بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of Higher Education and Scientific Research

University of Shendi

Faculty of Graduate Studies and Scientific Research

Comparison of Plasma Fibrinogen Level and Complete Blood Count among Glycomic Control Diabetic Mellitus Patients Type 2 in Shendi Town

A thesis Submitted for partial fulfillment of the Msc Degree in Haematology

By

Abd-Alhalim Hasab Alrasul Alamin

Bsc. (Shendi University-2007)

Supervisor

Dr: Hamza Ahmed Hassan Mohammed

Assistant Professor in Haematology, Medical Laboratory Science

Shendi University

August – 2018

الآيـــــــة

بسم الله الرحمن الرحيم

قال تعالى:

﴿ فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَى إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْماً ﴾

صدق الله العظيم

ســـــوره طـــــــه الآية (١١٤)

Dedication

To those

Who give me the best of life without payment

To my parents for their patience and

Support

To my wife

To my daughters

To my Brothers

To my sisters

my teachers

all my friends

Acknowledgements

First of all I thank the Almighty Allah who helped me to complete this study.

I would like to express endless thanks to my supervisor:

Dr: Hamza Ahmed Hassan Mohammed

For her great efforts and guidance..

I thank the staff of the Faculty of Medical Laboratory science Also I would like to thank the staff of El Mek Nimer University Hospital.

I would also like to thank all the participants (patients) who gave the allowance to give samples of this research and many others whom I could not mention here, but their direct and indirect supports had already contributed in this study.

List of abbreviations

Abbreviation	Term
CAD	Coronary artery disease
CBC	Complete blood count
CHD	Coronary heart disease
CVD	Cardiovascular diseases
DM	Diabetes mellitus
HTN	Hypertension
Hb	Hemoglobin
hsCRP	High- sensitivity C.reactive protein
IHD	Ischaemic heart disease
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
Plt	Platelet
PCV	Packed cell volume
RBCs	Red blood cells
WBCs	White blood cells

Abstract

Background Diabetes mellitus is actually group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, action, or both. Thrombosis is one of the complications of diabetes; the coagulation system may be one of the affected systems.

Objective To evaluate the effect of Diabetes mellitus on plasma fibrinogen level and glycomic control and complete blood count in Shendi town.

Methods: This is cross sectional study conducted in Shendi town in period between March and August 2018 to evaluate the effect of Diabetes mellitus on plasma fibrinogen level and glycomic control and complete blood count. Data collected, cleaned and analyzed using SPSS version (25.0).

Results: The study revealed that the mean of plasma fibrinogen among cases group was (398 mg/dl) and among control group (242 mg/dl). This difference was statistically significant ($p < 0.001$). These results were in agreement with other study. They found a Higher plasma fibrinogen levels were found in patients with poor control HA1C more than 6.8 (539 mg/dl) as compared to patients with good controls HA1C less than 6.8 (292 mg/dl) which were statistically significant.

Conclusion and recommendation: Fibrinogen level was slightly higher in patient who had poor glycemic control HbA1C more than 6.8(539mgdl) than those had good control HbA1C less than 6.8(292mgdl), also was statistically significant (p.value: 0.001). White blood cells hemoglobin hemotcrite and platelet were most statistically significant (p.value: 0.000).

Special attention should be given for patients who had the combination Diabetes and hyper-fibrinogenemia because that may increases the risk of developing micro and macro vascular complications. The control of diabetes should be assessed by further study to ensure if the elevation of fibrinogen level was significantly associated with uncontrolled diabetes in Sudan.

الخلاصـــة

الخلفية: مرض السكري هو مجموعة من الأمراض الأيضية التي تتميز بارتفاع السكر في الدم الناتج عن عيوب في إفراز الأنسولين، أو عمله، أو كليهما. تجلط الدم هو أحد مضاعفات مرض السكري. قد يكون نظام تجلط الدم أحد الأنظمة المتأثرة في الجسم وتؤدي إلى مضاعفات خطيرة.

الهدف: تقييم تأثير مرض السكري على مستوى الفيبرينوجين بالبلازما لسكان مدينة شندي المصابين بمرض السكري.

المنهجية: دراسة مقطعية أجريت بين المرضى بالسكري من سكان مدينة شندي بولاية نهر النيل في الفترة بين مارس وأغسطس ٢٠١٨ لتقييم تأثير مرض السكري على مستوى الفيبرينوجين بالبلازما وفحص صورة الدم الكامل. البيانات تم جمعها وإعدادها وتحليلها باستخدام برنامج تحليل الحزم الإحصائية للعلوم الاجتماعية الإصدار ٢٥,٠.

النتائج: كشفت الدراسة أن متوسط الفيبرينوجين بالبلازما في جميع المشاركين بالدراسة كان ٣٩٨ مجم/ ديسيلتر وبين مجموعة الكنترول ٢٤٢ملغ/ ديسيلتر. حيث نجد أن ارتفاع قيمة الفيبرونوجين عند مرضى السكري الذين يمتلكون مستوى مرتفع في هيموقلوبين A1C (٥٣٩ مجم/ديسليتر) ونجد المستوى المنخفض في هيموقلوبين A1C (٢٩٢ مجم/ديسليتر).

الخاتمة والتوصيات: حيث نجد أن ارتفاع قيمة الفيبرونوجين عند مرضى السكري الذين يمتلكون مستوى مرتفع في هيموقلوبين A1C (٥٣٩ مجم/ديسليتر) ونجد المستوى المنخفض في هيموقلوبين A1C (٢٩٢ مجم/ديسليتر).

ينبغي إيلاء اهتمام خاص للمرضى الذين لديهم السكري وفرط في مستوى الفيزيرينوجين بالدم لأنه قد يزيد من خطر الإصابة بمضاعفات الأوعية الدموية الدقيقة والكلية. يجب تقييم السيطرة على مرض السكري من خلال مزيد من الدراسة لضمان ما إذا كان ارتفاع مستوى الفيبرينوجين مرتبطا بشكل كبير مع مرض السكري غير المنضبط في السودان.

List of Contents

List of Tables

Chapter One

Introduction

Rationale

Objectives

Introduction, Rationale and Objectives

1.1 Introduction

Diabetes mellitus defined as “a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced.

Patients with type2 DM have been reported to be at increased risk of developing cardiovascular related diseases (Myocardial infarction, stroke, and atherothrombosis). Many studies elucidated that DM affects vascular integrity by its effect on endothelium, smooth muscle function, as well as propensity to thrombosis, in addition to increased level of coagulation procoagulant factors and decreased fibrinolytic activity.

Poor glycemic control has been reported to be associated with increased vascular complications in diabetics.

Fibrinogen is the major coagulation protein in the blood from which fibrin clot is formed. It is an important determinant of plasma viscosity, platelet aggregation and thrombus formation; also it is an acute-phase reactant that increases in inflammatory states.

High fibrinogen level has been described as independent risk factor for cardiovascular diseases. It has been suggested to be involved in the excess rate of cardiovascular diseases in patients with T2DM.

This study aimed to investigate the correlation between glycemic control and plasma fibrinogen level in Sudanese patients with T2DM.

1.2 Rationale

Diabetes mellitus is the most common non communicable diseases Characterized by hyperglycemia. It's widely distributed and effect on different organ. Thrombosis is one of the complications of diabetes; the coagulation system may be one of the affected systems. So this study was conducted to evaluate the effect of Diabetes mellitus on plasma fibrinogen level with glycomic control and assay the haematological parameter in D.M type 2.

1.3 Objectives

1.3.1. General objective

Comparison of Plasma Fibrinogen Level and Complete Blood Count among Glycomic Control Type 2 Diabetic Mellitus Patients in Shendi Town.

1.3.2. Specific objectives

1. To Compare fibrinogen level with diabetic mellitus and CBC.
2. To Compare between fibrinogen level with diabetic mellitus and Hb.
3. To Compare between fibrinogen level with diabetic mellitus and HbA_1C.
4. To Compare between fibrinogen level with diabetic mellitus and TWBs.
5. To Compare between fibrinogen level with diabetic mellitus and RBCs.
6. To Compare between fibrinogen level with diabetic mellitus and platelet.

Chapter Two

Literature Review

2. Literature review

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

2.1 Classification of DM

The current classification includes four main categories

Type 1 DM (formerly known as insulin-dependent) in which the pancreas fails to produce the insulin which is essential for survival. This form develops most frequently in children and adolescents, but is being increasingly noted later in life. This form accounts for 5–10% of all cases

Type 2 DM (formerly named non-insulin-dependent) which results from the body's inability to respond properly to the action of insulin produced by the pancreas. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well.

Certain genetic markers have been shown to increase the risk of developing Type 1 diabetes. Type 2 diabetes is strongly familial, but it is only recently that some genes have been consistently associated with increased risk for Type 2 diabetes in certain populations. Both types of diabetes are complex diseases caused by mutations in more than one gene, as well as by environmental factors.

Other: less common types of diabetes that are caused or associated with certain specific conditions and/or syndromes.

Gestational diabetes: Gestational diabetes mellitus (GDM) is defined as any degree of carbohydrate intolerance, with onset or first recognition during second or third trimester of pregnancy

2.2 Etiology and Pathogenesis

2.2.1 Type 1 Diabetes

In type 1a diabetes mellitus, a genetically susceptible host develops autoimmunity against his or her own beta cells. What triggers this autoimmune response remains unclear at this time. In some (but not all) patients, this autoimmune process results in progressive destruction of beta cells until a critical mass of beta cells is lost and insulin deficiency develops. Insulin deficiency in turn leads to the onset of clinical signs and symptoms of type 1 diabetes. At the time of diagnosis, some viable beta cells are still present and these may produce enough insulin to lead to a partial remission of the disease (honeymoon period) but over time, almost all beta cells are destroyed and the patient becomes totally dependent on exogenous insulin for survival. Over time, some of these patients develop secondary complications of diabetes that appear to be related to how well controlled the diabetes has been.. The natural history of type 1 diabetes involves some or all of the following stages:

- Initiation of autoimmunity.
- Preclinical autoimmunity with progressive loss of beta-cell function.
- Onset of clinical disease.
- Transient remission.
- Established disease.
- Development of complications.

2.2.2 Type 2 Diabetes Mellitus

Twin studies suggest that genetic makeup accounts for 60–90% of the susceptibility to type 2 diabetes. Type 2 diabetes and impaired glucose tolerance (IGT) cluster in families. Thus, most patients have a positive family history, and the lifetime risk for developing type 2 diabetes is increased up to 40% (more than five times the background rate) by having a first-degree relative with the disease. If both parents have type 2 diabetes, the risk to the offspring may be as high as 70%. Available evidence supports a polygenic mode of inheritance with a considerable environmental input.

Hyperglycemia is due to deficiency of insulin due to β-cell dysfunction. Possible causes of β-cell dysfunction in type 2 diabetes include a defect in β-cell glucose metabolism and glucose sensing, deficiency of some key stimulatory molecule, reduction in β-cell mass, and deposition of amyloid. Some defects may be hereditary while others are acquired. The number of β-cells may be reduced by 20–50% in patients with long-standing type 2 diabetes.39 However, in the absence of insulin resistance, more than 80% of β-cells must be lost before insulinopenic diabetes develops, and the function of the remaining β-cells must be impaired. Reduced number of β-cells could be a consequence of long-standing diabetes. Conversely, a decrease in β-cell numbers could lead to decreased function of the remaining β-cells.

2.2.3 Clinical features

1. Frequent and excessive urination
2. Excessive thirst
3. Greater than normal hunger.

2.3 Complication of diabetes

Diabetes increase the risk of heart disease and strokences of diabetes:

Over time, diabetes can damage the heart, blood vessels.

50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). Combined with reduce blood flow, neuropathy in the feet increase the chance of foot ulcer and eventual limb amputation.

Diabetic retinopathy an important causes of blindness, and occur as result of long term accumulated damage to small blood vessels in the retina. After 15years of diabetes approximately 2% of people become blind, and about 10% develop sever visual impairment. Diabetes is among the leading causing of kidney failure. 10 – 20% of people with diabetes die of kidney failure. Diabetic neuropathy is damage to the nerves as result of diabetes, and affect up to 50% of people with diabetes. Although many different problem can occur as result of diabetic ,neuropathy or weakness in the feet and hands.

2.3.1 Metabolic complications of diabetes

2.3.1.1 Ketoacidosis: may the presenting feature of type 1 DM, or may develop in a patient known to be diabetic who omits to take his insulin or whose insulin dosage becomes inadequate because of an increased requirement. Clinical and metabolic features of diabetic ketoacidosis:

Clinical: thirst, polyuria, dehydration, hypotension, tachycardia, vomiting hyperventilation, abdominal pain, coma. Metabolic: hyperglycemia, glycosuria, non-respiratory acidosis, ketonaemia and ketonuria, uraemia, hyper kalaemia, haemoconcentration, hypertriglyceridaemia. (Ketoacidosis miting stosigns; 1 fruity smelling breath2.nausea and vomit cramps 4.mental confusion.

2.3.1.2 Non-kenotic hyperglycemia

In type 2 DM, severe hyperglycemia can develop (blood glucose concentration >50 mmol/l) with extreme dehydration and a very high plasma osmolality, but with no ketosis and minimal acidosis.

1. Loss of appetite which may accompanied by weight loss.
2. Fatigue and weakness.
3. Shortness of breath.
4. Vision problems.
5. Skin 6.nerve problem and pain.
6. Changes.
7. Impotence (in men).

2.3.1.3 Lactic acidosis

Is an uncommon complication of diabetes? It was formally chiefly seen in patients treated with phenformin, oral hypoglycemic drug, but is now more usually associated with severe systemic illness, e.g.: shock and pancreatitis.

2.3.2 Diabetic nephropathy

Is a major cause of premature death in patient with diabetes, related with cardiovascular disease as well as renal failure .the earliest detectable abnormality is microalbuminuria, proteinuria, increasing plasma creatinine, hyperlipidemia and hypertension?

2.3.3 Hypoglycemia

Defined as a blood glucose concentration of less than 2.8mmol/l, hypoglycemia is a potentially dangerous.

2.3.3.1 Causes

Fasting hypoglycemia (hepatic and renal disease, endocrine disease, metabolic disorder, hyperinsulinism, alcoholic induced, septicemia).

Reactive hypoglycemia (post-prandial, drug induced, inherited metabolic disorder);

Hypoglycemia symptoms;

1. Headache.
2. Unsteadiness dizziness.
3. Tremor.
4. Great hunger.
5. Moodiness.
6. Clumsiness.
7. Mental confusion.
8. Irritability and anxiety.

2.4 Diagnosis

Criteria for the diagnosis of diabetes mellitus:

- Symptoms of diabetes + random plasma glucose concentration ≥ 200 mg/dl (11.1mmol/l)
- Fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/l)
- Two hour plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during a 75 g oral glucose tolerance test
- An HbA1c level of 6.5% or higher; the test should be performed in a laboratory using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay.

2.5 Complete blood count

A complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) or blood panel, is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC.

Alexander Vastem is widely regarded as being the first person to use the complete blood count for clinical purposes. Reference ranges used today stem from his clinical trials in the early 1960s.

The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdiction. A phlebotomist collects the specimen, in this case blood is drawn in a test tube containing an anticoagulant (EDTA, sometimes citrate) to stop it from clotting, and transported to a laboratory.

In the past, counting the cells in a patient's blood was performed manually, by viewing a slide prepared with a sample of the patient's blood under a microscope (a blood film, or peripheral smear). Nowadays, this process is generally automated by use of an automated analyzer, with only approximately 30% samples now being examined manually.[6]

2.5.1 Automated blood count

The blood is well mixed (though not shaken) and placed on a rack in the analyzer. This instrument has many different components to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The results are printed out or sent to a computer for review, Blood counting machines aspirate a very small amount of the specimen through narrow tubing. Within this tubing, there are sensors that

count the number of cells going through it, and can identify the type of cell; this is flowcytometry. The two main sensors used are light detectors, and electrical impedance. One way the instrument can tell what type of blood cell is present is by size. Other instruments measure different characteristics of the cells to categorize them. Because an automated cell counter samples and counts so many cells, the results are very precise. However, certain abnormal cells in the blood may be identified incorrectly, and require manual review of the instrument's results and identifying any abnormal cells the instrument could not categorize .In addition to counting, measuring and analyzing red blood cells, white blood cells and platelets, automated hematology analyzers also measure the amount of hemoglobin in the blood and within each red blood cell. This information can be very helpful to a physician who, for example, is trying to identify the cause of a patient's anemia. If the red cells are smaller or larger than normal, or if there's a lot of variation in the size of the red cells, this data can help guide the direction of further testing and expedite the diagnostic process so patients can get the treatment they need quickly.[6]

2.5.2 A complete blood count will normally include

Red cells

1. Total red blood cells — The number of red cells is given as an absolute number per litre.
2. Haemoglobin - The amount of hemoglobin in the blood, expressed in grams per decilitre. (Low hemoglobin is called anaemia.)
3. Hematocrit or packed cell volume (PCV) - This is the fraction of whole blood volume that consists of red blood cells.
4. Red blood cell indices
    a. Mean corpuscular volume (MCV) - the average volume of the red cells, measured in femtolitres. Anaemia is classified as microcytic or macrocytic based on whether this value is above or below the expected normal range. Other conditions that can affect MCV include thalassemia, reticulocytosis and alcoholism.

b. Mean corpuscular haemoglobin (MCH) - the average amount of haemoglobin per red blood cell, in picograms.
c. Mean corpuscular haemoglobin concentration (MCHC) - the average concentration of haemoglobin in the cells.

5. Red blood cell distribution width (RDW) - a measure of the variation of the RBC population

White cell

- Total white blood cells - All the white cell types are given as a percentage and as an absolute number per liter.

Differential leucocytes counts will also include

- Neutrophil granulocytes — May indicate bacterial infection. May also be raised in acute viral infections. Because of the segmented appearance of the nucleus, neutrophils are sometimes referred to as "segs." The nucleus of less mature neutrophils is not segmented, but has a band or rod-like shape. Less mature neutrophils — those that have recently been released from the bone marrow into the bloodstream — are known as "bands" or "stabs". Stab is a German term for rod.
- Lymphocytes — Higher counts with some viral infections such as infectious mononuclesis and. Also raised in chronic lymphocytic leukaemia (CLL). Can be decreased by HIV infection. In adults, lymphocytes are the second most common WBC type after neutrophils. In young children under age 8, lymphocytes are more common than neutrophils..
- Monocytes — May be raised in bacterial infection, tuberculosis, malaria, Rocky Mountain spotted fever, monocytic leukaemia, chronic ulcerative colitis and regional enteritis .
- Eosinophil granulocytes — increased in parasitic infections, asthma, or allergic reaction.
- Basophil granulocytes- May be increased in bone marrow related conditions such as leukaemia or lymphoma.

A manual count will also give information about other cells that are not normally present in peripheral blood, but may be released in certain disease processes.

Platelets

- Platelet numbers are given, as well as information about their size and the range of sizes in the blood.
- Mean platelet volume (MPV) - a measurement of the average size of platelets.

Many disease states are heralded by changes in the blood count

- leukocytosis can be a sign of infection.
- thrombocytopenia can result from drug toxicity.
- pancytopenia is generally as the result of decreased production from the bone marrow, and is a common complication of cancer chemotherapy.

2.6 Stage of human growth and development

2.6.1 Normal fetal development and growth

Determinant of birth weight are multi-factorial, and reflect the influence of the natural growth potential of the fetus and the intrauterine environment .the later is controlled by both maternal and placenta factors fetal growth is dependent on adequate transfer of nutrients and oxygen. This in itself is on appropriate maternal nutrition and placental perfusion . other factor are important in determining fetal growth ,for example fetal hormones ,they affect the metabolic rate ,growth of tissue and maturation of individual organs

2.6.2 Fetal blood formation

The first fetal blood cells are formed on the surface of the yolk sac from 14-19 days after conception. Haemopoiesis continues from this site until third post-conceptual month .during the fifth week of embryonic life, exteramedullary haemopoiesis begins in the liver and to lesser extent in the spleen .the bone marrow starts to produce red cells at 7 – 8 weeks and is the predominant source of red cells from 26 weeks gestation.

Most hemoglobin in the fetus is fetal hemoglobin (HbF) which has two gammas – chains (alpha -2, gamma-2) . this differs from the adult haemoglobins HbA and HbA2 ,which have two beta- chains(alpha -2, beta-2)and two delta – chains (alpha-2,delta-2) respectively. Ninety percent of fetal hemoglobin is HbF between 10and 28 weeks gestation. From 28 to 34 weeks, a switch to HbA occurs, and at term the ratio of HbF to HbA is 80:20 ;by 6 month of age ,only 1percent of haemoglobin is HbF. Akey feature of HbF is higher affinity for oxygen than HbA this in association with ahigher Hb concentration

2.6.3 Postnatal development

During infancy and childhood, there is active hematopoiesis in the medullary cavity of virtually every bone. With age, the hematopoietic ally Active marrow (red marrow) is gradually replaced by inactive marrow (yellow marrow), which consists predominantly of adipose tissue. In adults, hematopoiesis is restricted to the proximal long bones and the axial skeleton(skull, vertebral bodies, ribs, sternum, and pelvis). The yellow marrow can resume active hematopoiesis under conditions of chronic hematologic stress (chronic bleeding or hemolytic anemia).

2.6.4 Hemopoiesis during adult life

By about 25 years of age, the main site of hematopoiesis are the vertebrate ,ribs, sternum skull bones, pelvis and sacrum, and the proximal ends of the femur and humerus. At these sites about half the marrow is red active cell producing marrow and the remainder, non cell producing yellow fatty marrow .other bone marrow cavities in the body contain non haematopoietic fatty marrow .in certain blood disorders eg. Chronic dyserythropoietic, blood cell production can resume in the liver and speen (exteramedullary hematopoiesis) and the fatty marrow in some bones can become replaced by hematopoietic marrow.

2.7 Haemostasis

Requires normal haemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors an efficient and rapid mechanism for stopping bleeding from

sites of blood. The vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The haemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels.

2.8 Classification of Coagulation Factors

Coagulation factors may be categorized into substrates, cofactors, and enzymes. Substrates are the substance upon which enzymes act. Fibrinogen is the main substrate. Cofactors accelerate the activities of the enzymes that are involved in the cascade. Cofactors include tissue factor, factor V, factor VIII, and Fitzgerald factor. All of the enzymes are serine proteases except factor XIII which is a transaminase. [4]

2.8.1 Factor I, Fibrinogen

Substrate for thrombin and precursor of fibrin, it is a large globulin protein. Its function is to be converted into an insoluble protein and then back to soluble components. When exposed to thrombin, two peptides split from the fibrinogen molecule, leaving a fibrin monomer to form a polymerized clot. In general ,proteins are complex macro molecules composed of more than 40 amino acid linked by peptide bonds. Proteins are found in all cells and body fluid with the plasma alone containing more than 100 different species. The plasma proteins serve a large array of important function and exist in a variety of shape, size and structure.

The one can separate the proteins of plasma into three major groups fibrinogen, albumin and globulin by the use of varying concentration of sodium and ammonium sulfate. [5]

Fibrinogen (factor I, 340 kD) is a soluble plasma glycoprotein that consists of three non -identical pairs of polypeptide chains, All three chains are synthesized in the liver; the three structural genes involved are on the same chromosome. [5]

Is large ,stable globulin protein ,it is precursor of fibrin with form the resulting clot. When fibrinogen is exposed to thrombin ,2peptide split from the fibrinogen molecule, leaving a fibrin monomers .these monomers aggregate together to form the final polymerized fibrin clot product**.** [6]

The fibrinogen gene cluster is located on chromosome 4q31 in the order γ α β with β transcribed in opposite direction to γ and α. the over all structure of fibrinogen is asymmetrical dimer α2 β2 2the molecule is trinodualar with the outer two globular domain containing the carboxytermini of all 3 chains .connected to central globular domain which contain the N-termini of all 6 chains tethered together by disulphide bonds, the lateral and central globular domains are connected by soiled coil regions forming α-helical ropes. Polymerization of fibrinogen occur when thrombin cleaves 2 short negatively charged fibrinopeptide A and B from the N-termini of the α and β chain respectively. [7]

It is classified as a glycoprotein because it has considerable carbohydrate content. On plasma electrophoresis, fibrinogen is seen as a distinct band between the β- and -globulins. The function of fibrinogen is to form a fibrin clot when activated by thrombin; therefore, fibrinogen is virtually all removed in the clotting process and is not seen in serum. Fibrinogen customarily has been determined as clottable protein. Fibrinogen concentration is proportional to the time required to form a clot after the addition of thrombin to citrated plasma. Fibrin split products (degradation products of fibrinogen and fibrin) are determined by immunoassay methods such as radial immunodiffusion, nephelometry, and RIA.Fibrinogen is one of the acute-phase reactants, a term that refers to proteins that are significantly increased in plasma during the acute phase of the inflammatory process. Fibrinogen levels also rise with pregnancy and the use of oral contraceptives. Decreased values generally reflect extensive coagulation, during which the fibrinogen is consumed**.** [8]

2.9 Laboratory Model of Coagulation

Laboratory testing looks at the in vitro effect of the coagulation process which is measured by the prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrin degradation products (FDPs), and D-dimer. This section will focus on PT and a PTT. While the coagulation cascade does not reflect what goes on in vivo, it provides a model in which the laboratory relates to for testing. However, the coagulation cascade reflects the mechanisms that the laboratory uses for results. The screening tests provide a tremendous amount of information to the physician. They can be performed both quickly and accurately. [4]

2.10 Fibrinolysis

The fibrinolysis system is responsible for the dissolution of a clot. Fibrin clots are not intended to be permanent. The purpose of the clot is to stop the flow of blood until the damaged vessel can be repaired. The presence or absence of hemorrhage or thrombosis depends on a balance between the procoagulant and the fibrinolysis system. The key components of the system are plasminogen, plasminogen activators, plasmin, fibrin, fibrin/ FDP, and inhibitors of plasminogen activators and plasmin.6 Fibrinolysis is the process by which the hydrolytic enzyme plasmin digests fibrin and fibrinogen, resulting in progressively reduced clots. This system is activated in response to the initiation of the activation of the contact factors. Plasmin is capable of digesting either fibrin or fibrinogen as well as other factors in the cascade (V, VIII, IX, and XI). Normal plasma contains the inactive form of plasmin in a precursor called plasminogen. This precursor remains dormant until it is activated by proteolysis enzymes, the kinases, or plasminogen activators. Fibrinolysis is controlled by the plasminogen activator system. The components of this system are found in tissues, urine, plasma, lysosome Granules, and vascular endothelium. An activator, tissue-plasminogen activator (tPA) results in the activation of plasminogen to plasmin resulting in the degradation of fibrin. The fibrinolysis system includes several inhibitors. Alpha-2-antiplasmin is a rapid inhibitor of

plasmin activity and alpha- 2 macroglobulin is an effective slow inhibitor of plasmin activity. This system is in turn controlled by inhibitors to tPA and plasmin-plasminogen activator inhibitor 1 (PAI-1) and alpha-2-antiplasmin. Reduced fibrinolytic activity may result in increased risk for cardiovascular events and thrombosis. Pharmacologic activators are currently used for therapeutic thrombolysis, including streptokinase, urokinase, and tPA. Urokinase directly activates plasminogen into plasmin, and streptokinase forms a streptokinase plasminogen complex, which then converts plasminogen into plasmin.[2]

2.11 Coagulation Inhibitors

Inhibitors are soluble plasma proteins that are natural anticoagulants. They prevent the initiation of the clotting cascade. There are two major inhibitors in plasma that keep the activation of coagulation under control**.** [9]

These inhibitors are:

1. Protease inhibitors: inhibitors of coagulation factors, which include

• Antithrombin.

• Heparin cofactor II.

• Tissue factor pathway inhibitor.

• Alpha-2-antiplasmin.

• C1.

2. The protein C pathway: inactivation of activated cofactors, which includes

• Protein C and protein S.

Kinin System

Another plasma protein system in coagulation is the kinin system. This system is capable of vascular dilatation leading to hypotension, shock, and end-organ damage by its capability to increase vascular permeability.

The kinins are peptides of 9 to 11 amino acids. The kinin system is activated by factor XII. Hageman factor XIIa converts prekallikrein (Fletcher factor) into kallikrein, and kallikrein converts kininogens into kinins. The most important is bradykinin (BK). This is an important factor in vascular permeability as well as

a chemical mediator of pain. BK is capable of reproducing many characteristics of an inflammatory state such as changes in blood pressure, edema, and pain, resulting in vasodilation and increased microvessel permeability. [3]

Complement System

This system has a role in inflammation and the immune system as well as important thrombohemorrhagic disorders such as disseminated intravascular coagulation (DIC). Activated complement fragments have the capacity to bind and damage self-tissues. Regulators of complement activation are expressed on cell surfaces. These protect the cell from the effects of cell-bound complement fragments. If this regulation process is abnormal, it may participate in the pathogenesis of autoimmune disease as well as inflammatory disorders. This system includes 22 serum proteins that play a role in mediating immune and allergic reactions and the lysis of cells due to a production of membrane attack complex (MAC). The lysis and disruption of red blood cells and platelets lead to the release of procoagulant material. This system is a sequential activation pathway. Complement is activated by plasmin through the cleavage of C3 into C3a and C3b. C3 causes increased vascular permeability, and because of the degranulation or lysis of mast cells, which in turn results in the release of histamine, C3b causes immune adherence. The interrelationship between the complement, kinin, and the coagulation system is complex and revealing. Coagulation and the elements that contribute to the success of the hemostatic system are multifactorial, and with each decade, more knowledge about this versatile system is learned. Illustrates the important interrelationships between the coagulation, fibrinolytic, complement, and kinin systems. [3]

2.12 Previous studies

Correlation between Glycemic Control and Plasma Fibrinogen Level in Patients with Type2 Diabetes Mellitus

Nizar M. Abdeurahman, Elshazali W. Ali

Department of haematology, Faculty of medical laboratory sciences, Alneelain University, Khartoum, Sudan.

Abstract

Results: Mean plasma fibrinogen level in Sudanese patients with type2 diabetes mellitus was in the upper limit of the normal range (mean ± SD: 3.96 ± 0.92), and it was slightly higher in females than males but the difference was not statistically significant significant (Mean ± SD: 4.1 ± 0.92 & 3.7 ± 0.91 g/L respectively, P.value: 0.18).

Fibrinogen level was slightly higher in patient who had poor glycemic control (mean±SD: 4.0±0.93) than those had good control (mean±SD: 3.5±0.68), but the difference also was not statistically significant (p.value: 0.513)

2 Red cell distribution in type2 diabetic patients

Amal Mohamed Nada

Results: Patient and healthy control characteristics are RDW was significant higher in diabetic patient than healthy controls (p 0,008) whereas MCV was significantly smaller (p 0.036) no statistically significant different were noted between both group in MPV plate late count or WBC count comparing patient with HbAc>7

Chapter Three

Materials and Methods

3 Materials and Methods

3.1 Study Design

This is across sectional descriptive study to Comparison of Plasma Fibrinogen Level and Complete Blood Count among Glycomic Control T2 Diabetic Mellitus Patients in Shendi Town in the period from March to August 2018.

3.2 Study Area

The study was conducted in Shendi town which is located in the north of Sudan and north of the capital Khartoum and for about 173km, and to the south of Aldamer for about 127km, located in the east side of the river Nile, and covering area about 30km, the population about. Most of people are farming. It contain three hospitals and health centers.

3.3 Study Population

Thirty blood samples collected form study groups of diabetic patient and 10 blood samples collected as control without diabetic patients.

3.4 Sample

Venous blood collected using sterile disposable plastic syringe after cleaning the venepuncture area with 70% ethanol, the blood was add to the anticoagulant and gently mix.

Venous blood with EDTA to make CBC and HbA1C

Venous blood with 3.2 sodium citrate to make fibrinogen.

3.5 Data Collection Tools

The primary data was collected by using questionnaire.

3.6 Data Analysis

Data was entered, cleaned, and analyzed using SPSS version 25.0 using with test.

3.7 Method

The determination of fibrinogen in human plasma according to method developed by Clauss.

3.7.1. Principle of Clauss

Diluted plasma is clotted with a strong a high concentration of thrombin. The plasma is diluted 1:10to minimise the effect of inhibitory substances within the plasma heparin elevated level FDPs.

3.7.2 Contents

1. Thrombin Reagent (Bovine Thrombin).
2. IBS Buffer.
3. TE Control Normal.
4. TE control A.

3.7.3 Specimen Collection and Storage

1. Obtain venous blood by clean vein puncture.
2. Immediately mix 9 parts blood with 1 part 3.2% sodium citrate (0.105M) and mix well.
3. Centrifuge the specimen at 1500g for 10 min.
4. Separate plasma after centrifugation and store in plastic tube.
5. Use plasma within 4fours. Otherwise store frozen and thaw just prior to use.

3.7.4. Procedures:

Afibrinogen activity test is also known as a factor I assay . it used to determine the level of fibrinogen or factor I is a blood plasma protein that is made in the liver . fibrinogen is one of 13 coagulation factors responsible for normal blood clotting

3.7.4.1 Automated Method. Coatron A.

3.7.4.2 Manual Method (Coatron) M.

Pipette 50µl diluted standard or patient plasma ; (1:10) into test cuvette prewarm at 37ºC for 1-2 minutes .Add 25µl thrombin reagent and simultaneously start test.

For other instrument. Please refer to your instrument manual for more detailed instrument specific instructions.

Normal Range: 180-450mg/dl.

3.8 CBC was done by using Mindray Haematology Analyzer (mindray bc 3000)

Principle

Blood cells can be broadly divided into three categories .red blood cells, White blood cells and platelets. The analyzer measures the number of cells and distinguishing between their types according to size using sheath flow DC detection. Electrical current is passed through a solution; this method measures the changes in electrical resistance that occurs when blood cells pass through detection aperture. This instrument performs haematology analyses according to the RF/DC detection method, Hydro Dynamic Focusing (DC Detection), and sodium lauryl sulphate (SLS) haemoglobin method. The radio frequencies and direct current (RF/DC detection method) detects the volume of blood cells by changes in direct- current resistance.

3.8.1.2. Procedure

RBCs count, Hct, Hb concentration, haematimetric indices (MCV, MCH, and MCHC), RDW, WBCs and platelets counts were measured by using an automatic blood cell counter (Mindray -3000 analyzers). The assay was performed according to the instructions provided by the manufacturer. The analyzer was controlled by normal control, abnormal high and abnormal low.

the EDTA blood samples were aspirated into analyzer through a sample probe, and the counting was started automatically, the results were displayed on the screen within (20) second, the print key was pressed to print out the results.

3 .9 Ethical consideration

An informed consent was obtained from select individuals to be study is take after being informed with all objectives of the study.

Chapter Four

Results

4. Results

Table (4-1): Demographic data of the study group

		Frequency	Percent	Total
Study groups	Cases	30	70%	100%
	Controls	10	30%	
Sex	Male	15	50%	100%
	Female	15	50%	

Table (4.2): Demographic data of participate in Fibrinogen level:

Study group	Number	Mean	P. value
Cases	30	398mg dl	0.001
Controls	10	242mg dl	

Table (4.3): Mean of fibrinogen level among well and poor controlled T2 DM.

HbA1C	Mean	P.Value
≤6.8	292.8	0.000
≥6.8	539.6	

Table (4.4): Mean of Hb / TWBCs/ PCV/ PLTs/ RBCs among cases and controls

	Mean				
	TWBC $\times 10^3$/L	**Hb g/dl**	**PCV g/dl**	**Plt $\times 10^9$/L**	**RBC $\times 10^6$/L**
Cases	8.940	11.303	36.547	314.400	4.716
Controls	7.380	12.410	35.540	279.500	4.233
p.value	0.000	0.000	0.000	0.000	0.000

Chapter Five

Discussion

Conclusion

Recommendations

5.1 Discussion

In this study we aimed to measure the level of plasma fibrinogen among patients with T2DM glycomic control and CBC during the period from March to Augusts 2018. This study include 40 participants, divided into two groups, thirty participants who diagnosed with diabetes (cases) (70%) and 10 healthy participants (30%) acted as control group.

The study revealed that the overall plasma fibrinogen in all study participates, among cases group 398 mg/dl and among control group 242 mg/dl. This difference was statistically significant ($p < 0.001$). The study found a Higher plasma fibrinogen levels in patients with poor control HA1C more than 6.8 (539 mg/dl) as compared to patients with good controls HA1C less than 6.8 (292 mg/dl) which were statistically significant.

The results of our study was in agreement with other study done by *Nizar M.*Abdeurahman, Elshazali W. Ali. [21]

Also found that the mean plasma level of fibrinogen in female (404mg/dl) was higher than male (385 mg/dl).

White blood cells hemoglobin hemotcrite and platelet were most statistically significant (p.value: 0.000). The results of our study was in agreement with other study done Amal Mohamed Nada.

5-2 Conclusion

- Fibrinogen level was slightly higher in patient who had poor glycemic control HbA1C more than 6.8 (539mg/dl) than those had good control HbA1C less than 6.8(292mg/dl).
- Also was statistically significant (p.value: 0.001) white blood cells hemoglobin hemotcrite and platelet were most statistically significant (p.value: 0.000)

5.3 Recommendations

1- Special attention should be given for patients who had the combination Diabetes and hyper-fibrinogenemia because that may increases the risk of developing micro and macro vascular complications.
2- The control of diabetes should be assessed by further study to ensure if the elevation of fibrinogen level was significantly associated with uncontrolled diabetes in Sudan.
3- Based on our findings, we suggest increased plasma fibrinogen can be prevented by promotion of glycemic control.

Chapter Six

References

Appendix

6.1 References

(1) Joshi SK, Shrestha S. Diabetes mellitus: A review of its associations with ent environmental factors. *Kath Univ Med J* 2010; 8(29):109-115

(2) Zhao Y, Zhang J, Zhang J, Wu J. DM is associated with shortened activated partial thromboplastin time and increased fibrinogen values. *PLoSOne* 2011;6(1):e16470.

(3) Stec JJ, Silbershatz H, Tofler GH, Matheney TH, Sutherland P, Lipinska I, *et al*. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham offspring Population. *Circulation* 2000; 102(14):1634-8.

(4) Kuusisto J, Mykkanen L, Pyorala K, Laakso M. NIDDM and its metabolic control predict coronary heart disease in elderly subjects. *Diabetes* 1994; 43(8):960- 7.

(5) Kafle DR, Shrestha P. Study of fibrinogen in patients with DM. *Nepal Med Coll J* 2010; 12(1):34-7.

(6) Maple-Brown LJ, Cunningham J, Nandi N, Hodge A, O'Dea K. Fibrinogen and associated risk factors in a high-risk population: urban Indigenous Australians, the DRUID Study. *Cardiovasc Diabetol* 2010; 9:69.

(7) Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, *et al*. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet* 1986; 2(8506):533-7.

(8) Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study. *JAMA* 1987; 258(9):1183-6.

(9) Kannel WB, D'Agostino RB, Wilson PW, Belanger AJ, Gagnon DR. Diabetes, fibrinogen, and risk of cardiovascular disease: the Framingham experience. *Am Heart J* 1990;120(3):672-6.

(10) Lee AJ, Lowe GD, Woodward M, Tunstall-Pedoe H. Fibrinogen in relation to personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary heart disease, and family history: the Scottish Heart Health Study. *Br Heart J* 1993; 69(4):338-42.

6.2 Appendix

Questionnaire

SHENDI UNIVERSITY

FACULITY OF GRADUATE STUDIES & SCI ENTIFIC RESEARCH

Research questioner:

Comparison of Plasma Fibrinogen Level and Complete Blood Count among Glycomic Control Diabetic Mellitus Patients Type 2 in Shendi Town

1-Name:……..……NO ( )

- No. of Case ☐
- Age ☐
- Occupation ☐
- Address ☐
- WT ☐

- Gender

  Female ☐

  Male ☐

Results

- Fibrinogen

  HbA1C ≥ 6.8 ☐

  HbA1C ≤ 6.8 ☐

- CBC finding

  Hb g/dl ☐

  RBCS count ☐

  TWBCS ☐

  PCV ☐

  Platelet count ☐

Appendix II

إقـــرار بالموافقـــة

الاسم :----------

العمر :---------- العنوان :----------

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة الفيبرونوجين وفحص صورة الدم الكاملة لدى مرضى السكري النوع الثاني.

الباحث/ عبد الحليم حسب الرسول الأمين

بعد أن شرح لي بأنه لا يترتب عليه أي أذى جسدي أو نفسي واعلم أن المشاركة في هذا البحث لن تؤثر بأي حال من الأحوال في الرعاية الطبية التي أتلقاها كما أنه يحـــق لـــي بدون إبداء أسباب الانسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشراف:

د. حمزة أحمد حسن

التوقيع: ---------- لتاريخ :----------