



Sudan University of Science and Technology



College of Graduate Studies

**Assessment of Serum Levels of C-reactive protein
and Troponin I in Sudanese Patients Reside in UAE with
Type 2 Diabetes Mellitus**

**تقويم مستويات وبروتين سي التفاعلي وتروبونين اى للسودانيين المقيمين بدولة
الإمارات العربية المصابين بداء السكري النوع الثاني**

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medical laboratory science (Clinical Chemistry)

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الآية

قال تعالى:

قَالَ نَزِعُونَ سَبْعَ سِنِينَ دَأَبًا فَمَا حَصَدْتُمْ فَذَرُونَهُ فِي سُنْبُلِهِ إِلَّا قَلِيلًا مِمَّا نَأْكُلُونَ

صدق الله العظيم

سورة يوسف الآية (47)

Dedication

To

My father Soul..... who worked hardly for us.

To

My mother Who taught me

How I could be as I am now

To

My lovely husband Ayman and our Daughters' Namatallah and Maryam

To

My beloved brothers, sisters and colleagues for their continuous support

To

My Teachers

The people whom I respect and appreciate

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With all due humbleness and gratitude, I render ultimate thanks and special praise to GOD (Almighty) who gave me health, power and patience to accomplish and conduct this work.

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Abstract

Diabetes mellitus is one of the most common metabolic diseases worldwide. This metabolic disorder contributes greatly to the significant proportion of the burden of cardiovascular problem the aim of the study was to investigate the cardiovascular biomarker of the diabetic patients who reside in United Arab of Emirates, diagnosed as diabetes mellitus type -2.

A cross-sectional study conducted during the period from July 2015 to June 2016 to determine and to evaluate the plasma CRP and Troponin I in Sudanese resides in UAE. a total of 100 subjects 97 (97%) of them were males and 3 (3%) were females, 50 Sudanese with type 2 diabetes mellitus were selected as a test group and 50 Sudanese healthy volunteers as a control group, age from 35-70 y mean of age is $(48.16 \pm 11.2y)$. Demographic data as well as medical history were obtained through the administration of a questionnaire. . Obtained results were analyzed using soft were SPSS version 16.0.

There was a statistically significant difference between the mean of glucose, Hba1c, CRP and troponin I among the test group when compared with control group. There was a positive correlation between plasma C- reactive protein and serum troponin I levels ($r=0.320$, $p=0.023$), also there was strong positive correlation between plasma levels of Glucose and Hba1c % ($r=0.531$, $p=0.000$) and negative correlation between serum glucose levels and troponin level ($r=0.059$, $P=0.63$). It was concluded that our results on the parameters measured; show that the diabetic population was experiencing mild increase in CRP and troponin compare to non-diabetic

المستخلص

داء السكري هو أحد الأمراض الأيضية الأكثر شيوعا في جميع أنحاء العالم. هذا الاضطراب الأيضي يساهم إلى حد كبير في نسبة كبيرة من عبء تلف القلب واختلاله الوظيفي. وكان الهدف من هذه الدراسة التحقق في وظائف القلب في السودانيين الذين يقيمون في دولة الإمارات العربية المتحدة تم تشخيصهم بداء السكري النوع-2.

أجريت هذه الدراسة المقطعية خلال الفترة من يوليو 2015 إلى يونيو 2016 لتحديد وتقييم تربونين السيرم وبروتين سي النشط في السودانيين الذين يعانون من مرض السكري النوع-2. 2 يقيمون في دولة الإمارات العربية المتحدة. وقد تم اختيار 50 سودانيا مصاب بداء السكري النوع-2 كمجموعة اختبار مقارنة مع 50 سودانيا متطوعا من الأصحاء كمجموعة تحكم.

تم الحصول على البيانات الديموغرافية وكذلك التاريخ الطبي بواسطة استبيان تم إجراؤه من خلال أندية التجمعات المحلية للسودانيين المقيمين بدولة الإمارات العربية المتحدة، كما تم جمع عينات الدم وتحليلها للجلوكوز، نسبة الهيموقلوبين المجلکز، التربونين والبروتين سي النشط. وقد تم تحليل البيانات التي تم جمعها بواسطة البرنامج الإحصائي SPSS نسخة رقم 16.

أجريت الدراسة في 100 مشارك منهم 50 مرضى السكري النوع-2 كمجموعة اختبار و 50 متطوعين من الأصحاء كمجموعة تحكم، الذين تتراوح أعمارهم بين 17-70 سنة. 97 منهم كانوا من الذكوراي بنسبة 97% مع 3% من الإناث كان الانحراف المعياري لمتوسط الأعمار $(SD \pm 48.16)$ (11.2 ± 48.16)

وكان هناك فروق ذات دلالة إحصائية بين متوسط مجموعة الاختبار مقارنة مع مجموعة التحكم وكانت قيمة $(P=0.000)$ في كل المتغيرات للجلوكوز، نسبة الهيموقلوبين المجلکز، التربونين وبروتين سي النشط)، كانت هناك علاقة سلبية بين نسبة التربونين في الدم ومستويات الجلوكوز البلازما $(r=0.059, P=0.68)$ ، كما كان هناك ارتباط إيجابي قوي بين مستويات البلازما من الجلوكوز ونسبة الهيموقلوبين المجلکز (HbA1c) $(r=0.531, p=0.000)$ ، وجود علاقة ايجابية ضعيفة بين مستويات التربونين وبروتين سي النشط $(r=0.320, P=0.023)$.

تخلص الدراسة إلى أن هنالك تأثير ضعيف لداء السكري نوع-2 ومستويات التربونين و بروتين سي النشط كما تبين أن مرضي السكري النوع الثاني لا يتأثرون بخلل في القلب ، مقارنة مع الأصحاء وغير المصابين بداء السكري. وذلك لأخذ عدد قليل من العينات.

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Chapter one

1.1. Introduction

Diabetes Mellitus (DM) is defined as a clinical syndrome characterized by hyperglycemia disease that occurs when the pancreas is no longer able to make insulin, or when the body cannot make good use of the insulin it produces. The metabolism of carbohydrates, fats and proteins is affected by the lack of insulin thus leading to a disturbance of water and electrolytes homeostasis. Cells of the vascular system are particularly susceptible to diabetes leading to the development of complications which characteristically affect the heart, eye, kidney and the nervous system (Frier& Fisher, 2006).

Diagnosis of diabetes is by detecting hyperglycemia, which if left untreated may lead to micro vascular diseases, like retinopathy, nephropathy and neuropathy, as well as macro vascular diseases, like coronary heart disease and atherosclerosis. A less severe form of hyperglycemia is called “impaired glucose tolerance”. Patients with impaired glucose tolerance have a high risk of developing macro vascular diseases, but a lower risk of developing micro vascular diseases (Frier& Fisher, 2006).

Nowadays, DM is one of the most challenging health problems of the 21st century. Prevalence of diabetes worldwide was 8.3% in 2014 and is estimated to rise to 10.1% in 2035 (International diabetes Federation 2014). DM type II accounts for approximately 90 percent of all cases of DM diagnosed in older individuals worldwide. Diabetes incidence is increasing due to population growth, aging, urbanization and the increasing prevalence of obesity and physical inactivity the world health organization (WHO) deems the prevention, diagnosis, and treatment of type ii dm a priority (WHO, 2008). The international diabetes federation has recently release estimates of the numbers of people with diabetes for 2014 and forecasts for 2035 with 378.3 million and 583.7 million, respectively (International Diabetes Federation, 2014)

The current epidemiological profile of population health in the United Arab Emirates (UAE) and consequently health care needs for the country are characterized by an increased burden of chronic diseases. Social and economic advances in UAE following the Union in 1972 have been accompanied by many cultural changes, which contributed to environmental and behavioral changes such as the adoption of new dietary habits and a sedentary lifestyle, and the stress of urbanization and of working conditions. All these changes contributed to the rise in diabetes risk factors (Gerald Reaven, 2004)

C-reactive protein (CRP) is an acute-phase protein that serves as an early marker of inflammation or infection. The protein is synthesized in the liver and is normally found at concentrations of less than 10 mg/L in the blood. During infectious or inflammatory disease states, CRP levels rise rapidly within the first 6 to 8 hours and peak at levels of up to 350–400 mg/L after 48 hours. CRP binds to phosphocholine expressed on the surface of damaged cells, as well as to polysaccharides and peptidoglycans present on bacteria, parasites and fungi. This binding activates the classical complement cascade of the immune system and modulates the activity of phagocyte cells, supporting the role of CRP in the opsonization (i.e. the process by which a pathogen is marked for ingestion and destruction by a phagocyte) of infectious agents and dead or dying cells. When the inflammation or tissue destruction is resolved, CRP levels fall, making it a useful marker for monitoring disease activity. CRP has been most widely measured using enzyme-linked protein. (World Health Organization 2014)

Serum levels of cardiac enzymes and isozymes are essential to the diagnosis or exclusion of myocardial damage. A new set of serum assays have been developed for the detection of cardiac injury. Cardiac troponin I is specific for cardiac tissue and is detected in the serum only if myocardial injury has occurred. The troponin I assay allows early identification and stratification of patients with chest pain suggestive of ischemia, allows identification of patients that present 48 hours to 6 days after infarction, and identifies patients with false positive elevations in CK-MB (such as in rhabdomyolysis). (William. Herman, *et al* 2015)

1.2. Rationale:

The purpose of this study is to evaluate the levels of CRP and troponin I in patients of diabetes type 2. Chronic inflammatory processes are thought to play a key role in the development of micro- and macro vascular complications in type 2 diabetes mellitus. This study aims to evaluate type 2 diabetic patient and the complication of diabetes in Sudanese people live on U.A.E which will help authorities to evaluate the problem more objectively and implement appropriate measures to reduce morbidity and mortality of diabetes in Sudanese people. To determine whether elevated levels of the inflammatory markers C-reactive protein (CRP) and troponin I are associated with development of type 2 DM in Sudanese men aged between 30-70 years. Too high glucose in the blood for a long time can cause diabetes problems; can damage many parts of the body, such as the heart, blood vessels,

eyes, and kidneys. Heart and blood vessel disease can lead to heart attacks and strokes, the leading causes of death for people with diabetes. The link between hyperglycemia, enhanced free-radical activity, and the complications of diabetes is unknown. In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. In Sudan, also diabetes continues to be a particularly serious medical and social problem like anywhere else in the world. Moreover, children are falling victims to it increasingly often. A lot can be done to prevent or slow down diabetes problems such as follow the healthy lifestyle, take medicines as directed and check blood glucose regularly with record. Also the study aims to set a group of data concerning complication and control of diabetes in Sudanese.

1.3 Objectives

1.3.1 General objective:

To study the level of C - reactive protein and troponin I level in Sudanese patient with type 2 diabetes mellitus.

1.3.2 Specific objectives:

1. To measure and compare the glycated hemoglobin (Hb A1C), CRP, troponin I and plasma glucose level among Sudanese patients with Type 2 Diabetes Mellitus with normal control.
2. To correlate between the serum CRP with the level of troponin I.
3. To correlate between glycated hemoglobin (Hb A1C) and plasma glucose level.
4. To correlate between plasma glucose and troponin I level.

Chapter Two

Literature Review

2.1. Diabetes Mellitus (DM)

Diabetes Mellitus (DM) is defined as a metabolic disorder of multiple etiology and characterized by hyperglycemia, which is caused by relative or absolute deficiency of insulin, which is normally produced by the Beta (β -) cells in the pancreas. The metabolism of carbohydrates, fats and proteins is affected by the lack of insulin, thus leading to a disturbance of water and electrolytes homeostasis (WHO. 1999).

In 1979, the National Diabetes Data Group developed a classification and diagnosis scheme for diabetes mellitus (Michael, *et al.* 2005, American diabetes association 2005). This scheme included dividing diabetes into two broad categories are type 1 insulin dependent diabetes mellitus (IDDM), and type 2, non-insulin dependent diabetes mellitus (NIDDM). Established in 1995, The International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, working under the sponsorship of the American Diabetes Association (ADA) was given the task of updating the 1979 classification system. They proposed eliminating the older terms of IDDM and NIDDM. Therefore, the ADA and World Health Organization (WHO) guidelines recommended these categories, Type 1 diabetes, Type 2 diabetes, other specific type of diabetes, Gestational diabetes. (Michael, *et al.* 2010)

2.1.1. Types of Diabetes:

2.1.1.1. Type I diabetes:

This type of diabetes is known as juvenile-onset diabetes. It may affect people of any age, but usually develops in children or young adults. It is usually caused by an auto-immune reaction where the body's defense system attacks the cells that produce insulin. Therefore, the body will produce very little or no insulin. Patients with this form of diabetes need injections of insulin every day in order to control the levels of glucose in their blood (International Diabetes Federation 2013).

2.1.1.2. Type II diabetes:

This type of diabetes is also called non-insulin dependent diabetes or adult-onset diabetes. It accounts for at least 90% of all cases of diabetes. It is caused by insulin resistance and/or relative insulin deficiency. Type II diabetes can occur at any age. This Type is very serious because it may remain undetected for many years and the diagnosis is often made when a

complication appears or a routine blood or urine glucose test is done. It is regularly, but not at all times, associated with overweight or obesity, which itself can cause insulin resistance and lead to high blood glucose levels. People with type II diabetes can often initially manage their condition through exercise and diet. Though, over time most people will require oral drugs or insulin to control the high blood glucose levels (International Diabetes Federation 2013.)

2.1.1.3. Gestational diabetes mellitus (GDM):

This is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy, usually during their third trimester. Every one in 25 pregnancies worldwide develop GDM. This type is considered serious because it is associated with complications to both mother and baby. GDM generally disappears after pregnancy but women with GDM and their children are at an increased risk of developing type II diabetes within five to ten years after delivery. Pregnant women who are overweight, have been diagnosed with Impaired Glucose Tolerance, or have a family history of diabetes are all at increased risk of developing GDM (International Diabetes Federation 2013).

2.1.2. Pathogenesis of DM:

2.1.2.1. Type I DM:

Diabetes type I is an autoimmune disease which is caused primarily by T lymphocytes reacting against beta cell antigens, causing cell damage, and resulting in reduction of the beta cell mass in the pancreas. The T cells include CD4+ T cells of the T helper1 subset, which cause tissue injury by activating macrophages, and CD8+ cytotoxic T lymphocytes, which directly kill beta cells and also produce cytokines that activate macrophages and damage the beta cells. The cytokines implicated in cell injury are Interferon- α produced by T cells and tumor necrosis factor and interleukin -1, produced by macrophages that are activated during the immune reaction (Maitra *et al.* 2007).

2.1.2.2. Type II DM:

Type II DM has some environmental risk factors, such as sedentary life style and obesity, as well as genetic risk factors. There are two possible metabolic defects that lead to type II DM:

Insulin resistance: This is defined as resistance to the effects of insulin on glucose uptake, metabolism or storage. There is a link between obesity and diabetes, as the risk of getting diabetes increases more with body mass index.

Beta cells' dysfunction: This is defined as the inability of beta cells to adapt themselves to the long-term demands of peripheral insulin resistance and increased insulin secretion. Beta cells dysfunction in type II diabetes may encompass both qualitative and quantitative aspects. (Maitra *et al.* 2007)

2.1.3. Signs and Symptoms of Diabetes:

According to the American Diabetic Association the development of type I diabetes is usually sudden and dramatic, whereas in people with type II diabetes, the symptoms can often be mild or absent, making this type of diabetes unnoticed. However, early detection and treatment of diabetes can decrease the risk of developing complications of diabetes dramatically. Frequent urination, excessive thirst, increased hunger, weight loss, tiredness, Lack of concentration, frequent infections, Slow-healing wounds, Weight loss, even though one is eating more (more common with type I), tingling, pain, or numbness in the hands/feet (more common with type II). (American Diabetes Association 2013)

2.1.4. Risk factors for Diabetes:

The increased prevalence of diabetes has been linked to increased body mass index, particularly in middle-income countries (Asia, Latin America, and Middle East), where obesity is the fifth most common cause of disease burden. About 90% of type II diabetes is attributable to excess weight. Furthermore, approximately 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the associated metabolic syndrome. This number is expected to increase to 420 million by 2025. Rates of obesity have tripled in developing countries that adopted a westernized life style involving a daily intake of fast food and very little physical activity. On the other hand, diabetes has shown a lower prevalence in countries where a traditional lifestyle has been preserved (Hossain, Kavar & El Nahas 2007).

According to the International Diabetes Federation (2013), not all type I diabetes' risk factors are known yet. Though, having a family member with type I diabetes will increase the risk of developing the disease. Environmental factors and exposure to some viral infections have also been associated with developing type I diabetes. Whereas several risk factors have been linked to type II diabetes, such as family history of diabetes, Overweight,

Unhealthy diet, Physical inactivity, Increasing age, High blood pressure, Ethnicity, Impaired Glucose Tolerance (IGT), History of gestational diabetes, Poor nutrition during pregnancy(Philip, *et al.* 2001, Carroll and Matz, 1983).

2.1.5. Complications of diabetes:

As mentioned before, diabetes has microvascular (damage to small blood vessels) and macro vascular (damage to large blood vessels) complications. Microvascular complications affect the eye (retinopathy) and might lead to blindness, the kidneys (nephropathy) and might cause renal failure, and the nervous system (neuropathy) leading to peripheral neuropathy, diabetic foot, and impotence. On the other hand, macro vascular complications affect the cardiovascular system leading to heart attacks, strokes and heart failure. There is evidence from large randomized-controlled trials that good metabolic control in both type I and II diabetes can delay the onset and progression of these complications (WHO, 2014).

Several DM complications include heart disease and stroke, kidney disease, neuropathy, foot amputations, dental disease and retinopathy that may lead to blindness (NDIC, 2012). These could decrease productivity level and increase work absenteeism, the rate of health care utilization and patients' and governments' medical expenses because health care needs at individual and community levels are high (Disdier *et al.* 2001).

2.1.5.1 Acute glycemic complication:

In diabetes mellitus, severe hyperglycemia may result from absolute or relative insulin deficiency. In some patients, the condition may culminate in diabetic ketoacidosis or hyperglycemic hyperosmolar nonketotic coma. Profound hypoglycemia may result from a relative excess of insulin. Symptoms associated with acute hyperglycemia generally develop more slowly (over hours or days) than do symptoms associated with an acute fall in the level of blood glucose (over minutes). Philip, *et al.* 2001, Carroll and Matz, 1983)

2.1.5.1.2 Hypoglycemia:

Hypoglycemia is the medical term for a state produced by a lower than normal level of blood glucose level below approximately 60 mg/dL. Hypoglycemia can produce a variety of symptoms and effects but the principal problems arise from an inadequate supply of glucose as fuel to the brain, resulting in impairment of function (neuroglycopenia), other symptoms associated with adrenergic symptoms (apprehension, tremors, sweating, or

palpitations). Usually, the symptoms of low blood glucose are mild, related to catecholamine release, and easily treated by the patient. Effects can range from vaguely "feeling bad" to seizures, unconsciousness, and (rarely) permanent brain damage or death. The most common forms of moderate and severe hypoglycemia occur as a complication of treatment of diabetes mellitus with insulin or oral medications. Hypoglycemia is less common in non-diabetic persons, but can occur at any age, from many causes. Among the causes are excessive insulin produced in the body, inborn errors of carbohydrate, fat, amino acid or organic acid metabolism, medications and poisons, alcohol, hormone deficiencies, certain tumors, prolonged starvation, and alterations of metabolism associated with infection or failures of various organ systems (Philip, *et al.* 2001, Carroll and Matz, 1983).

2.1.5.2. Cardiovascular disease:

Cardiovascular disease is the most common cause of death and disability among people with diabetes. The cardiovascular diseases that accompany diabetes include angina, myocardial infarction (heart attack), stroke, peripheral artery disease, and congestive heart failure. In people with diabetes, high blood pressure, high cholesterol, and other risk factors contribute with high blood glucose to the increased risk of cardiovascular complications. The potential health benefits of screening and early treatment for type 2 diabetes have been debated. Clinical trials of screening and early intervention versus screening and delayed intervention could address this question but have not been undertaken because of the ethics of not informing individuals when they are found by screening to have previously undiagnosed diabetes (1). (IDF, 2013).

2.1.5.3. Diabetic nephropathy:

High blood glucose levels can damage small blood vessels in the kidneys leading to renal impairment or malfunction. Research shows that kidney disease is much more common in people with diabetes than in those without diabetes; and diabetes is one of the leading causes of chronic kidney disease. Maintaining near normal levels of blood glucose and blood pressure can greatly decrease the risk of kidney diseases (IDF, 2013).

Diabetic nephropathy has been shown to have a rising incidence in the developing world affecting about a third of diabetic patients, with the Asia-Pacific region being the most severely affected. According to a survey published in 2003, diabetic nephropathy was the most common cause of end-stage renal disease in 9 of 10 Asian countries, with an

incidence that had increased from 1.2% of the overall population with end-stage renal disease in 1998 to 14.1% in 2000 (Hossain, Kawar & El Nahas 2007).

2.1.5.4. Diabetic neuropathy:

Diabetes can affect and damage the nerves throughout the body when blood glucose is too high. Extremities are among the most commonly affected areas, especially the feet. Nerve damage in these areas is called peripheral neuropathy, and can lead to pain, tingling, and loss of feeling. Loss of feeling is very dangerous because it can allow injuries to occur without being felt, leading to serious infections and possible amputations (IDF 2013).

Diabetic foot ulcer has been shown to precede 84% of lower extremity amputations in diabetic patients and to increase the risk of death by 2-4 folds (Goodridge, *et al*, 2005).

2.1.5.5. Diabetic retinopathy

Many patients with diabetes develop some form of eye disease called retinopathy that causes reduced vision or blindness. Consistently, high levels of blood glucose, together with high blood pressure and high cholesterol, are the main causes of retinopathy. It can be managed through regular eye checks and keeping glucose and lipid levels at or close to normal (IDF 2013).

2.1.5.6. Pregnancy complications:

Women with any type of diabetes during pregnancy risk a number of complications if they do not carefully monitor and manage their condition. Uncontrolled high blood glucose during pregnancy can lead to fetus extra weight and size, which can lead to problems in delivery, trauma to the child and mother, and a sudden drop in blood glucose for the child after birth. Furthermore, children who are exposed for a long time to high blood glucose during pregnancy are at higher risk of developing type II diabetes in the future). In general, controlling blood glucose levels, blood pressure and cholesterol levels can assist in delaying or preventing diabetes complications. (IDF 2013)

2.1.6. Monitoring of blood glucose:

Self-monitoring of blood glucose may not improve outcomes in some cases, that is among "reasonably well controlled non-insulin treated patients with type 2 diabetes". Nevertheless, it is very strongly recommended for patients in whom it can assist in maintaining proper glycemic control, and is well worth the cost (sometimes considerable)

if it does. It is the only source of current information on the glycaemic state of the body, as changes are rapid and frequent, depending on food, exercise, and medication (dosage and timing with respect to both diet and exercise) and secondarily, on time of day, stress (mental and physical) and infection). The National Institute for Health and Clinical Excellence (NICE), UK released updated diabetes recommendations on 30 May 2008. They indicate that self-monitoring of blood glucose levels for people with newly diagnosed type 2 diabetes should be part of a structured self-management education plan. However, a recent study found that a treatment strategy of intensively lowering blood sugar levels (below 6%) in patients with additional cardiovascular disease risk factors poses more harm than benefit, and so there appear to be limits to benefit of intensive blood glucose control in some patients (Andrew *et al* 2000).

2.1.7 Glycated hemoglobin (HbA1C)

Also named as glycosylated hemoglobin, is formed by a post- translational, non-enzymatic, substrate-concentration dependent irreversible process of combination of aldehyde group of glucose and other hexoses with the amino-terminal valine of the alpha-chain of hemoglobin and the rate of combination is directly proportional to the plasma glucose concentration. Because the average red blood cell lives approximately 120 days, the glycated hemoglobin level at any one time reflect the average blood glucose level over the previous 2-3 months (Michael, *et al.* 2005). Glycated hemoglobin is recommended for both, checking blood sugar control in people who might be pre-diabetic and monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. There are a significant proportion of people who are unaware of their elevated HbA1c level before they have blood lab work. The American Diabetes Association guidelines are similar to others in advising that the glycosylated hemoglobin test be performed at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycaemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycaemic goals, also it added the $A1c \geq 6.5\%$ as another criterion for the diagnosis of diabetes (Walid, *et al.* 2009, American Diabetes Association 2007). Laboratory results may differ depending on the analytical technique, the age of the subject, and biological variation among individuals. Two individuals with the same average blood sugar can have A1C values that differ by as much as 3 percentage points. In general, the reference range (that found in healthy persons), is about 4%–5.9%. Higher levels of HbA1c are found in people with persistently elevated blood sugar, as in diabetes mellitus. While diabetic patient treatment goals vary, many include a target range of HbA1c values. A

diabetic person with good glucose control has a HbA1c level that is close to or within the reference range (Nathan, *et al.* 2008, Ruchi and William, 2007).

In autoimmune hemolytic anemia, concentrations of hemoglobin A1 (HbA1) is undetectable. Administration of prednisolone (PSL) will allow the HbA1 to be detected. The alternative fructosamine test may be used in these circumstances and it similarly reflects an average of blood glucose levels over the preceding 2 to 3 weeks (Bay, *et al.* 2001).

2.1.7.1 Hb1c and fructose amine in control diabetic

Measurement of hemoglobin A1C (HbA1c) or fructosamine levels gives additional information. HbA1c levels reflect average blood glucose levels over the preceding three weeks. Glucose in plasma reacts chemically with amino groups on proteins. It diffuses freely into red cells and reacts with hemoglobin; in plasma it reacts with albumin and other plasma proteins. The unstable products formed initially then undergo further rearrangement to form very stable modified proteins. The different information derived from the two assays is related to the average half-life of the proteins concerned. Hemoglobin has a relatively long life in blood, the average life span of red cells being 120 days. Thus HbA1c levels reflect glucose levels over a relatively long period. The major glycosylated protein measured by the fructosamine assay is albumin, with a half-life of about 19 days. This assay therefore reflects glucose levels over a much shorter period. Both assays can give misleading low results in situations where the rate of turnover of the protein concerned is increased. HbA1c levels are low in patients with hemolytic anemia, and fructosamine levels are low in patients with heavy proteinuria causing hypoalbuminemia. (American Diabetes Association (2004).

2.1.8. Diabetes and mortality:

Diabetes remains the 7th leading cause of death in the United States in 2010, with 69,071 death certificates listing it as the underlying cause of death, and a total of 234,051 death certificates listing diabetes as an underlying or contributing cause of death (ADA 2014). According to IDF (2014), worldwide, 387 million people (8.3%) are living with diabetes. In the year of 2014, estimation mortalities from diabetes were 4.9 million people of the world mortality. In individuals with diabetes younger than 35 years, 75% of all deaths were attributable to diabetes; in individuals with diabetes aged 35–64 years, 59% of deaths

were attributable to diabetes; while in individuals with diabetes and older than 64 years, 29% of all deaths were attributable to diabetes.

2.2. Cardiovascular disease:

Cardiovascular diseases are diseases affecting the heart and circulatory system, which, for example, can result in heart attack, stroke and amputation of the lower limbs. Diabetes is closely associated with cardiovascular disease and therefore an increased risk of heart attack, stroke and amputation of the lower limbs. Indeed, heart attack and stroke are the major causes of premature death in people with diabetes. IDF considers cardiovascular disease to be one of the most serious problems facing people with diabetes, and intends to lead the fight against it from the front. (IDF2014)

2.2.1 Troponin:

Troponins are regulatory proteins that are part of the contractile apparatus of skeletal and cardiac muscle tissue. They are not present in smooth muscle tissue. With the proteins actin and tropomyosin, they are part of the thin filaments within the myofibrils and are essential for the calcium-mediated regulation of muscle contraction. The troponin complex consists of 3 interacting and functionally distinct proteins (troponin I, T, and C).⁷ Tissue-specific isoforms exist for each type of troponin.⁸ Within the thin filament, tropomyosin dimers form a continuous chain along the groove of the actin helix. The troponin complex lies at regular intervals along the filament. Tropomyosin acts to block the myosin binding sites on actin. Each troponin protein has specific functions that regulate muscle contraction. Integrity and therefore will not cause leakage of troponins.¹⁸ Troponin release kinetics are consistent with 2 separate intracellular populations. After acute cardiac injury, the cytosolic pool is released resulting in an early rise in blood levels. This is followed by the slower release of structurally bound troponin that results in sustained elevation. The half-life of troponin and its complex in the circulation is about 2 hours.²⁰ In humans with acute myocardial infarction (AMI), cTn levels begin to rise 4–12 hours after the infarction and reach peak values at 12–48 hours. The levels remain elevated for 7–10 days (cTnI) and 10–14 days (cTnT). (Scott M. Wells, et al 2008)

2.2.2 Troponin and diabetes

A retrospective analysis was performed to evaluate the cardiac troponin I in patients admitted with diabetes and without evidence of acute coronary syndrome. The analysis done for 872 patients but only 264 met the criteria, so cardiac troponin I (cTnI) measured

for them within 24 hours of hospital admission and a follow up period of at least 18 months was set. 24 patients were found to have elevated. Patients with elevated CK-MB had increased lengths of hospitalization compared to the other group ($p < 0.001$). Thus, elevated troponin I during metabolic defects identify a group of patients at an increased risk for poor long-term. Consequences. Clinical significance of troponin elevations in acute decompensate diabetes without clinical acute coronary syndrome (Aruna, *et al* 2001).

2.3 C-creative protein

C-reactive protein is a special type of protein produced by the liver that is only present during episodes of acute inflammation. The most important role of CRP is its interaction with the complement system, which is one of the body's immunologic defense mechanisms. C-reactive protein is produced in the liver by pro-inflammatory cytokines called interleukin-1B, interleukin-6 and tumor necrosis factor alpha. Substance but the Cardiac test is like looking through a high powered microscope – you get a much better and more accurate view with the amount of the substance found being higher for the same amount of sample. This is especially useful when evaluating chronic as opposed to acute inflammation. There is risk factor reported with the Cardiac results for future MI and stroke events. In the “normal, healthy” individual CRP would be 0.0-4.9 mg/L but it has now been established that there are risk factors within the so-called normal range. If you want to know cardiac risk or you are evaluating chronic inflammation, use the cardiac CRP test. Since the CRP is a general test, a positive CRP may indicate any of a number of things: Rheumatoid arthritis, Rheumatic fever, Cancer, Tuberculosis, Pneumococcal pneumonia Myocardial infarction, SLE. (American Diabetes Association (2004).

2.3.1 CRP and the risk of cardiovascular disease:

C-reactive protein, among other systemic inflammatory mediators, has been widely accepted as a potent risk indicator, independently predicting future cardiovascular events. The impact of C-reactive protein on cardiovascular outcome has been corroborated by large number of observational studies and meta-analyses. These studies show that an elevated C-reactive protein has a clear prognostic value for major cardiovascular events and mortality, whereas the lowering of C-reactive protein is associated with a reduction in cardiovascular risk. Combining these findings with experimental observations has led to a paradigm shift in which C-reactive protein is no longer merely a marker, but is increasingly considered as a mediator of cardiovascular disease. In the present issue of ‘controversies in cardiovascular medicine’, we will focus on the emerging evidence

supporting a potentially causal role of C-reactive protein I cardiovascular disease(Radjeshet *al* 2010)

2.3.2High-sensitivity C - reactive protein:

The role of inflammation in the pathogenesis of atherosclerosis has been firmly established in the past two decades. Numerous studies, both observational (nested case control and prospective cohort) and randomized controlled trials (RCTs) have shown an association of pro-inflammatory biomarkers with incident hypertension, metabolic syndrome, coronary artery disease (CAD), acute coronary syndrome(ACS), peripheral artery disease, stroke and recurrent coronary and cerebrovascular events¹⁻⁴. Approximately 25 large observational studies published since the 1990s have established high sensitivity C-reactive protein (hsCRP), a biomarker of inflammation, as an independent predictor for CAD. A meta-analysis of these observational studies showed that people in the top quartile for hsCRP levels had an odds ratio (OR) of 1.5 compared with those in the lowest quartile for major cardiovascular events, after adjusting for established risk factors⁵. (Deepak, *et al*,2014)

Chapter Three

Materials and Methods

3.1 materials

3.1.1 Study design:

This is a quantitative, descriptive, analytic, cross-sectional study.

3.1.2 Study area and period:

This study was conducted in U.A.E Abu Dhabi and Dubai patient enrolled in this study came to Sudanese clubs

3.1.3 Study population and sample size:

Population of this study was categorized into a study group of 50 diabetic patients who attended in Sudanese clubs and a control group of 50 healthy subjects (non diabetic).

3.1.4 Ethical consideration:

Firstly, the permission of this study was obtained from Sudan embassy in Dubai.

3.1.5 Inclusion criteria:

Study group include patients complain from type 2 dm for 5 years and above, while control group include non-diabetic persons.

3.1.6 Exclusion criteria:

Study and control group did not include diabetic type 1 patient or diabetic pregnant women.

3.1.7 Data collection and clinical examination:

Clinical data for every patient was collected by questionnaire.

3.1.8 Sample collection:

Blood samples "5ml" were collected from subjects of the study group after fulfillments of questionnaire as well as control group, using disposable syringe and sprit for sterilization the area of collection. Collected blood was drawn in three containers with delta, fluoride oxalate and plan container without anticoagulant, blood in all containers was gently mixed with anticoagulant to obtain plasma and whole blood consecutively, hemolysis samples

were rejected and excluded from the study, whole blood was used immediately after collection for testing glycosylated hemoglobin, serum samples were preserved at -20 c prior to processing, plasma from fluoride oxalate for testing blood glucose, while serum samples for testing troponin and c - reactive protein.

3.1.9 Quality control:

Three level of control run (low, medium, high).

3.2 Methods:

3.2.1 Measurement of troponin I:

3.2.1.1 Principle:

The architect stat troponin-I assay is a two-step immunoassay to determine the presence of troponin I in human serum and plasma using cmia technology with flexible assay protocols, referred to as chemiflex, sample, assay diluents and anti-troponin-I antibody-coated paramagnetic micro particles are combined, troponin-I present in the sample binds to the anti-troponin-I coated micro particles, after incubation and wash, anti-troponin-I acridinium-labeled conjugate is added in the second step, following another incubation and wash, pre-trigger and trigger solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as relative light units (rlus), a direct relationship exists between the amount of troponin-I in the sample and the rules detected by the architect system optics. (appendix III)

3.2.2 Measurement of C-RP:

3.2.2.1 Principle: C-RP reagent is used to measure c-reactive protein concentration by a turbid metric method, in the reaction, an anti-C-RP antibody-coated particle binds to c-reactive protein in the patient sample resulting in the formation of insoluble aggregates causing turbidity, the synchron system(s) automatically proportions the appropriate sample and reagent volumes into a cuvette, the ratio used is one part sample to 50 parts reagent, the system monitors the change in absorbance at 600 nanometers, this change in absorbance is proportional to the concentration of C-RP in the sample and is used by the system to calculate and express C-RP concentration based upon a single-point adjusted, predetermined calibration curve. (appendix V)

3.2.3 Measurement of glucose:

3.2.3.1 Principle: Glucose reagent is used to measure the glucose concentration by a timed endpoint method, in the reaction, hexokinase (hk) catalyses the transfer of a phosphate group from adenosine triphosphate (atp) to glucose to form adenosine diphosphate (adp) and glucose-6-phosphate, the glucose-6-phosphate is then oxidized to 6-phosphogluconate with the concomitant reduction of β -nicotinamide adenine dinucleotide (nad) to reduced β -nicotinamide adenine dinucleotide (nadh) by the catalytic action of glucose-6-phosphate dehydrogenase (g6pdh), the synchron system(s) automatically proportions the appropriate sample and reagent volumes into the cuvette, the ratio used is one part sample to 100 parts reagent, the system monitors the change in absorbance at 340 nanometers, this change in absorbance is directly proportional to the concentration of glucose in the sample and is used by the system to calculate and express glucose concentration. (appendix VI)

3.2.4 Measurement of Glycated hemoglobin:

3.2.4.1 Principle: The system utilizes two unique cartridges, hb2 and a1c2, to determine hemoglobin A1c concentration as a percentage of total hemoglobin, hemoglobin reagent is used to measure total hemoglobin concentration by a colorimetric method, the system monitors the change in absorbance at 410 nanometers, this change in absorbance is directly proportional to the concentration of total hemoglobin in the sample and is used by the system to calculate and express total hemoglobin concentration. A1c2 reagent is used to measure the hemoglobin A1c concentration by a turbid metric immunoinhibition method, in the reaction, hemoglobin a1c antibodies combine with hemoglobin a1c from the sample to form soluble antigen-antibody complexes, polyhapten from the reagent then bind with the excess antibodies and the resulting agglutinated complex is measured turbid metrically, the system monitors the change in absorbance at 340 nanometers, this change in absorbance is inversely proportional to the concentration of hemoglobin a1c in the sample and is used by the system to calculate and express hemoglobin A1c concentration as a percentage of total hemoglobin. (appendix VII).

Chapter Four

Results

The study applied on 100 participants were divided to 50 individual with type 2 diabetes as test group and 50 healthy volunteer as control group. The age and gender of test group was matched with the control group. The male account 96 % (n=48) in the test group and 98 % (n=49) from control group, while females account 4% (n=2) from test group and 2% (n=1) from control group.

Fig (4.1) A scatter plot shows the relationship between levels of Glucose in mmol/L and HbA1c in %

Fig (4. 2) A scatter plot shows the relationship between CRP in mmol/L and Troponin I in ug/l

Fig (4.3) A scatter plot shows the relationship between levels of Glucose in mmol/L and Troponin I in ug/l

Table (4-1) shows significant difference between means of blood glucose levels, Hb A1C, CRP and Troponin in test group and controls groups $p < 0.001$ as below

Table (4.1) Comparison of means plasma levels Glucose, Hba1c, CRP and Troponin between test group and control group (n=50)

Variable	Test Group)	Control Group	P .value
Glucose mmol/l	(9.7±3.0)	(5.3± 0.7)	<0.001
Hba1c %	(8.5± 1.9)	(5.5± 0.7)	<0.001
CRPmg/dl	(5.3±4.0)	(2.7±2.6)	<0.001
Troponin ug/l	(0.07±0.01)	(0.002±0.005)	<0.001

- The table shows the mean±Std deviation in brackets and probability (P).
- Independent t-test was used for comparison
- P-value ≤ 0.05 is considered significant

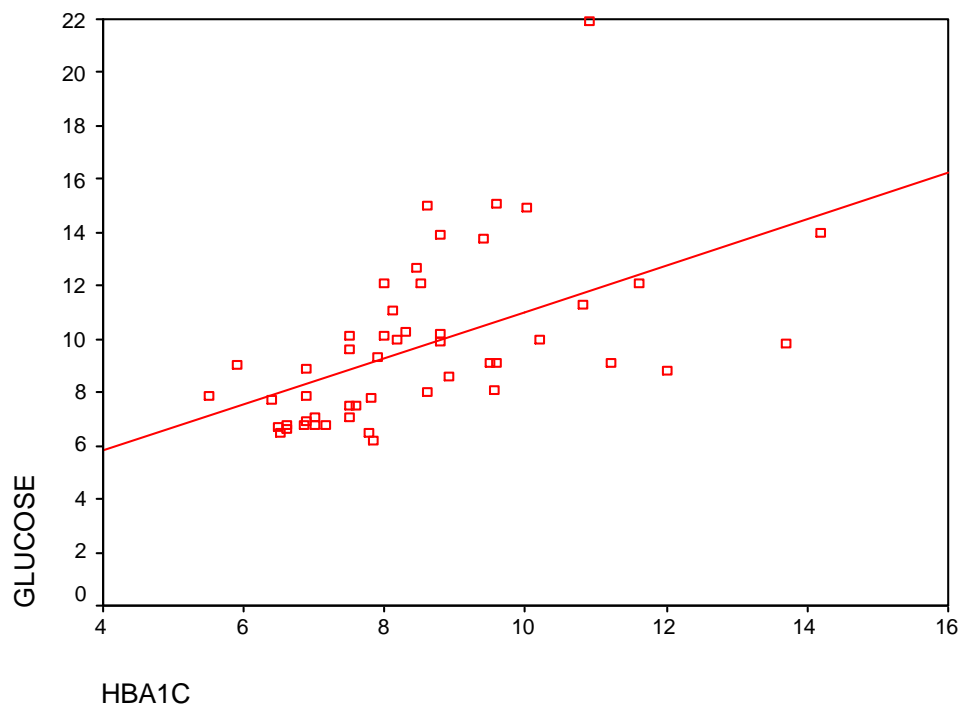


Fig (4.1) A scatter plot reflects the relationship between levels of glucose in mmol /l and Hba1C in % ($r=0.531$ $p<0.001$)

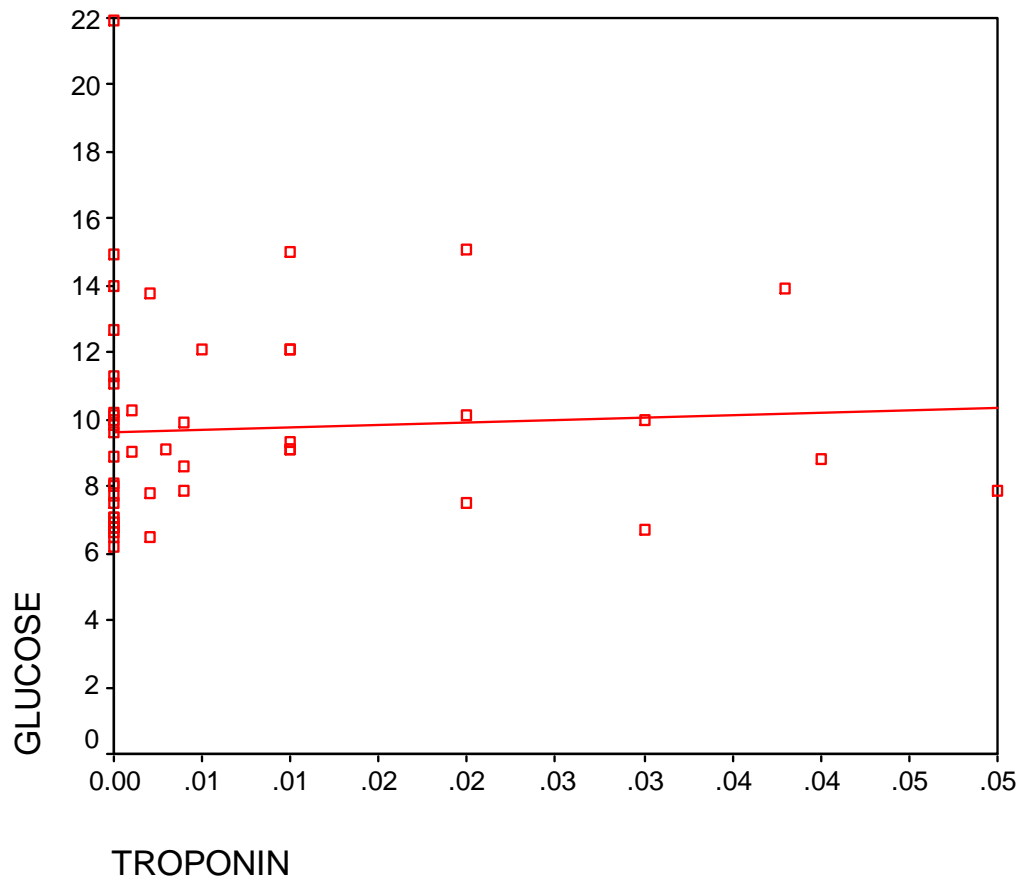


Fig (4.1) a scatter plot shows the relationship between levels of Troponin I in ug/l and Glucose mmol/.(r=0.059,p=0.68)

Chapter Five

Discussion, Conclusion and Recommendation

5.1 Discussion

This is across sectional study performed among Sudanese patients with type 2 DM (50 subjectas tested group and 50 as control group). The results showed there was a significant increasing in the mean of CRP of the test group when it compared with control group ($p < 0.001$), this result is in agree with the results reported in study performed in *JAMA* among diabetic subjects which reported there is a significantly higher level of CRP and positively associated with type 2 diabetes (Aruna D. Pradhan, *et al* 2001)

The present study showed there is a significant increasing in the mean of HbA1c of the test group when it compared with control group ($p < 0.001$) this agree with study done by (Wu, Dorn *et al.* 2002) ($p < 0.05$)

In the present study Troponin was significantly increasing in diabetic patient when compared with control and this agree with study done by (Wilson, *et al.* 2005) ($p < 0.05$). and study done by (Libby, *et al.* 2002) ($p < 0.05$).

The result of current study shows significant and positive correlation between the CRP and Troponin I ($r = 0.320$) the significant level is < 0.001 . The present study correlate with previous study reported by Robbert J de Winter, *et al* 1999 who concluded that--An abnormal CRP (> 5 mg/l) and an abnormal TnI (> 0.4 μ g/l) were more frequent in patients that suffered a major cardiac eventt (CRP: 93 vs. 35%, $P < 0.0001$; TnI: 73 vs. 26%, $P < 0.001$).

The current study show significant and strong positive correlation between Hba1c and glucose ($r = 0.53$, $p < 0.001$) this study agree with (Mannarino, 2005 *et al.*).

The present study reported that there is no significant association between the glucose level and troponin I. ($r = 0.059$, $p = 0.68$) This study disagreement with study done by Carlos *et al.* 2015) who concluded that DM with coronary artery disease had higher levels of troponin (median values, 12.0 pg/mL (95 % CI: 10–16) vs 7.0 pg/mL (95 % CI: 5.9–8.5), respectively; $p = 0.0001$) plasma glucose (0.03) and Troponin I ($p = 0.01$) had independent statistical significance (Carlos 2015). This might be due to the reduction in sample size and the subjects were not known case of CVD.

5.2 Conclusion

This study concluded that:

1. The CRP and Troponin I levels were significantly raised in type-2 diabetes mellitus compared with normal control.
2. There is no association between HBA1C (CRP and troponin) respectively in patient with type -2 diabetes mellitus.

5.3 Recommendations

From the results of this study, it is recommended that:

1. Diabetic patients should be monitored at regular bases to achieve good control in order to delay or minimize development of disease and complications.
2. Study with large sample size with specific selection criteria should be done to determine the association between CVD and DM.

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Appendices

Appendix-I

بسم الله الرحمن الرحيم

التبرع بعينة دم لأغراض البحث والدراسة
الموافقة علي المشاركة

أنا الباحث / ثناء إبراهيم حسن سعد

أرغب في أخذ عينة دم لغرض البحث والدراسة

موضوع البحث / تقييم مستويات التروبين وبروتين سي النشط للسودانيين المقيمين بدولة الإمارات
العربية

ومصابين بداء السكري النوع الثاني

اسم المتبرع

لأوافق

أوافق

التوقيع

Appendix -II

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Evaluation of Serum levels of CRP level and troponin I in Sudanese Reside in UAE Diagnosed with Diabetes Mellitus Type 2

Name:Tele: Ageyears Gender: male () female ()

Duration of diabetes since diagnosis Years

Complications of diabetes 1

According to medical records and physical examination:

Heart attach Yes () Stroke Yes ()

Foot problem Yes () Eye disease Yes ()

Hypertension Yes () Others

Treatment and Drug history:

1-Diet control () 2- Insulin () 3- Oral hypoglycemic drugs () 4-Lipid lowering
drugs () 5-Multivitamins ()

Investigations:

- Random Blood Glucose mmol/l

- Hemoglobin A_{1c}%

- Serum Troponinug/L

- Serum CRPmg/ dl

Appendix-III Troponin

Reagent content: 1 or 4 bottles (6.6 ml/27.0 ml) anti-troponin-I (mouse, monoclonal) coated micro particles in tris buffer with protein (bovine and goat) stabilizers, preservatives: antimicrobial agents, 1 or 4 bottles (5.9 ml/26.3 ml) anti-troponin-i (mouse, monoclonal) acridinium-labeled conjugate in mes buffer with protein (bovine) stabilizer, preservative: proclin 300, 1 or 4 bottles (10.0 ml/50.9 ml) troponin-i assay diluents, containing protein (bovine and goat) stabilizers in phosphate buffer, preservative: proclin 300.

Other reagents: pre-trigger solution containing 1.32% (w/v) hydrogen peroxide, trigger solution containing 0.35 n sodium hydroxide and wash buffer containing phosphate buffered saline solution, preservatives: antimicrobial agents.

Sample: Blood was collected using plan container, then centrifuged at 700-1,000 x g for 10 minutes.

Procedure: Ensure that the specimen for processing is properly labeled. Acceptable specimen type must be processed for the test, check for its quality and quantity as mentioned in the rejection of sample. You may need to start up, pause, shut down, cycle power to, or power off the system and its components to: Load samples, reagents, and solutions, perform maintenance or diagnostic procedures and Replace components, always check consumable inventory before processing samples, use the Supply status screen to check inventory, always check reagent inventory before processing samples, from the Reagent status and Reagent status all screens you can view the status of reagents on board. manual patient order: select the sampling priority: stat option on the patient order screen to display the "s (stat) code for the sample orders and results, (optional), select the carrier or carousel button, if displayed, enter a carrier or carousel id in the c data entry box, if displayed ,enter a position in the p data entry box, if displayed, enter the side (sample identification) in the side data entry box, select the desired panel(s) from the panels list and/or select an assay(s) from the assays list.

Calculation: If using the automated dilution protocol, the system performs a 1:9 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Reference value: Stat troponin-i - (s) level < 0.3 ug/l

Appendix-V CRP

Sample: Blood was collected using plain container, then centrifuged at 700-1,000 x g for 10 minutes.

Reagent Content: Each kit contains the following items Two C-RP reagent cartridges (2 x 200 tests), one lot-specific parameter card, C-RP antibody (particle bound goat and mouse anti-C-RP antibody), reaction buffer and bovine serum Albumin. Reagent preparation: no preparation is required, invert cartridge several times prior to loading on to the synchron dxc600 system, check for bubbles or foam in compartments; break any bubbles. Acceptable reagent performance: The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within the facility's acceptance criteria.

Procedure: Acceptable specimen type must be processed for the test, check for its quality and quantity, processing samples manually: select samples from the menu bar, type the sample id, type the numbers in the rack and pos fields, select sample type from the pull-down menu, (serum, plasma, csf or urine), do not use any other sample type, to enter demographics, select demog f2, type the information provided and select next f10 to return to the program sample screen, select C-RP, select next f10, result reporting, click on accession result entry icon, enter the number of the specimen to be result, result will appear then print it.

Calculation: Synchron dxc600 system performs all calculations internally to produce the final reported result.

Reference value: Serum C-RP (0-7.5) mg /dl.

Appendix-VI GLUCOSE

Sample: Freshly drawn plasma fluoride oxalate, then centrifuged at 700-1,000 x g for 10 minutes, whole blood is not recommended for use as a sample, the use of fluoride as a glycolysis inhibitor is recommended.

Reagent: Adenosine triphosphate, NAD⁺, hexokinase, glucose-6-phosphate dehydrogenase and also non-reactive chemicals necessary for optimal system performance, no preparation is required for reagent, acceptable reagent performance: the acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within the laboratory acceptance criteria. Reagent storage and stability: glu reagent when stored unopened at +2°c to +8°c will obtain the shelf-life indicated on the cartridge label, once opened, the reagent is stable for 30 days unless the expiration date is exceeded.

Procedure:

Sample preparation: sample preparation is not required prior to analysis. Sample volume: the optimum volume, when using a 0.5 ml sample cup, is 0.3 ml of sample.

Sample processing: select samples from the menu bar. If the sample has no bar code or bar code cannot be read, type the sample id type the numbers in the rack and pos fields, select sample type from the pull-down menu, select sample comment from the pull-down menu, to enter demographics, select demog f2, type the information provided and selects next f10 to return to the program sample screen, select each chemistry (glucose), select next f10, then press run to run start machine to run, result printed automatically

Calculation: Synchron dxc600 system performs all calculations internally to produce the final reported result; the system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

Reference value: New born is 25-80mg/dl and 70-110 for adult and child (Caraway and Watts.1987, Tietz.1995, Howanitz and Howanitz, 1984).

Appendix-VII Glycated hemoglobin

Reagent: Tetradecyltrimethyl ammonium bromide (ttab), antibody buffer, anti-hba1c antibodies (sheep), 2-morpholino-ethanesulfonic acid buffer (ph 6.2), polyhapten buffer, hba1c polyhapten, hemoglobin buffer, phosphate buffer (ph. 7.4).

Procedure:

Select samples from the menu bar, type the sample id, type the numbers in the rack and pos fields, select sample type from the pull-down menu (serum, plasma, csf or urine), select sample comment from the pull-down menu, to enter demographics, select demog f2, type the information provided and select next f10 to return to the program sample screen, select chemistry (hba1c), to access additional chemistries, use the up and down arrows to move to other pages, 8, select next f10; don't run any other chemistry while processing hba1c2 samples, instrument must be in standby before processing hba1c2 samples, use only the racks assigned in the "reserved racks for hba1c" field to run hba1c2 assay, sample type must be selected as "blood" and result must be printed automatically.

Calculation: Synchron dxc600 system performs all calculations internally to produce the final reported result. the system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

National Glycohemoglobin Standardization Program (NGSP):

$$\% \text{HbA1C2 (NSGP)} = 91.48 * (\text{A1C2} / \text{Hb2}) + 2.152$$

Reference value: no diabetic 4.4 – 6.7% and controlled one 6.7 – 7.3%. (Tietz.1990), (Roberts.2002).