بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of Higher Education and scientific Research

University of Shendi

Faculty of Graduate Studies and Scientific Research

Determination of Fibrinogen level in Recurrent Abortion in Shendi Town

Athesis submitted for the partial fulfillment of Msc in haematology

By

Amal Awad Elshader Gamer Alden

Bsc. (Shendi University-2006)

Supervisor

Dr: Hamza Ahmed Hassan

Assistant Professor in Haematology,Medical Laboratory

Sciences Shendi University

October - 2018

الآيـة

Quran VERSE

بسم الله الرحمن الرحيم

قال تعالى:

﴿ وَقَضَى رَبُّكَ أَلاَّ تَعْبُدُواْ إِلاَّ إِيَّاهُ وَبِالْوَالِدَيْنِ إِحْسَاناً إِمَّا يَبْلُغَنَّ عِندَكَ الْكِبَرَ أَحَدُهُمَا أَوْ كِلاَهُمَا فَلاَ تَقُل لَّهُمَا أُفٍّ وَلاَ تَنْهَرْهُمَا وَقُل لَّهُمَا قَوْلاً كَرِيماً (23) وَاخْفِضْ لَهُمَا جَنَاحَ الذُّلِّ مِنَ الرَّحْمَةِ وَقُل رَّبِّ ارْحَمْهُمَا كَمَا رَبَّيَانِي صَغِيراً (24) ﴾

صدق الله العظيم

سـورة الإسراء الآية (23-24)

Dedication

To those

Who give me the best of life without payment

To my mother for their patience and

Support

To my Brothers

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

For her great efforts and guidance..

I thank the staff of Algawda Centre for Medical Analysis.

I thank the staff of the Faculty of Medical Laboratory science and I would like to thank the staff of El Mek Nimir 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:

APSAC	activator plasmenogen Strepto Kinase anisolyated Complex.
DIC	Disamineted intravascular Coagulation.
ER	endoplasmic reticulum
FDPs	fibrindegradation Products .
FPA	Fibrinogen peptide A .
FP**B**	Fibrinogen peptide **B**.
HMW	High Molecular – Weight
-INR	international normalizing ratio
PTT	partial thromboplastin time
PAl-1	plasmenogen activator 1
PAl-2	plasmenogen activator 2
PT	Prothrombin Time
PTT	partial thromboplastin time
RSA	recurrent spontenous abortion.
Scu – PA	Single Chain uro Kinase plasmenogen activator
t-PA	tissue plasmenogen activator
t-PA	Tissue plasmenogen activator
U – PA	uro Kinase plasmenogen activator
UPA	Urokinese plasmenogen activator
VwF	Von will brand factor

Abstract

fibrinogen is one of factors that change during pregnancy .this study conducted to detect fibrinogen level in caces with recurrent abortion.

This is a cross-sectional study conducted at El-Mek Nimir University Hospital in Shendi town to determine the fibrinogen level in ladies with recurrent abortion in the period between (April 2018- August 2018). The study included (33) patients whom diagnosed with recurrent abortion and the study groups were compared with (20) ladies with normal pregnancy.

Data was collected using a structured face to face questionnaire and samples were collected from the two groups. Fibrinogen level were measured and the (SPSS) version (11.5) program was used for data analysis.

This study found elevated fibrinogen level in recurrent abortion

Women compared with control group. Diabetic mellitus , contraceptive drugs ,hyper tent ion and age this factor affected in fibrinogen level.

ملخص البحث

يمثل الفبرينوجين عوامل احد التجلط التي تتغير إثناء الحمل وقد أجريت هذه الدراسة لتحديد مستوى الفبرينوجين في حالات تكرر الإجهاض .

أجريت الدراسة بمستشفي المك نمر في مدينة شندي في الفترة ما بين (ابريل – أغسطس 2018م) على 33 سيدة مصابة بالإجهاض و 20 سيدة حمل طبيعي.

جمعت العينات على كل من المجموعتين وتم قياس مستوى الفبرينوجين في الدم تم جمع البيانات عن طريق الاستبيان وتم تحليلها عن طريق برنامج الحزمة الإحصائية للعلوم الاجتماعية.

هذه الدراسة وجدت ارتفاع في مستوى الفبرينوجين لدى السيدات ذوات الإجهاض المتكرر مقارنة بمجموعة الضبط. مرض السكري واستعمال الأدوية المانعة للحمل وارتفاع ضغط الدم والعمر عوامل مؤثرة في مستوى الفبرينوجين.

List of Contents:

List of Tables

Chapter one

Introduction

Rationale

Objectives

1.1 Introduction

During normal pregnancy the hemostatic balance changes in the direction of hypercoagulability , thus decreasing bleeding complications in connection with initial factor for acute hemostasis at delivery is, delivery. The most important however , uterine muscle contractions. Which interrupt blood flow. Global tests such suchas sonoclot signature , the thromboelastogram , and a new method analyzing overall entative of hypercoagulability during plasma hemostasis , all show changes repres pregnancy. increased endogenous thrombin generation, acquired activated protein C resistance, slightly decreased activated partial thromboplastin time (a PTT)

And intnational normalized ratio increased prothrombin complex level (PT) measurd as have been reported as well. in normal pregnanay , the platelet INR of less than count is within normal range except during the the third trimester when benign platelet turnover, can be odserved 80 –150 $\times 10^9$ gestational thrombocytopenie , beta thromboglobulin and is usually normal. Activation of platelets and release of are reported. The bleading time is unchanged during normal Pregnancy.

normal most blood coagulation factors and fibrinogen increase during pregnancy .

Factor (F) Xl is the only blood coagulation factor that decreases Blood coagulation inhibitors are mainly unchanged put the level of free protein S decreases markedly and the level of tissue factor pathway inhibitor increases . Thrombomodulin levels increase during pregnancy.

Fibrinolytic capacity is diminished during pregnancy mainly because of markedly increased levlis of plasminogen activator inhibitor from the (PAI - 2) from endothelial cells and plasminogen activator inhibitor (PAI - 1) activated Fidrinolysis inhibitor is reported to be unaffected.

The –Thrombin placenta hemostatic balance has been studied by analyses of prothrombin fragment D- dimer , and antithrombin complex.
Fibrinopeptide A soluble fibrin, thrombin compiex. There is activation of blood coagulation and a antiplasmin – plasmin
Simultaneous increase in fibrinolysis without signs of organ dysfunction during normal pregnancy.
These changes increase as pregnancy progresses . During delivery , there blood coagulation factors , including fibrinogen is consumption of platelets and fibrinolysis improves and increases fast following childbirth and expulsion of the limitindg at D – dimer levels. These changes are self – placenta , resulting in increased noted during pregnancy.
Normalize after normal delivery. The haemostatic changes can be 4 to 6 weeks. Platelet count and free protein S , however delivery within 3months following abnormal longer. Homeostasis should not be tested earlier than and influences of pregnancy. PAl -1 delivery and after terminating lactation to rule has been detected up to 2 - 8 weeks levels decrease fast postpartum , but PAI 2- PAI
antiplasmin, urokinase, and kallikrein inhibitor levels have been – postpartum. Alpha 2- postpartum 6 weeks reported to be increased [1]
The determination of total fibrinogen and high molecular weight (HMW) fibrinogen was performed with the glycine precipitation method. Ninety patients in different trimesters of pregnancy and 37 non-pregnant controls participated in the study. Total fibrinogen was found to increase during pregnancy until delivery, after which it declined gradually, peaking once more on the 3rd day of puerperium. HMW fibrinogen showed a similar pattern during pregnancy and puerperium, but markedly increased at the time of delivery. These elevated values during pregnancy indicate a significant change in the fibrinolytic system, which is believed to be

depressed during pregnancy, enhanced during the postpartum period, and stabilized after the 3rd day of puerperium [2]

1.2.Rationale

The recurrent abortion associated with thrombosis in women in fertility age.Fibrinogen level associated with establish thrombosis. It has been debated whether women with recurrent abortion should be treated as aggressively in risk of thrombosis , however we need more studies to clarify relationship between recurrent abortion and fibrinogen level that causes thrombosis.

1.3 Objectives

1.3.1 General objective:

- To determine of Fibrinogen level in recurrent abortion in shendi town.

1.3.2 Specific objectives:

1- To estimate fibrinogen in woman with recurrent abortion.

2- To determine the correlation between fibrinogen level and other diseases

3- To determine the correlation between fibrinogen level and contraceptive drugs

Chapter two

Literature Review

2. Literature review

2.1 Homeostasis

When the continuity of the vascular endothelium is disrupted, platelets and fibrin seal off the defect. Haemostatic processes are classified as primary (mainly involving platelets) and secondary (mainly related to fibrin formation or blood coagulation). When the blood clot is no longer required for haemostasis, the fibrinolytic system will dissolve it. The pivotal ligand for initial platelet recruitment to injured vessel wall components is von Willebrand factor (vWF), a multimeric protein present in the subendothelium and in plasma, where it is conformationally activated by shear forces. Adhering activated platelets recruit additional platelets, which are in turn activated and form a platelet aggregate. Coagulation is initiated by a reaction, activating factors IX and X. Once critical amounts of factor Xa are generated, thrombin generation is initiated and soluble fibrinogen is converted into insoluble fibrin. Excessive thrombin generation is prevented via inhibition by antithrombin and also via downregulation of its further generation by activation of the protein C pathway. Activation of the fibrinolytic system results from conversion of the proenzyme plasminogen into the active serine proteinase plasmin by tissue-type or urokinase-type plasminogen activators. Plasmin digests the fibrin component of a blood clot. Inhibition of the fibrinolytic system occurs at the level of the plasminogen activator (by plasminogen activator inhibitors) or at the level of plasmin (by alpha2-antiplasmin). Together, these physiological processes act to maintain normal functioning blood vessels and a non-thrombotic state[3]

2.2Fibrinolytic agents: mechanisms of activity and pharmacology

Fibrinolytic (thrombolytic) agents activate the fibrinolytic system by conversion of the inactive proenzyme, plasminogen into the active

enzyme plasmin, that degrades fibrin. Agents available for clinical use are: the physiologic tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) either in a single chain (scu-PA, prourokinase) or a two-chain (tcu-PA, urokinase) form, and the bacterial activator plasminogen streptokinase or its anisoylated complex with plasminogen (APSAC). Despite their widespread use, mainly in patients with acute myocardial infarction, all these agents suffer from a number of significant limitations, including resistance to reperfusion, the occurrence of acute coronary reocclusion and bleeding complications. Several lines of research towards improvement of thrombolytic agents are being explored, including the construction of mutants and variants of plasminogen activators, chimeric plasminogen activators, or plasminogen activators from animal (e.g. vampire bat) or bacterial (e.g. staphylokinase) origin. Pilot studies in patients with acute myocardial infarction have been performed with a few selected agents. Definition of their relative therapeutic benefit, or lack thereof, will require more detailed dose-finding studies, followed by randomized clinical trials against presently available thrombolytic agents.[4]

2.3The physiology of pregnancy

Pregnancy is the time from fertilization of an egg, also known as conception, to birth. Getting pregnant and growing a human from scratch is a very complicated biological process that takes a lot of resources. As a result, pregnancy can have a wide range of effects on the mother, both physically and emotionally.

Each egg that is released during a menstrual cycle travels to your uterus. However, unlike unfertilized eggs that proceed unaltered and then disintegrate when they get there, a fertilized eerrgg develops into a tiny human embryo on the way. On reaching the uterus, the embryo implants itself in the uterine wall, develops into a fetus, and steadily grows, until

about nine months later it is ready to emerge into the outside world as a newborn baby.

2.3.1 Signs and symptoms

If you are fertile, sexually active, and become pregnant, the first thing you are likely to notice is a late or missing mens7trual period. Fertilization of an egg triggers changes in the production of various hormones almost immediately, and hormone changes evolve and persist throughout your pregnancy to help you grow a he
althy baby. Unfortunately, these changes may also cause unpleasant side effects. As a result, in addition to a missed period, many women experience tender, swollen breasts, fatigue, nausea and vomiting, or morning sickness during the first few weeks of becoming pregnant.

2.3.2 Diagnosis

If you are experiencing some or all of the early signs and symptoms of pregnancy, or if you suspect you might be pregnant, you may want to take a home pregnancy test. These tests are designed to detect the presence of human chorionic gonadotropin in a sample of your urine. This hormone becomes detectable in urine once the embryo has implanted in the uterus, typically about 8 or 9 days after fertilization.[5]

2.4 Re current Abortion

More consecutive pregnancy losses.[6]

In fertility differs because it's the inability to concave … In many Cases the cause of RPL is unknown . After three or more losses a through evaluation is recommended by American Society of Reproductive Medicine . .[7]

About 1% of couples trying to have children are affected by recurrent miscarriage .[8] .[9]

Cases :-

There are various causes for recurrent miss carriage and some can be treated . some couples never have a cause identified .

Often after extensive investigation. .[10]

About 50-75% of cases are un explained .[7]

2.5 Fibrinogen

2.5.1 Fibrinogen (***factor I***) is a glycoprotein that in vertebrates circulates in the blood. During tissue and vascular injury it is converted enzymatically by thrombin to fibrin and subsequently to a fibrin-based blood clot. Fibrinogen functions primarily to occlude blood vessels and thereby stop excessive bleeding. However, fibrinogen's product, fibrin, binds and reduces the activity of thrombin. This activity, sometimes referred to as antithrombin I, serves to limit blood clotting. Loss or reduction in this antithrombin 1 activity due to mutations in fibrinogen genes or hypo-fibrinogen conditions can lead to excessive blood clotting and thrombosis. Fibrin also mediates blood platelet and endothelial cell spreading, tissue fibroblast proliferation, capillary tube formation, and angiogenesis and thereby functions to promote tissue revascularization, wound healing, and tissue repair.

Reduced and/or dysfunctional fibrinogens occur in various congenital and acquired human fibrinogen-related disorders. These disorders represent a clinically important group of rare conditions in which individuals may present with severe episodes of pathological bleeding and thrombosis; these conditions are treated by supplementing blood fibrinogen levels and inhibiting blood clotting, respectively. Certain of these disorders may also be the cause of liver and kidney diseases.

Fibrinogen is a "positive" acute-phase protein, i.e. its blood levels rise in response to systemic inflammation, tissue injury, and certain other events.

It is also elevated in various cancers. Elevated levels of fibrinogen in inflammation as well as cancer and other conditions have been suggested to be the cause of thrombosis and vascular injury that accompanies these conditions.

2.5.2 Structure

The Aα, Bβ, and γ chains are transcribed and translated coordinately on the endoplasmic reticulum (ER) with their peptide chains being passed into the ER while their signal peptide portions are removed. Inside the ER, the three chains are assembled initially into Aαγ and Bβγ dimers, then to AαBβγ trimers, and finally to $(AαBβγ)_2$ heximers, i.e. two AαBβγ trimers joined together by numerous disulfide bonds. The heximer is transferred to the Golgi where it is glycosylated, hydroxylated, sulfated, and phosphorylated to form the mature fibrinogen glycoprotein that is secreted into the blood. Mature fibrinogen is arranged as a long flexible protein array of three nodules held together by a very thin thread which is estimated to have a diameter between 8 and 15 Angstrom (Å). The two end nodules (termed D regions or domains) are alike in consisting of Bβ and γ chains while the center slightly smaller nodule (termed the E region or domain) consists of two intertwined Aα alpha chains. Measurements of shadow lengths indicate that nodule diameters are in the range 50 to 70 Å. The length of the dried molecule is 475 ± 25 Å.

The fibrinogen molecule circulates as a soluble plasma glycoprotein with a typical molecular weight (depending on its content of Aα verses AαE and γ versus γ' chains) of ~340 kDa. It has a rod-like shape with dimensions of 9 × 47.5 × 6 nm and has a negative net charge at physiological pH (its isoelectric point is pH 5.8). The normal concentration of fibrinogen in blood plasma is 150–400 mg/dL with levels appreciably below or above this range associated with pathological

bleeding and/or thrombosis. Fibrinogen has a circulating half-life of ~4 days.

2.5.3 Blood clot formation

During blood clotting, thrombin attacks the N-terminus of the Aα and Bβ chains in fibrinogen to form individual fibrin strands plus two small polypeptides, fibrinopeptides a and b derived from these respective chains. The individual fibrin strands then polymerize and are cross-linked with other fibrin stands by blood factor XIIIa to form an extensive interconnected fibrin network that is the basis for the formation of a mature fibrin clot.[5][12][13] In addition to forming fibrin, fibrinogen also promotes blood clotting by forming bridges between, and activating, blood platelets through binding to their GpIIb/IIIa surface membrane fibrinogen receptoer

Fibrin participates in limiting blood clot formation and lysing formed blood clots by at least two important mechanisms. First, it possesses three low affinity binding sites (two in fibrin's E domain; one in its D domain) for thrombin; this binding sequesters thrombin from attacking fibrinogen. Second, fibrin's Aα chain accelerates by at least 100-fold the mount of plasmin activated by tissue plasminogen activator; plasmin breaks-down blood clots.[11][13][5][12] Plasmin's attack on fibrin releases D-dimers (also termed DD dimers). The detection of these dimers in blood is used as a clinical test for fibrinolysis.

A fibrinogen activity test is also known as a Factor I assay. It’s used to determine the level of fibrinogen in your blood. Fibrinogen, or factor I, is a blood plasma protein that’s made in the liver. Fibrinogen is one of 13 coagulation factors responsible for normal blood clotting.

When you start to bleed, your body initiates a process called the coagulation cascade, or clotting cascade. This process causes coagulation factors to combine and produce a clot that will stop the bleeding. If you

don’t have enough fibrinogen or if the cascade isn’t working normally, clots will have difficulty forming. This can cause excessive bleeding.

Low fibrinogen levels can also cause thrombosis due to an increase in coagulation activity. Thrombosis refers to the formation of a blood clot inside of a blood vessel. The clot blocks the normal flow of blood through the circulatory system. This can lead to serious medical conditions such as heart attack and stroke.

Clinical analyses of the fibrinogen disorders typically measure blood clotting using the following successive steps: Higher levels are, amongst others, associated with cardiovascular disease (>3.43 g/L). It may be elevated in any form of inflammation, as it is an acute-phase protein; for example, it is especially apparent in human gingival tissueduring the initial phase of periodontal disease.

- Blood clotting is measured using standard tests, e.g. prothrombin time, partial thromboplastin time, thrombin time, and/or reptilase time; low fibrinogen levels and dysfunctional fibrinogens usually prolong these times whereas the lack of fibrinogen (i.e. afibrinogenemia) renders these times infinitely prolonged.
- Antigenic levels of fibrinogen levels are measured in the plasma isolated from venous blood by immunoassays with normal levels being about 1.5-3 gram/liter, depending on the method used. These levels are normal in dysfibrinogenmia (i.e. 1.5-3 gram/liter), decreased in hypofibrinogenemia and hypdysfibrinogenemia (i.e. <1.5 gram/liter), and absent (i.e. <0.02 gram/liter) in afibrinogenima.
- Functional levels of fibrinogen are measured on plasma induced to clot. The levels of clotted fibrinogen in this test should be decreased in hypofibrinogenemia, hypodysfibrinogenemia, and dysfibrinogenemia and undetectable in afibrinogenemia.

Functional fibrinogen/antigenic fibrinogen levels are <0.7 in hypofibrinogenemia, hypodsyfibrinogenemia, and dysfibringognemia and not applicable in afibringenemia.

Fibrinogen analysis can also be tested on whole-blood samples by thromboelastometry. This analysis investigates the interaction of coagulation factors, their inhibitors, anticoagulant drugs, blood cells, specifically platelets, during clotting and subsequent fibrinolysis as it occurs in whole blood. The test provides information on hemostatic efficacy and maximum clod firmness to give additional information on fibrin-platelet interactions and the rate of fibrinolysis Scanning electron microscopy and confocal laser scanning microscopy of in vitro-formed clots can give information on fibrin clot density and architecture.

- The **fibrinogen uptake test** or **fibrinogen scan** was formerly used to detect deep vein thrombosis. In this method, radioactively labeled fibrinogen, typically with radioiodine, is given to individuals, incorporated into a thrombus, and detected by scintigraphy.

A fibrinogen activity test may be ordered alone or as part of a series of tests to determine the cause of abnormal bleeding.

Your doctor may order a fibrinogen activity test if you're experiencing any of the following:

- excessive bruising
- excessive bleeding from the gums
- frequent nosebleeds
- hemorrhage of the gastrointestinal tract
- blood in the urine
- blood in the stool
- bleeding in the head
- rupture of the spleen

Tests may also be ordered if you have:

- abnormal results from a prothrombin time test or partial thromboplastin time test
- symptoms of disseminated intravascular coagulation, which is a condition in which small clots form throughout the body
- signs of an abnormal breakdown of fibrinogen (fibrinolysis)
- a possible acquired or inherited factor deficiency that affects how your blood clots

A fibrinogen activity test may also be part of a general evaluation of your risk of cardiovascular disease. People with clotting disorders can have an increased risk of heart disease and storks

There are no special preparations necessary for this test. Your doctor may advise you to stop taking certain medications before this test. It's very important that you inform your doctor if you're taking any blood thinners.

A healthcare provider will take a sample of blood from your arm. They'll clean the site with a swab of rubbing alcohol. They'll insert the needle into a vein, and a tube will be attached to collect the blood. The needle will be removed when enough blood has been drawn. The site will then be covered with a gauze pad.

This blood sample will be sent to a laboratory for analysis.

2.6 Normal Results

The normal level of fibrinogen in the blood is between 1.5 to 3.0 grams per liter.

Abnormal Results

Abnormal results may be higher or lower than the reference range. Abnormal results can be caused by:

- excessive fibrinogen use
- acquired or inherited fibrinogen deficiency
- abnormal fibrinolysis
- hemorrhage

The three types of fibrinogen deficiency are afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia:

2.6.1Afibrinogenemia

Afibrinogenemia is the total absence of fibrinogen. This disorder affects 5 out of every 10 million people. This disorder causes the most severe bleeding out of the three forms of fibrinogen deficiency.

2.6.2Hypofibrinogenemia

Hypofibrinogenemia is an abnormally low level of fibrinogen. In this case, the test would show a level between 0.2 and 0.8 grams per liter. This form of the deficiency is less common than afibrinogenemia and it can cause mild to severe bleeding.

2.6.3Dysfibrinogenemia

Dysfibrinogenemia is a condition in which fibrinogen levels are normal, but the protein doesn't function properly. Dysfibrinogenemia affects only about one in every 1 million people. The condition rarely causes a bleeding problem and instead is more likely to cause thrombosis

Fibrinogen defects may be quantitative (hypo- or hyper-fibrinogenaemia) or qualitative (dysfibrinogenaemia). Inherited dysfibrinogenaemia is rare with only 250-300 patients reported worldwide but an acquired defect of fibrinogen function is more common, especially in liver disease when the fibrinogen molecule is excessively glycosylated impairing its activity. Elevated levels of fibrin degradation products (FDPs) also impair the action of fibrinogen. Fibrinogen levels are a useful as part of the investigation of a bleeding tendency or an unexplained prolongation of the APTT or PT. Elevated levels may correlate with increased risk of thrombosis in epidemiological studies although the significance in individual patients is unclear.

Fibrinogen consists of three pairs of polypeptide chains: two Aα, two Bβ and two γ. These are linked together by 29 disulphide bonds in such a

way that N-terminal regions of the 6-polypeptide chains meet to form a central E-domain. The C-terminal regions [Aα, Bβ and γ] for the D-domain and these are joined by α-helical ropes to the central E-domain to give the characteristic fibrinogen structure.

Activation of fibrinogen by thrombin [IIa] cleaves the two short peptides from the N-terminal regions of the Aα and Bβ chains - these peptides are known as Fibrinopeptide A [FpA] and B [FpB] respectively. Removal of the N-terminal sequences from Aα and Bβ chains chains reveals new N-terminal sequences in the Aα and Bβ chains located within the E domain knows as 'knobs.' These knobs can interact spontaneously with the D-dimer regions to form fibrin polymers. Under the influence of factor XIIIa, cross-linking of these fibrin polymers then occurs to form cross-linked fibrin polymers.

Principles

There are a number of assay for measuring fibrinogen levels in plasma although in practice most laboratories use the Clauss method.

2.7 Fibrinogen Assays

Clauss	A functional assay based upon the time for fibrin clot formation
PT-derived Fibrinogen Assays	A derived fibrinoge n based upon the prothrombin time
Immunological	An immunological method which measures fibrinogen antigen rather than functional fibrinogen
Gravimetric Method	A method based upon clot weight

The four methods for measuring fibrinogen are summarised below:

.7.1 Clauss Assay :

Diluted plasma is clotted with a high concentration of thrombin. The plasma is diluted (usually 1:10 but this may vary if the fibrinogen concentration is very low or very high) to minimise the effect of 'inhibitory substances' within the plas ma e.g. heparin, elevated levels of FDPs.

The use of a high concentration of thrombin (typically 100 U/ml) ensures that the clotting times are independent of thrombin concentration over a wide range of fibrinogen levels.

The test requires a reference plasma with a known level of fibrinogen calibrated against a known international standard. A calibration curve is constructed using this reference plasma by preparing a series of dilutions (1:5 –1:40) in buffer to give a range of fibrinogen concentrations. The clotting time of each of these dilutions is established (using duplicate samples) and the results (clotting time(s)/fibrinogen concentration (g/L) are plotted on log-log graph paper. The 1:10 concentration is considered to be 100% i.e. normal. There should be a linear correlation between clotting times in the region of 10-50s.

The test platelet poor diluted plasma (diluted 1:10 in buffer) is incubated at 37°C, phospholipid and thrombin are added followed by calcium (all pre-warmed to 37°C). On the addition of the calcium timing begins. The time taken for the clot to form is compared to a calibration curve and the fibrinogen concentration deduced.

Most laboratories use an automated method in which clot formation is deemed to have occurred when the optical density of the mixture has exceeded a certain threshold.

2.7.2 PT – derived Fibrinogen Assays

The PT is determined by optical density change for a range of plasma dilutions with known fibrinogen levels. The optical change for each different fibrinogen level is plotted as a calibration curve. A PT is performed on the patient's platelet poor plasma and the fibrinogen derived from the change in optical density compared to the calibration curve.

The derived fibrinogen is a simple and inexpensive test and is widely used. However, the test can give misleading results in some disorders and is not recommended for routine laboratory use.

2.7.3 Immunological Assays

Assays based on enzyme linked immunoabsorbant assays (ELISA), radial immunodiffusion and electrophoresis are the most commonly employed. Immunological assays measure protein concentration rather than functional activity.

They are of value in the investigation of congenital dysfibrinogenaemias where there is a discrepancy between functional activity and antigen level.

2.7.4 Gravimetric Assays

2.7.4.1. Clot Weight

Similar to the Clauss method - a fibrinogen clot is formed by the addition of thrombin and calcium to dilute patient plasma. However, instead of using the time to clot formation to derive the fibrinogen

the clot is compressed (to extrude plasma and unused reagents), washed, dried then weighed. This assay is technically difficult and time consuming.

2.7.4.2 Clottable protein

Thrombin is added to plasma without calcium and the clot formed is washed then dissolved in an alkaline reagent then spectrophotometry is performed (e.g. typically absorbance at 282nm). The clot is almost all fibrin and so the measured protein concentration is taken as equivalent to the fibrinogen concentration.

2.7.4.3 TEG

The thromboelastogram has been used to measure functional fibrinogen levels

Investigation of bleeding	Clauss
Suspected dysfibrinogenaemia	Clauss *and* clottable protein *and* immunoassay
Bleeding disorders affecting factors in addition to fibrinogen (e.g. DIC)	Clauss
Thrombolytic therapy	Clauss
Very high fibrinogen levels	Clauss or immunoassay

Interpretation

Fibrinogen Level	Interpretation
Fibrinogen	DIC due the the consumption of clotting

levels are reduced in:	factors Liver disease due to decreased synthesis. An abnormal fibrinogen may be also be found in patients with liver disease due to an abnormal (increased) sialic acid content Massive transfusion leading to a dilutional coagulopathy Inherited deficiencies e.g. Hypofibrinogenaemia, afibrinogenaemia and dysfibrinogenaemia [the latter is often associated with reduced fibrinogen levels as well as activity] Following thrombolytic therapy In some patients following treatment with asparaginase
Fibrinogen levels are increased in:	Increasing age Female sex, pregnancy, oral contraception In post-menopausal women Acute phase reaction Disseminated malignancy [but may also be decreased if this is associated with DIC [14]

2.8 previous studies

2.8.1 Altered Levels of Fibrinogen in Relation to the Path physiology of Recurrent Spontaneous Abortions Dr.MonikaGandhi,India the role of fibrinogen levels in the RSA has been established apart from the other anatomical and immunological factors. Thus fibrinogen protein can help in devising an optimal diagnostic strategy to evaluate patients with RSA

and normalize this phenomemon by supplementation of fibrinogen to elevate its levels in the RSA pregnant females.[15]

Chapter three

Materials and methods

3. Materials and Methods

3.1. Study design:

This is a descriptive cross-sectional study to determine the fibrinogen level in recurrent abortion in Almek Nimir University Hospital during a period of (march 2018—August 2018).

3.2. Study area:

The study was conducted at Almek Nimir University Hospital which located in Shendi town in Sudan. Shendi is a town in Northern Sudan, situated on the east bank of the Nile (150 km) Northeast of Khartoum. Shendi is also about (45 km) southwest of the ancient city of Meroe. Located in the River Nile state, Shendi is the centre of the Ja'aliin tribe and an important historic trading centre. Its principal suburb on the west bank is Al-Matamma. A major traditional trade route across the Bayuda desert connects Al-Matamma to Marawi and Napata, (250 km) to the Northwest. The majority of population profession is farming and trading beside other.Shendi their is Shendi university with various faculties and many hospitals such as :Almak Namer, Altalimy and other.

3.3. Study population:

Women with recurrent abortion .

3.4. Data collection tools:

Data was collected using self-administrated per-coded questionnaire which specifically designed to obtain information that helped in study.

3.5. Blood Sampling:

Venous blood collected using sterile disposable plastic syringe after cleaning the venipuncture area with (70%) ethanol, the blood added to the anticoagulant and gently mix.The sample centrifuge at (1300 rpm) for (15min) to obtain plasma.

3.6. Inclusion criteria:

Women with recurrent abortion were included in the study.

3.7. Exclusion criteria:

Women with once time of abortion were excluded from study.

3.8. Methods:

3.8.1 Principle:

Fibrinogen in the sample precipitate in the presence of anti-human fibrinogen antibodies. the light scattering of the antigen antibody complex is proportional with fibrinogen concentration and can be measured turbedmetery.

3.8.2Procedure:

Sample dilution

The stander don't require pre-treatment

1-Pipette in the test tube

Human plasma	200mlμL
Distilled water	400mlμL

2Mix gently. the deluted sample is staple for8h at15-25c and24h in2-8 c

Turbidmetry

1-Being reagent and instrument to37 c

2-Zero the instrument with desalted water

3-Pipette in to curvet

Reagent A	0,8mlμL
Distilled water in blank ,SDT , test	16mlμL
Reagent B	0,2 mlμL

4-Mix and insert curvet in to instrument . start stop watch

5-Read absorbance at 340 nm at 5 minutes of reagent B addition [12]

3.9. Ethical consideration:

The consent of the selected individuals to the study was taken after being informed with all detailed objectives of study and it is health emphasis in the future.

3.10. Data analysis:

The collected data code in master sheet and proceed for analysis using SPSS version 11.5 using t.test. (mean, standard deviation, , P.value).

Chapter four

Results

4.Result

A total of (33) samples collected of Study group of women with recurrent abortion and (20) samples collected of healthy individuals as control group.

Table (4-1): Distribution of study population according to age :

Age	**Frequency**	**Percent**
20-30 years	5	15.2%
31-40 years	23	69.7%
41-50 years	5	15.2%
Total	**33**	**100%**

Table (4-2): Distribution of study population according to number of abortion:

number of abortion	**Frequency**	**Percent**
2-4	29	87.9%
more than 4	4	12.1%
Total	**33**	**100%**

Table (4-3): Distribution of study population according to Gestational age

Gestational age	**Frequency**	**Percent**
first trimester	17	51.5%
Second trimester	16	48.5%
Third trimester	0	0.0%
Total	**33**	**100%**

Table (4-4): Distribution of study population according to contraceptive drugs

contraceptive drugs	**Frequency**	**Percent**
Yes	20	60.6%
No	13	39.4%
Total	**33**	**100%**

Table(4-5): Distribution of study population according Diabetic disease

Diabetic disease	**Frequency**	**Percent**
Yes	20	60.6%
No	13	39.4%
Total	**33**	**100%**

Table (4-6): Distribution of study population according to Hypertension disease

Hypertension dis ease	**Frequency**	**Percent**
Yes	18	54.5%
No	15	45.5%
Total	**33**	**100%**

Table (4-7): Distribution of study population according to Drugs intake

Drugs intake	**Frequency**	**Percent**
Yes	8	24.2%
No	25	75.8%
Total	**33**	**100%**

Table(4-8): comparsion between the study group in fibrinogen level

	N	**Mean**	**Std. Deviation**	**P.value**
Test	33	237.76	33.016	0.000
Control	20	202.60	9.694	

Table(4-9): correlation between the study group Age and number of abortion Cross tabulation

Age	number of abortion Cross tabulation		Total	P.Value
	2-4	more than 4		
20-30 years	15.2%	0.0%	15.2%	0.044
31-40 years	63.6%	6.1%	69.7%	
41-50 years	9.1%	6.1%	15.2%	
Total	87.9%	12.1%	100.0%	

Table(4-10): correlation between the study group Number of abortion and Diabetic disease

Number of abortion	Diabetic dis ease		Total	P.Value
	Yes	No		
2-4	54.5%	33.3%	87.9%	0.041
more than 4	6.1%	6.1%	12.1%	
Total	60.6%	39.4%	100.0%	

Table (4-11): correlation between the study group Number of abortion and Hypertension **disease**

Number of abortion	Hypertension dis ease		Total	P.Value
	Yes	No		
2-4	48.5%	39.4%	87.9%	0.056
more than 4	6.1%	6.1%	12.1%	
Total	54.5%	45.5%	100.0%	

Chapter five

Discussion

conclusion

Recommendations

5.1 Discussion

Recurrent abortion associated with thrombosis in women in fertility age.

The mean of fibrinogen level in women with recurrent abortion was 237.76.

The result of these study obtain demonstrated that was significant increase in fibrinogen level in women with recurrent abortion compared to control (p value < 0.05).

The results showed that women with recurrent abortion and suffering from Diabetic mellitus was significant increased in fibrinogen level . (p value 0.041)

women with recurrent abortion.

also results showed that women with recurrent abortion and suffering from hypertension was significant increased in fibrinogen level . (p value 0.056)

The results showed that women with recurrent abortion and using contraceptive drug was significant increased in fibrinogen level .

Result of present study are similar to study done by Dr. Monika Gandhi in India. [10]

5.2 Conclusion

-Plasma fibrinogen level elevated in recurrent abortion women when compared with healthy women in control group.

-Plasma fibrinogen level in recurrent abortion women affected by Diabetic mellitus , Hypertension , contra captive drugs .

5.3 Recommendation

1- Hematological and biochemical test should be checked regularly in recurrent abortion.

2. More investigation should be done for women with recurrent abortion to decrease the chance of abortion.

Chapter *six*

References

Appendices

6.1.Referencees

1.Hellgren M. Semin Thromb Hemost. Hemostasis during normal pregnancy and puerperium. 2003.

2.Nakamura Y, et al. Biol Res Pregnancy Perinatol. Fibrinogen and its fraction during pregnancy and puerperium. 1984.

3.Arnout J, et al. Handb Exp Pharmacol. Haemostasis. 2006.

4. Lijnen HR, et al. Thromb Haemost..Fibrinolytic agents: mechanisms of activity and pharmacology. 1995

5. Taber, Clarence Wilbur;venesm ,Donald taberscyclopedic medical dictionary. F .A .Davis co .PP .1018- 23. (2009) ISBN 0-8036-1559-

6."Jere , yadava B , Davies , William (2014-07-01) ".Evidence

-based management of recurrent misscarriges , " – journal of Human Reproductive sciences ,7 (3) .

doi : 10.4103/0974- 1208. 142475.

7." Archired copy .Archived from the original on April 3,2015 Retrieved April 2,2015.

8. American college of obstetricians and Gynecologists (A COG) Repeated misscarrige FAQ .

9-Royad college of obstertricians and Gynaeco logists (RCOG) (April 2011) . the investigation and treatment of couples with recurrent first and second trimester miss carriage

(PDF) . GREEN Top Guideline No. 17.Royal college of obstetricians and Gynae cologists (Rcoa)

Archirved from the original (PDF) on 31/December 2013- Retrieved 2 / July / 2013.

10.The investigation and treatment of couples with Recurrent miscarriage. Guideline No 17 (PDF). Royal College of obstetricians and Gynaecologists . Archived from the original (PDF) o n 2013-12-31.

11. "Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy" (PDF). Obstet. Gynecol. *122 (5):* 1122–31. *Nov 2013.* doi*:*10.1097/01.AOG.0000437382.03963.88. PMID 24150027

12. Henderson, JT; Whitlock, EP; O'Connor, E; Senger, CA; Thompson, JH; Rowland, MG (May 20, 2014). "Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: a systematic evidence review for the U.S. Preventive Services Task Force.". Annals of Internal Medicine. 160 (10): 695–703. doi:10.7326/M13-2844. PMID 24711050.

13. GBD 2015 Mortality and Causes of Death, Collaborators. (8 October *2016).* "Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015". *Lancet. 388 (10053):* 1459*–1544.* doi*:*10.1016/s0140-6736(16)31012-1. PMC 5388903. PMID 27733281.

14. *Innes, KE; Byers, TE (1999*). "Preeclampsia and breast cancer risk". Epidemiology. 10: 722–32. doi*:*10.1097/00001648-199911000-00013. JSTOR 3703514. PMID 10535787.

15. Dr. Monika Gandhi : Altered Levels of Fibrinogen in Relation to the Path physiology of Recurrent Spontaneous Abortions in India

Appendix I
Questinnare
University ofshendi
Faculty of post Graduate studies
Estimation of fibrinogen level in the Recurrent Abortion

1/ Name

bortion : ……..

4/ Gestational age.

a/ first trimester . b/ second trimester.c/ third trimester.

5/ History of thrombosis :

Yes ( ) No ( )

6/ family history:

Yes ( ) No ( )

7/ contraceptive drugs :

Yes ( ) No ( )

8/ Diabetic dis ease :

Yes ( ) No ( )

9/ Hypertension dis ease:

Yes ( ) No ( )

10/ Drugs intake :

Yes ( ) No ( )

Result :

Fibrinogen level : ( )

Appendix II

إقـــرار بالموافقـــة

الاسم : ----------

العمر :---------- العنوان :----------

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة قياس مستوى الفبرينوجين عند السيدات ذوات الاجهاض المتكرر في مستشفى الملك نمر الجامعي

الباحثة:امال عوض الخضر قمر الدين

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 يحق لي بدون إبداء أسباب الانسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشراف :

د.حمزه احمد حسن

التوقيع : ---------- التاريخ :----------