتلة الجسم والعمر(P-value =0.59 , P- =0.15 value) على التوالي.

خلاصة الدراسة: لخصت الدراسة الى ان بروتين سي المتفاعل اظهر ارتفاعا بدلالة معنوية في مرضى السكري خاصة الغير متحكمين.لذلك يمكن الاستفادة منه كواسم لامراض الاوعية القلبية.

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Chapter One

(Introduction and Literature review)

1.1 Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. The prevalence of diabetes has been increasing in all countries during the last century. In 2010, 285 millions in the world were estimated with the disease, and the number is expected to grow to 438 million by 2030. 70% of the global prevalence of cases occurs in low and middle income countries. According to WHO project diabetes is one of four non communicable diseases which are responsible for the 63% number of deaths worldwide (Tietz *et al.,*2005; Bishop *et al.,*2006; Abbaso *et al.,* 2000; Bisal, 2011).

Much of mortality of diabetes results from long term of microvascular complications (DN, neuropathy and retinopathy) and macrovascular complications (heart disease), the progression of these complications correlated with glycemic control (life style and pharmaceutical) and with presence of non glycemic influence factors which are hypertension and hyper lipidenia (Malandrino and Robert , 2010).

Diabetic kidney disease or DN is one of the principle causes of end stage renal disease (ESRD) or renal failure, together with age, hypertension and increase BMI. So blood glucose management strategies together with therapy of other risk factors contributed to reduction in the incidence of diabetic nephropathy (Malandrino and Robert, 2010).

An early sign of diabetic nephropathy is presence of small amount of albumin in urine called microalbuminuria (MAU), so measurement of MAU is useful to assist in diagnosis at early stage of renal disease (Bishop *et al.,* 2006).

1.1.1 Definition of Diabetes Mellitus (DM)

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin action, or both. Hyperglycemia it is an increase in plasma glucose level, in healthy subjects during hyperglycemia, insulin secreted, and enhances entry of glucose to cells where they take normal pathways. So hyperglycemia is caused by imbalance of hormones (Bishop *et al.,* 2006).

1.1.2 Hormonal control of Blood Glucose

1.1.2.1 Insulin: Insulin is a protein hormone produced by the β-cells of the islets of langerhans in the pancreas. It is an anabolic hormone that stimulates uptake of glucose into fat and muscle, promotes the conversion of glucose to glycogen or fat for storage, inhibits glucose production by liver, stimulates protein synthesis, and inhibit protein breakdown. Insulin secretion stimulated by glucose and amino acid, inhibited by hypoglycemia and somatostatin (Tietz *et al.,* 2005).

1.1.2.2 Other hormones

1.1.2.2.1 Glucagon: Is a small protein produced by β-cells of islets of langerhans in the pancreas, releases during fasting and stress. Is increase plasma glucose by glycogenlysis in the liver and increase glucoeogonsis (Bishop *et al.,* 2006).

1.1.2.2.2 Catecholamines: Are epinephrine and nor epinephrine increase plasma glucose by inhibiting insulin secretion increasing glycogenlysis and promoting lipolysis (Bishop *et al.,* 2006).

1.1.2.2.3 Glucocorticoids: Primary cortisol increase plasma glucose by decreasing entry into cell, increasing gluconeogensis, liver glycogen and lipolysis (Bishop *et al.,* 2006).

1.1.2.2.4 Growth Hormone: Stimulated by decrease blood glucose level, promotes increase it by decreasing entry of glucose to the cell and increasing glycolysis (Bishop *et al.,* 2006).

1.1.3 Classification of DM

The ADA and WHO guide lines recommend the following categories:

Type I diabetes mellitus, type II diabetes mellitus, other specific types of diabetes mellitus and gestational diabetes mellitus (Bishop *et al.,* 2006).

1.1.3.1 Type I DM

It is a previous term was insulin- dependent diabetes mellitus (IDDM), it result from cellular-mediated autoimmune destruction of the β-cells of the pancreas; causing absolute deficiency of insulin .Type I constitutes 10 to 20% of all cases, more commonly in youth, characteristics include abrupt onset, ketosis tendency and insulin dependence (Bishop *et al.,* 2006).

1.1.3.2 Type II DM

It is previous terms was non insulin dependent (NIDDM), it result from resistance to insulin with an insulin security defect, causing relative insulin deficiency, characteristics are constitutes the majority of cases, commonly in older one) associated with genetic predisposition, increase risk with patient with increase age, obesity and lack of exercise, move likely to go with hyperosmolar state and increase risk developing complications (Bishop *et al.,* 2006). .

1.1.3.3 Other specific types

Formerly known as secondary diabetes are associated with certain conditions, including pancreatic disease, endocrine disease, drug and chemical induced and other genetic syndromes (Bishop *et al.,* 2006).

1.1.3.4 Gestational DM

It is glucose intolerance with onset first recognition during pregnancy which caused by metabolic and hormonal changes (Bishop *et al.,* 2006).

1.1.4 Symptoms of common types

1.1.4.1 Symptoms of type I DM

The symptoms of type-1 DM are frequent urination, unusual thirst and extreme hunger, extreme fatigue and irritability.

1.1.4.2-Symptoms of type II DM:

The symptoms of type-II DM are frequent urination, unusual thirst and extreme hunger.
Extreme fatigue, irritability, frequent infection, blurred vision, cuts and numbness in hand and feet, reoccurring skin and gum and bladder infections (Cooke and Plotnick, 2012).

1.1.5-Diagnosis and management of DM:

The clinical laboratory has a vital role in both the diagnosis and management of diabetes.

1.1.5.1 Diagnosis

1.1.5.1.1 Preclinical diagnosis: Measuring of islet cell antibodies screening functional abnormalities of glucose insulin secretion, hyperinsulinemia and loss of normal palstile insulin secretion demonstration; before appearance of symptoms can delay onset of disease or prevent its development (Tietz *et al,* 2005).

1.1.5.1.2 Clinical diagnosis: by measurement and demonstrating hyperglycemia. There are three methods any one of it is diagnostic:

(1) Classical symptoms and unequivocal elevation of plasma glucose.

(2) Elevated fasting glucose more than one occasion, venous plasma >or=140mg/dl.
(3) Elevated glucose during the oral glucose tolerance test on more than one occasion 2hr postprandial > or =200 mg/dl (Tietz *et al.,*2005).

1.1.5.2 Management

1.1.5.2.1 Acute case: in diabetic ketoacidosis, hyperosmolar non ketotic coma and hypoglycemia, the laboratory has essential role in both diagnosis and monitoring of therapy.

1.1.5.2.2 Chronic case : aim of diabetes management is to maintain blood glucose within or near normal range, measuring of glucose and HA1c level provides an index of short term and long term control, the monitoring of complications are achieved by assessing urinary albumin excretion (UAE), urea, creatinine and serum lipids(Tietz *et al.,*2005) .

1.1.6- Complications of DM:

1.1.6.1 Acute complications: hypoglycemia, non ketotic hyperosmolar state common with type II patients and diabetes ketoacidosis with type I diabetic patients.

1.1.6.2 Chronic complications: are long term complications and involve three underlying mechanisms: Formation of advanced glycosylation end products which accumulate overtime in vessel wall, activation of protein kinase (which is leading to increase deposition of extracellular matrix and basement membrane materials and accumulation of sorbital as product of polyol pathways which is causing all injury special in some tissues not need insulin for glucose transport (e.g: nerves, lens, kidneys, blood vessels) all these processes are activated by intracellular hyperglycemia (Abbaso *et al.,*2000).

Table 1.1: Complications of DM (Abbaso *et al.,* 2000).

Micro vascular complications	**Presentations**
- Retinopathy - Cataract	- Impaired vision - Impaired vision
- Nephropathy	- End stage renal disease
- Foot disease	- Ulceration and infection
- Neuropathy	-Sensor loss and motor weakness
Macrovascular complications	**Its Presentations**
- Coronary artery disease	-Angina and myocardial infarction.
- Cerebral ischemia	- Stroke
- Peripheral vascular.	- Claudication

1.2 C reactive protein

1.2.1 Definition-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation, it is an acute phase protein. Its physiological role is to bind to to phosphocholine expressed on the surface of dead or dying cells and some type of bacteria, in order to activate the complement system the C1Q complex (Thompson *et al*., 1999).

CRP is synthesized by the liver in response to factors released by fat cells (adipocyte). It is a member of the pentraxins family of proteins. It is not related to C-peptide or protein C. C-reactive protein was the first pattern recognition receptor (PRR) to be identified (Mantovani *et al*., 2008).

1.2.2 History and nomenclature of CRP

CRP was so named because it was first discovered as a substance in the serum of patient with acute inflammation that reacted with the C- (capsular) polysaccharide of pneumococcus. Discovered by Tillet and Francis in 1930, it was initially thought that CRP might be a pathogenic secretion as it was elevated in people with a variety of illness including cancer; however discovery of hepatic synthesis demonstrated that it is a native protein (Faraj and Salem, 2012).

1.2.3 Genetic and biochemistry of CRP

The CRP gene is located on the first chromosome (1q21-q23). CRP is a 224-residue protein with a monomer molar mass of 25106 Da. The protein is an annular pentameric disc in shape and a member of the small pentraxins family (Faraj and Salem, 2012).

1.2.4 Function of CRP

The acute phase response develops in a wide range of acute and chronic inflammatory conditions like bacterial, viral, or fungal infections; rheumatoid and other inflammatory disease; malignancy; and tissue injures and necrosis. These conditions cause release of interlukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver. During the acute phase response, levels of CRP rapidly increase within two hours of acute insult, reaching a peak at 48 hours. With resolution of the acute phase response, CRP declines with a relatively short half-life of 18 hours. Measuring CRP level is screen for infectious and inflammatory diseases. Rapid and marked increases in CRP occur in inflammation, infection, trauma, tissue necrosis, malignances and auto immune disorders. Because there are a large number of disparate

conditions that can increase CRP production, and an elevated CRP production, an elevated CRP level dose not diagnose specific disease. An elevated CRP level can indicate support for the presence of an inflammatory disease, such as rheumatoid arthritis, polymalgia rheumatica or giant cell arthritis (Pepys and Hirschfield, 2003).

The physiological role of CRP is to bind to phosphocholine expressed on the surface of dead or dying cells, in order to activate the complement system. CRP binds to phosphocholine on microbes and damaged cells and enhances phagocytosis by macrophages. Thus, CRP participates in the clearance of necrotic and apoptotic cells (Pepys and Hirschfield, 2003).

CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory process occurring in the body. This increment is due to arise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocyte. CRP assist complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin mediated phagocytosis), which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections. CRP rises up to 50,000-fold in acute inflammation, such as infection. It is rise above normal limit within six hours, and peaks at 48 hours. Its half-life is constant, and therefore its level is mainly determined by the rate of production, hence severity of precipitating cause. Serum amyloid A is a related acute-phase marker that responds rapidly in similar circumstances (Pepys and Hirschfield, 2003).

1.2.5 Clinical significance of CRP

Scleroderma, polymyositis, and dermatomyositis often elicit little or no CRP response. CRP levels also tend not be elevated in SLE unless serositis or synovitis is present. Elevation 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 sever peripheral vascular disease (Danesh *et al*., 2004).

1.2.6 Role of CRP in cardiovascular disease

Present researches suggest that patients with elevated basal levels of CRP are at increased risk of diabetes, hypertension and cardiovascular diseases (Pradhan *et al*., 2001). Although one group of researchers indicate that CRP may be only a moderate risk factor for cardiovascular diseases

(Danesh *et al*., 2004), in this study known as 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, 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 a high CRP levels might reflect a large benefit from statins. This is based on the JUPITER trial that found elevated CRP levels without hyperlipidemia benefited. Statins were selected because they have been proven to reduce levels of CRP (Ridker *et al*., 2008). A subsequent trial however failed to find if CRP was useful for determining statin benefit (Emberson *et al*., 2011).

To clarify whether CRP is a by stander or active participant in atherogenesis, at 2008 a study compared people with various genetic CRP variants. Those with a high CRP due to genetic variation had no increase risk of cardiovascular diseases compare to those with a normal or low CRP (Zacho *et al*., 2008).

1.2.7 Diagnostic use of CRP

There are two different tests for CRP. The standard test measures a much wider range of CRP levels but is less sensitive in the lower ranges. The high-sensitivity CRP (hs-CRP) test can more accurately detect lower concentrations of the protein (it is more sensitive), which makes it more useful than the CRP test in predicting a healthy person's risk for cardiovascular disease (Faraj and Salem, 2012). CRP is used mainly as a marker of inflammation, a part from liver failure; there are few known factors that interfere with CRP production (Pepys and Hirschfield, 2003). Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments. Blood, usually collected in a serum-separating tube, and analyzed in a medical laboratory or at the point of care. Various analytical methods are available for CRP determination, such as ELISA, immunoturbidimetry, rapid immunodiffusion, and visual agglutination. A high sensitive CRP (hs-CRP) test measures low levels of CRP using laser nephlometry, the test gives results in 25 minutes with sensitivity down to 0.04 mg/L, immunoturbidimetry (Immunoturbidimetric Method) this reagent is intended for the in vitro

quantitative determination of CRP concentration in serum or plasma on automated clinical chemistry analyzers. Normal concentration in healthy human serum is usually lower than 10mg/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 – 200mg/L), sever bacterial infection and burns (more than 200mg/L) (Clyne and Olshaker, 1999). CRP is more sensitive and accurate reflection of the acute phase response than the ESR. The half-life of CRP is constant. Therefore CRP levels are mainly determined by the rate of production. In the first 24hours, ESR may be normal and CRP elevated. CRP returns to normal more quickly than ESR in response to therapy (Clyne and Olshaker, 1999).

1.2.8 Cardiological diagnostic test of CRP

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. Nevertheless, a level above 2.4mg/L has been associated with a doubled risk of a coronary event compared to levels below 1mg/L (Lloyd-Jones *et al*., 2006).

1.2.9 CRP and Diabetes Mellitus

Inflammatory markers such as CRP have been related to the development of insulin resistance and type 2 diabetes .Previous researches has also established that CRP levels are higher in people with diabetes and associated with HbA1c in people without diabetes . The results of the study go a step further with the finding that among people with established diabetes, at successively higher levels of HbA1c the percent of people with CRP >0.30 mg/dl is significantly higher. The main implication of these findings is that inflammation may not only be implicated in the development of diabetes, but also in ongoing levels of hyperglycemia once diabetes is established. If poor glycemic control leads to inflammation, then better glycemic control should lower inflammation and therefore lower the risk of cardiovascular complications. If inflammation leads to poor glycemic control, then treatment of inflammation with NSAIDs or hydroxymethylglutaryl-CoA reductase inhibitors may help improve glycemic control. In light of recent findings of an association among inflammatory proteins, endothelial dysfunction, and insulin resistance, the results of the current study provide additional support for a relation between glycemic control and systemic inflammation in people with established diabetes.

Previous research evidence supports a link between hyperglycemia and inflammation. CRP is known to be higher in people with impaired glucose tolerance and frank diabetes. Furthermore, increased CRP has been found to be a risk factor for later development of diabetes. Other studies have related hyperglycemia to inflammation by demonstrating simultaneous inflammation, endothelial dysfunction, and insulin resistance at the physiologic level. One of the several mechanisms proposed is oxidative stress on the endothelium, which promotes inflammation and is enhanced by hyperglycemia (Danesh *et al.*, 2003).

1.3 Rationale

In Sudan diabetes mellitus disease is in increase in both sex's males and females and occurs in different age groups, it can cause many organ damages and dysfunctions. Diabetes mellitus is a major risk factor for stroke, peripheral vascular disease, heart failure and chronic kidney disease. HbA1c is one of the factors that monitor glycemic in diabetic patients. High level of HbA1c for long time leads to many chronic diseases.

Several studies hypothesis that, CRP may induce insulin resistant is a potential factor for diabetes mellitus. Reverse causation might also be implicated, whereby poor glycemic control leads to high level of CRP and inflammation, and therefore increase the risk of cardiovascular complications., several workers have reported elevated levels of CRP in diabetic individuals.

In the Sudan little is known about the association between HbA1c, CRP and cardiovascular complications in uncontrolled diabetic patient, accordingly present research conducted to study CRP as predictor markers for cardiovascular complications in uncontrolled diabetic patients.

1.4 General objective

To evaluate hs-CRP level as a predictor marker for cardiovascular diseases among uncontrolled type II Sudanese diabetic patients in Khartoum State.

1.5 Specific objectives

1- To estimate HbA1c and hs-CRP levels in study groups.

2- To compare means concentration of hs-CRP in cases and control groups.

3- To compare mean concentration of hs-CRP in control and uncontrolled type II diabetic patients.

4- To correlate between hs-CRP level and study variables (Hb A1C, gender, BMI, age and duration of DM).

Chapter Two

(Materials

and

Methods)

2 Materials and Methods

2.1 Materials

2.1.1 Study Design

This is a descriptive analytic cross-sectional study, conducted during the period of March to August 2015.

2.1.2 Study Area

This study was carried out in Diabetes specialize Hospital, Zeenam specialize hospital, Almotakamil medical center in Khartoum state.

2.1.3 Study Population

One hundred and twenty individuals were enrolled in this study, and classified into two groups, 60 type II diabetic patients as case group (Hb A1c 6.0-6.9% consider as good controlled and Hb A1c > 7% consider as poor controlled) and 60 healthy individuals as control group.

2.1.4 Inclusion criteria

Type II diabetic patients were included in this study

2.1.5 Exclusion criteria

Patients with chronic inflammatory diseases such as rheumatoid arthritis (RA), osteoarthritis (OA), systemic lupus erythromatosus (SLE), autoimmune diseases, tuberculosis, hypertension, and stroke, any hepatic or renal diseases and malignancies were excluded from this study.

2.1.6 Collection of Samples

Samples were collected using dry, plastic syringes, tourniquet was used to make the veins more prominent, 5ml blood samples was collected in plane containers and (3ml) in EDTA containers from each volunteer was collected under septic condition. All blood samples in plane containers were allowed to clot at 25°, and then they were centrifuged at 4000 rpm to obtain the serum samples, and stored in -20° until the analysis. While blood samples in EDTA containers were immediately analyzed.

2.1.7 Ethical Considerations

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

2.2 Methods

2.2.1 CRP Estimation

2.2.1.1 Principle

Particle enhanced immunoturbidimetric assay.

Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

2.2.1.2 Procedure

Dispensing and all processes done automatically by using Cobas C 311 automated chemistry analyzer.

2.2.1.3 Calculations

Roche/Hitachi Cobas c systems automatically calculate the analyte concentration of each sample. Conversion factors:

$$mg/L \times 9.52 = nmol/L$$

$$mg/L \times 0.1 = mg/dL$$

2.2.2 HbA1c Estimation

2.2.2.1 Principle

Ichroma HbA1c is based on florescence immunoassay technology, specifically the sandwich immune-detection method.

Whole blood is added to the mixture of hemolysis buffer and detection buffer, which result in hemolysis of red blood cells. Such that by mixing detecting buffer with blood specimen in test tube, the florescence-labeled detector anti-HbA1c antibody in buffer binds to HbA1c antigen in blood specimen. The sample mixture is loaded and migrates on the matrix of test cartilage; the

complexes of detector antibody and HbA1c are captured to anti-HbA1c sandwich pair antibody that has been immobilized on test matrix. As a result, the higher concentration of HbA1c produces a higher florescence signal from HbA1c-antibody complexes. The signal is interpreted and the result display on ichroma Reader in units of percentage, mmol/mol and mg/dl.

2.2.2.2 Procedure

- 100 µl from hemolysis buffer was added into detection buffer tube.
- 5 µl whole blood was added to detection buffer tube then sacked 15 times.
-75 µl from sample mixture was taken and dispensed into sample well on the test cartridge.
- Test cartridge was inserted into ichamber for 12 minutes then inserted into ichroma™ Reader for scanning.
- The button (select) was pressed to start scanning process and the test result read on display screen.

2.2.3 Calculation of BMI

BMI obtained by calculation according to formula:

$$\text{weight(kg)} \div \text{hight}^2\text{(m)}$$

2.2.4 Statistical Analysis

The data was analyzed using statistical package of social science (SPSS) computer program using frequencies, independent t-test and Pearson correlation , results was expressed as percentage (%) and (mean ± SD), and significance difference was consider as (*P*-value <0.05).

Chapter Three

Results

3. Results

One hundred and twenty randomly samples were collected from type II diabetic patients to evaluate the level of hs-c reactive protein, HbA1c and BMI among study groups, classified as 60 healthy apparently as control group and 60 type II DM as case, males account 23(38.33%) and female 37 (61.67%) with ratio Of 1:1.6, and participants average age is (51±11SD) years.

Figure.3.1 Shows frequencies of gender among diabetic patients.

Table.3.1 Shows frequencies of BMI, classified as normal weight (BMI ≤ 26.5 kg/m^2) and over weight (BMI > 26.5 kg/m^2) among gender (males and females).

Table.3.2 Presenting the mean concentration of HbA1c among males (9.90 ± 3.85%) and females (9.20 ± 3.93%), with P-value = 0.466.

Table 3.3 Shows mean concentration of hs-c reactive protein in case (3.10 ± 1.30 mg/l) and control group (1.60 ± 0.70 mg/l), with P-value = 0.000.

Table 3.4 Shows mean concentration of hs-c reactive protein in males (2.47 ± 1.34 mg/l) and females (2.28 ± 1.22 mg/l), with P-value = 0.423.

Figure 3.2 Shows mean concentration of hs-c reactive protein in HbA1c good control (2.00 ± 0.76 mg/l) and poor control groups (3.30 ±1.30 mg/l), result expressed as (mean ± SD) and significance deference (P-value 0.006).

Figure 3.3 Pearson correlation results shows no correlation between hs-c reactive protein and age with R-value 0.13 and P-value 0.15

Figure 3.4 Pearson correlation results show correlation between hs-CRP and duration of DM type II with R-value 0.399 (positive correlation) and P-value 0.002.

Figure 3.5 Pearson correlation results show no correlation between hs-c reactive protein and BMI with R-value 0.049 and P-value 0.59.

Figure 3.6 Pearson correlation results shows correlation between hs-c reactive protein and HbA1c with R-value 0.97 and P-value 0.000.

Fig. 3.1 frequencies of gender among diabetic patients

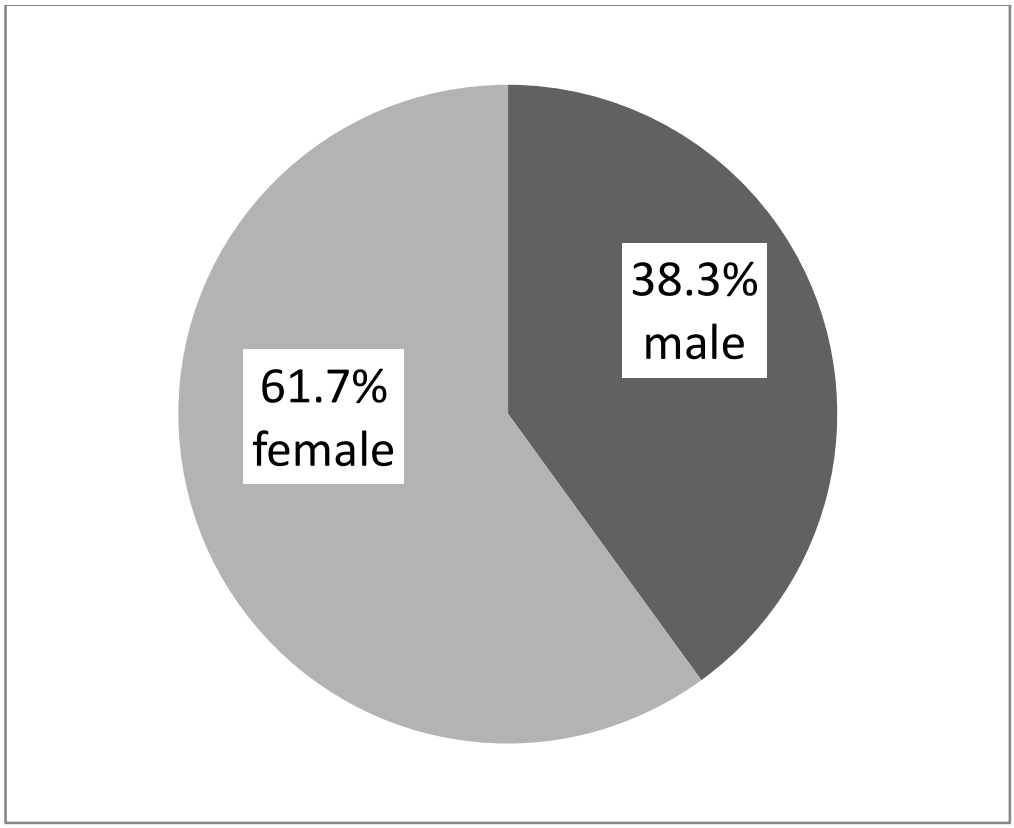

Fig. 3.1 Shows frequencies of gender among diabetic patients, results expressed as percentage (%).

Table.3.1: Frequencies of normal and overweight among gender variation

BMI	Gender	
	Male	Female
Normal weight	64.6%	58.3%
Over weight	35.4%	41.7%
Total (%)	100%	100%

Table.3.1 Shows frequencies of BMI according to gender, classified as normal weight (BMI ≤ 26.5 kg/m^2) and over weight (BMI > 26.5 kg/m^2) among gender (male and female).

Table.3.2: Mean concentration of HbA1c among gender variation

Variable		Mean ± SD	P-value
HbA1c (%)	**Males**	9.90 ± 3.85%	0.466
	Females	9.20 ± 3.93%	

Table.3.2 Shows mean concentration of HbA1c in males versus females, Significance difference considered as P-value < 0.05

Table 3.3: Mean concentration of hs-CRP in case and control groups

Variable		Mean ± SD	P-value
HsCRP (mg/l)	**Case**	3.10 ± 1.30 mg/l	0.000 **
	Control	1.60 ± 0.70 mg/l	

Table 3.3 Show mean concentration of hs-CRP in case and control group.

-** indicate highly significance

Table 3.4: Mean concentration of hs-CRP among gender variation

Variable		Mean ± SD	P-value
HsCRP (mg/l)	**Males**	3.27 ± 1.34 mg/l	0.261
	Females	2.90 ± 1.32 mg/l	

Table 3.4 Shows mean concentration of hs-CRP in males versus females, significance difference consider as P-value < 0.05

Figure 3.2 Mean concentration of hs-CRP in HbA1c good and poor control groups

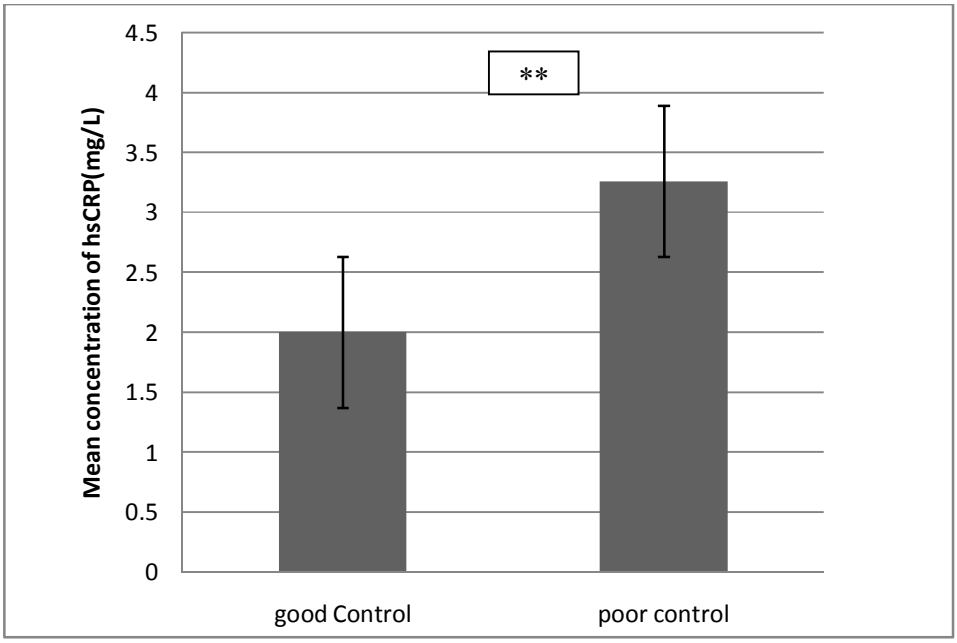

Figure 3.2 Shows mean concentration of hs-CRP in HbA1c good control (2.00 ± 0.76) and poor control groups (3.30 ± 1.30).

Figure 3.3 Correlation of hs-CRP and age

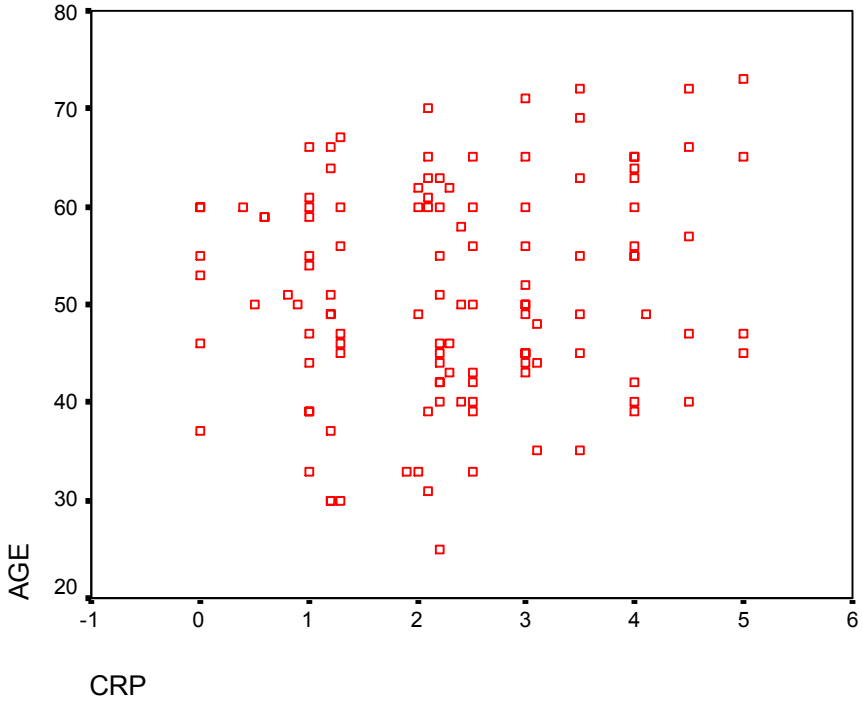

Figure 3.3 Shows Personal correlation results no correlation between hs-c reactive protein and age with r-value 0.13 and *P*-value 0.15

Figure 3.4 Correlation of hs-CRP and duration of diabetes mellitus

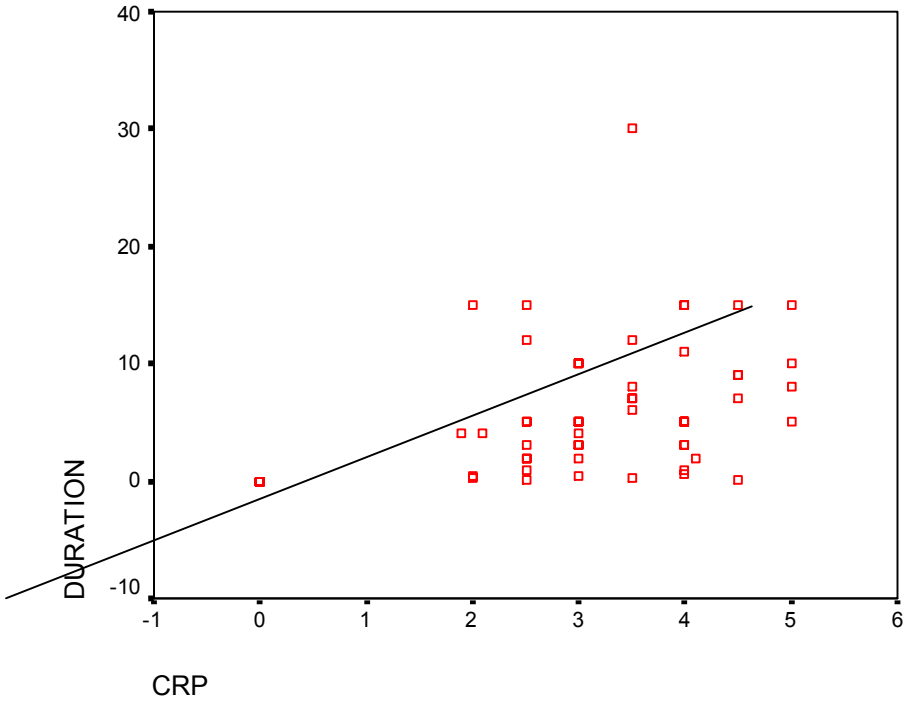

Figure 3.4 personal correlation results between hs-c reactive protein and duration of type II DM with r-value 0.399 and *P*-value 0.002

Figure 3.5 Correlation of hs-CRP and BMI

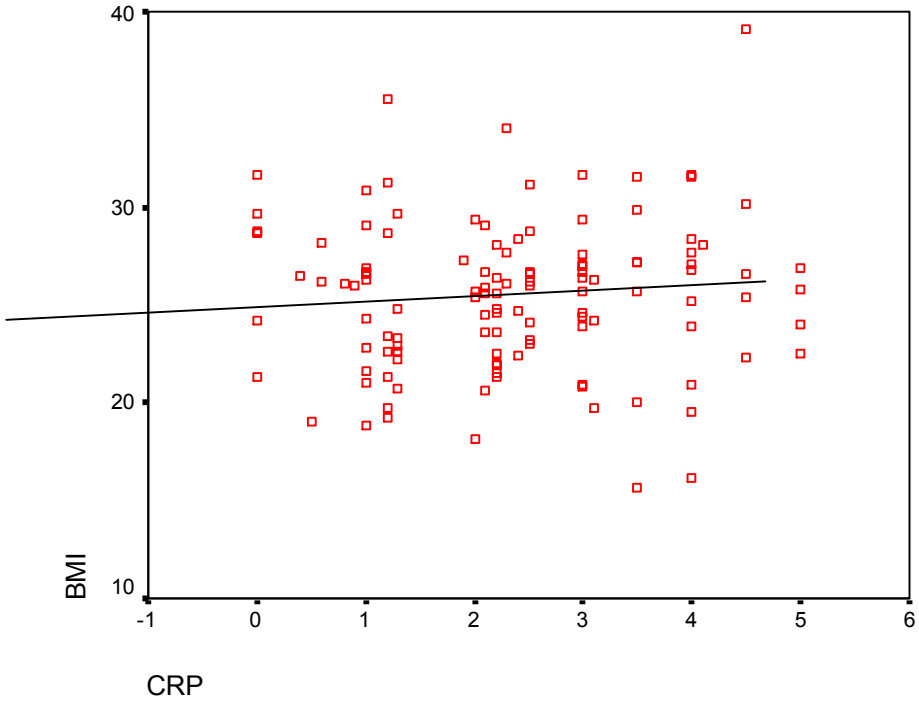

Figure 3.5 Shows no correlation between hs-c reactive protein and BMI with r-value 0.049 and *P*-value 0.59

Figure 3.6 Correlation of hs-CRP and Hb A1C

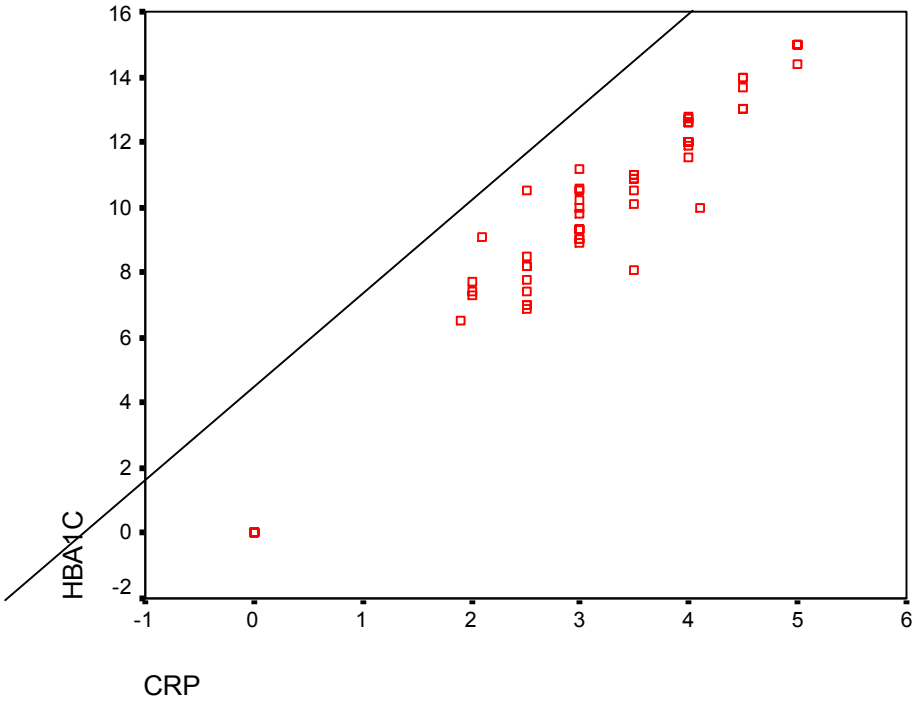

Figure 3.6 personal correlation results shows positive correlation between hs-c reactive protein and HbA1c with r-value 0.97 and *P*-value 0.000

Chapter Four

(Discussion

Conclusion, Recommendation)

4.1 Discussions

Nowadays, diabetes mellitus is one of the most important causes of death all over the world because of its adverse effects on cardiovascular system and is one of the important factors that may influence metabolic status. Many studies have shown the modulatory effect of hs-CRP on insulin resistant **(Ataru *et al.*, 2002).** Therefore this descriptive cross-sectional study was carried out to study hs-CRP levels as predictor marker for cardiovascular disease among diabetic patients in Khartoum State, in addition was correlated between hs CRP levels and study variables (HbA1c, gender, BMI, age and duration).

Concerning gender, frequencies showed that, the gender variation is one to two third (38.4% male and 61.7% female),with approximately 1:1.6 which indicated that type II DM females is higher than males, this finding was similar to previous reports in America (39.3%) were males, and (60.7%) were females **(Huffman *et al.*, 2010*)***, and in conflict with other study which stated that, male are more affected by DM than females, as reported in Japan as (62%) were male, and (38%) were female **(Ataru *et al*, 2002)** which justified by community gender variation.

According to BMI, the present study revealed that, diabetic overweight females were more (58.4%) than males (49.7%), these results indicate that diabetic female are more susceptible to complications of obesity, which may contributed to insulin resistance that, obesity and diabetes are closely linked, obesity is a potent risk factor for diabetes and diabetes may predispose to obesity **(Huffman *et al.*, 2010)**. Possible explanation for this result is that males are more physically active compared to females.

The present study results showed significant increase in mean CRP level in diabetic patients compared with control group with (*P*-value 0.000), results indicates that CRP is useful predictor marker for cardiovascular disease in diabetic patients. Since both diabetes mellitus and inflammation have been linked to cardiovascular diseases, these differences strengthen the association between CRP and increasing incidence of vascular inflammation. Similar observation was made that, Diabetes mellitus is independently associated with elevated hs-CRP **(Sarinn and Wanicagool, 2008)**.

The present study revealed that, there was insignificant difference between mean concentrations of hs-CRP in males in comparison with females with (*P*-value 0.261). This findings was agreed with study performed in Japan, among diabetic subjects, there were no significant difference in the level of C-reactive protein between the males and the females, also was observed agreed with

study performed in America where, the male diabetic patients did not show any significant difference in hs-CRP levels as compared to female diabetic subjects **(Huffman *et al.*, 2010, Ataru *et al*, 2002).** It has been reported that DM is in part an metabolic disorder and several workers have reported elevated levels of CRP in diabetic individuals when compared to healthy individuals which confirm the role of CRP in diabetes mellitus or vice versa, several studies hypothesis that CRP may induce insulin resistant and a potential risk factor for DM, reverse causation might also be implicated, whereby high blood glucose may induce inflammation and raise CRP levels **(Shoichiro *et al*, 2002)**.

The present study revealed that, there was significant increase in mean levels of hs CRP among poor controlled versus good controlled type II DM patients with P-value 0.006. In fact that C-reactive protein is significant higher in poor compared with good glycemic patients **(Sarinn and Wanicagool, 2008)**.

Pearson's correlation results showed that, there was no correlation between CRP level and age of the patients, with (*P*-valve 0.150), this evidence confirmed by India which showed that, hs-CRP obtained in male and female with different age group were not correlated with these factors **(Sarinn and Wanicagool., 2008)**.

The present study provide experimental evidence that, there was positive correlation between hs-CRP level and duration of diabetes, with (*P*-value 0.002), these findings agreed with a study performed in America which showed that, the elevation levels of hs-CRP was found to be depended on duration, patients with how long duration of diabetes history were found to have significantly elevated levels of hs-CRP compared to those with shorter duration of diabetes history **(Huffman *et al.*, 2010)**.

The present study revealed that there was no correlation between hs CRP level and BMI, with (*P*-value 0.59), these findings agreed with a study in India which reported that, the elevation levels of hs-CRP was independent on BMI **(Sarinn and Wanicagool., 2008).**

Finally, this study showed positive correlation between HbA1c and hs-CRP with (*P*-value 0.00, R-0.97). Similar observation was reported by previous studies as mentioned earlier that diabetic patients showed significant positive associations between Hb A1C and the inflammatory markers CRP **(Fatma *et al.*, 2010; Sarinn *et al.*, 2008)**.

4.2 Conclusion

The study concludes that, hs- CRP level is significant higher in type II DM specially uncontrolled DM patients, and positive correlation between hs- CRP, duration and HbA1C,while no correlation with BMI and age, as hs-CRP a marker for cardiovascular disease it could be useful predictor marker for early detection of atherosclerosis especially for long duration and uncontrolled DM patients.

4.3 Recommendation

As hs-CRP was proposed as promising alternative marker of cardiovascular diseases owing to better specificity and sensitivity for detecting mild decrease in GFR. Our study recommends that, DM patients should be monitoring for micro and macrovascular complications using serum hs-CRP every 3 months.

DM patients should be aware about importance of control blood glucose.

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4.5 Appendixes:

Appendix

(I)

Sudan University of Science and Technology

College of Graduate Studies

Msc of medical Laboratory

Questionnaire

Date: …… ID NO: …….....

Patient Name: ……..

Age: …….

Sex: Male ☐ Female ☐

Phone Number: …….

Weight: ……kg Height: ……meter

Body Mass Index (BMI): ……kg/m^2

Duration of Disease: ……..

Diseases Others:…….

Hb A1C Result: ……%

Hs CRP Result: ……mg/l

Appendix

(II)

Methodology sheets