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Evaluation of Protein C among Ladies with Placental complicated Pregnancy in Shendi Town

A thesis submitted in partial fulfillment of the degree of MSc in medical laboratory sciences (heamatology)

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الايـة

قال تعالي:

(لَقَدْ مَنَّ اللَّهُ عَلَى الْمُؤْمِنِينَ إِذْ بَعَثَ فِيهِمْ رَسُولاً مِنْ أَنْفُسِهِمْ يَتْلُوا عَلَيْهِمْ آيَاتِهِ وَيُزَكِّيهِمْ وَيُعَلِّمُهُمْ الْكِتَابَ وَالْحِكْمَةَ وَإِنْ كَانُوا مِنْ قَبْلُ لَفِي ضَلالٍ مُبِينٍ).

صدق الله العظيم

سورة آل عمران
الاية 164

Dedication

I have dedicated this research to dear **father** and

Mather

Who gave me all efforts and facilities to my study

from childhood until adulthood.

To all my **teachers**.

Who are teaching me give without take and

Patience without tedium

Also I would like to dedicate it to my brothers and sisters and all

my family for their continuous

Assistance and helps.

To my all friends

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All thanks to allah from the start to the end.

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I would like to thank my **family** and **friends** who

help me in this research

List of Abbreviation:-

Abbreviation	Term
AA	Arachidonic acid
APC	Activated protein c
ECs	Endothelial cells
EPCR	Endothelial protein c receptor
HMWK	High molecular weight kininogen
ICAMs	Intercellular adhesion molecules
NSAIDs	Non-steroidal anti-inflammatory drugs
PECAMs	Platelet endothelial cell adhesion molecules
PK	prekallikrein
RPL	Rrecurrent pregnancy loss
RRAS	Renin-angiotensin –aldosterone system
TF	Tissue factor
TM	thrombomodulin
VTE	Venous thromboembolism
vWF	Von willebrand factor

الخلاصة

هذه دراسة وصفية هدفت لمعرفت مستوي بروتين سي عند مضاعفات الحمل التي تنتج من وجود تخثرات في المشيمة .

اجريت هذه الدراسة لمجموعة من النساء بمدينة شندي بولاية نهر النيل خلال الفترة من فبراير الي اغسطس 2018 . تم جمع 33عينة في تراي صوديوم سيتريت و18عينة من نساء في مرحلة الحمل الطبيعي كعينة ضابطة ثم اخذت المعلومات من المرضي والمجموعة الضابطة ثم تم قياس مستوى بروتين سي باستخدام القياس اللوني .

الاجهاض المتكرر هو حاله شائعة يصيب حوالي70% من مضاعفات الحمل التي تنتج من وجود تخثرات في المشيمة .

كان الوسط الحسابي للعينات 72 وللمجموعة الضابطة 44, اظهرت النتائج وجود علاقه ذات دلالة بين انخفاض بروتين سي مع النساء اللاتي لديهن اجهاض متكرر (P=0.042). بالنسبه للتاريخ الايجابي العائلي للتخثر كانت له علاقه ذات دلالة احصائية مع الاجهاض المتكرر .

ونلاحظ وجود ارتباط ملحوظ بين نقص بروتين سي والولادة المبكرة;كما نتج من الدراسة ان هنالك علاقة بين التاريخ الايجابي العائلي للتخثروهوعامل خطر للإجهاض المتكرر.

Abstract

This is a descriptive study that aimed to identify protein C deficiency in women with placental complicated pregnancy. Our study was conducted in Shendi town during a period from February to august-2018.

33 citrated blood samples and 18 healthy pregnant women as control group were collected.

Full history was taken from patients and controls. Blood investigations were done for protein C level by using chromogenic method.

Recurrent pregnancy loss (RPL) is a common disorder that affects around 70% of placental complicated pregnancy.

The mean of the test sample was 44.0 and the mean of the control sample was 72.0. Which showed a significant relation of low protein c with recurrent miscarriage (P =0.042). Positive family history for thrombosis was significantly associated with recurrent abortions.

There is a significant association of protein c deficiency with placental complicated pregnancy especially in the pre-term labor; the positive family history of thrombosis is considered risk factor for recurrent miscarriage.

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Chapter one

1.1. Introduction

Abortion is the ending of pregnancy by removing a fetus or embryo before it can survive outside the uterus, an abortion that occurs spontaneously is also known as a miscarriage, an abortion may be caused purposely and is then called an induced abortion, or less frequently, "induced miscarriage", The word abortion is often used to mean only induced abortions, similar procedure after the fetus could potentially survive outside the womb is known as a "late termination of pregnancy [(1)].

Recurrent abortion is pregnancy loss two or three consecutive abortion in the first or early second trimester, many factor can be involved in recurrent miscarriages including gentic chromosomal, age, hormones metabolic abnormalities, immunological problems, clottingdisorder, and in many cases it can remain unexplained, the drug mifepristone in combination with prostaglandin appears to be as safe and effective as surgery during the first and second trimester of pregnancy [(2)].

Birth control, such as the pill or intrauterine devices, can be used immediately following abortion When performed legally and safely, induced abortions do not increase the risk of long-term mental or physical problems[(3)].

The World Health Organization recommends safe and legal abortions be availabl to all women, historically abortions have been done using herbal medicines, sharp tools, with force, or through other traditional methods [(4)].

Abortion laws and cultural or religious views of abortions are different around the world. In some areas abortion is legal only in specific cases such as rape, problems with the fetus, poverty, risk to a woman's health, or incest [(5)].

In many places there is much debate over the moral, ethical, and legal issues of abortion,Those who favor the legality of abortion often hold that a woman has a right to make decisions about her own body, The most common cause of spontaneous abortion during the first trimester is chromosomal abnormalities of the embryo or fetus, accounting for at least 50% of sampled early pregnancy losses, Other causes include vascular disease (such as lupus), diabetes, other hormonal problems, infection, and abnormalities of the uterus[3].

Advancing maternal age and a women's history of previous spontaneous abortions are the two leading factors associated with a greater risk of spontaneous abortion, A spontaneous abortion can also be caused by accidental trauma intentional trauma or stress to cause miscarriage is considered induced abortion or feticide[1].

Accumaltion of high level of homocysteine that subsequently promotes thrombophilia ,and plasminogen activator inhibitor -1(PAI-1) is an enzyme involved in the process of breaking down blood clots ,elevated level of this enzyme are associated with excessive clotting and pregnancy complication such recurrent pregnancy loss(RPL) pre-eclampsia, fetal growth retardation and fetal death[4].

Protein c a potent anticoagulant that play major role in the body's ability to stop or regulate blood clotting, Protein c is a vitamin k-dependent serine protease of MW62KDa and key component of the natural anticoagulant pathway, Protein c is converted by thrombin in to its active form (APC) which with it is cofactor protein s, degrades factor Va and factor VIIIa[6].

1.2. Objective:-

1.2.1. Genaral objective:-

To evaluation of protein c level among ladies with placental completed pregnancy.

1.2.2. Specific objective:-

- To compare protein c level with types of placental completed pregnancy.
- To compare protein c level in our study group with age
- To compare protein c level in our study group with other diseases
- To compare protein c level with number of abortion in placental complicated ladies.

1.3. Rationale:-

Placental completed pregnancy is most commonly complications that occur during pregnancy, may be due to many causes. Thrombophilia is one of the causes and protein c is an important in protein c system

Defects in protein c system may be qualitative, so we want use protein as a laboratory marker in our study group for thrombophilia.

Chapter tow

2. LITRETURE REVIEW

2.1- Hemostasis:-

Hemostasis is the physiological process that helps to maintain blood in the fluid state and prevent the escape of blood from damaged blood vessels through clot formation, many coagulation proteins are involved in reactions that precipitate the hemostatic process, deficiencies in any of the coagulation protein may lead to bleeding.[7-8]

Laboratory tests can monitor the hemostatic status of individual including the prothrombin time, which monitors the extrinsic pathways and the partial thromboplastin time, which monitors the intrinsic pathways [7-8].

Hemostasis is derived from a Greek word, which means stoppage of blood flow, The process is a combination of cellular and biochemical events that function together to keep blood in the liquid state within the veins and arteries and prevent blood loss following injury through the formation of a blood clot.[7-8]

It consists of a complex regulated system which is dependent on a delicate balance among several systems, The systems involved in thehemostatic process include the vascular system, coagulation system, fibrinolytic system, platelets, kinin system, serine protease inhibitors, and the complement system. [9-10]

The systems work together when the blood vessel endothelial liningis disrupted by mechanical trauma, physical agents, or chemical trauma to produce clots, the clots stop bleeding and are eventually dissolved through the fibrinolytic process, as a result, there is a delicate balance between the production and dissolution of clot during the hemostatic process, a disruption of this balance may precipitate

thrombosis or hemorrhage as a result of hyper coagulation or hypo coagulation, respectively .[7,10]

Hemostasis is categorized as either a primary or secondary process, Primary hemostasis involves the response of the vascular system and platelets to vessel injury [10].

It takes place when there are injuries to small vessels during which the affected vessels contract to seal off the wound and platelets are mobilized, aggregate, and adhere to components of thesubendothelium of the vasculature Platelet adhesion requires the presence of various factors such as von Willebrand factor *(vWF)* and platelet receptors IIb/IIIa and Ib/IX), Additional platelets are attracted to the site of injury by the release of platelet granular contents, such as adenosine diphosphate *(ADP)*, The platelet plug is stabilized by interaction with fibrinogen, Thus a defect in platelet function or von Willebrand's disease*(VWD*) may result in debilitating and sometimes fatal hemorrhage.[7]

Secondary hemostasis involves the response of the coagulation system to vessel injury.[10]

2.1.1-The Vascular System

The vascular system has procoagulant, anticoagulant, and fibrinolytic properties and is made up of blood vesselsthe vascular system. The vascular system has procoagulant, anticoagulant, and fibrinolytic properties and is made up of blood vessels, The innermost lining of the blood vessels is made up of endothelial cells (*ECs*) which form a smooth, unbroken surface that promotes the fluid passage of blood and prevents turbulence that may trigger activation of platelets and

plasmaproteins, The *ECs* are supported by a collagen-rich basement membrane and surrounding layers of connective tissues.[7]

A breakdown in the vascular system is rapidly repaired to maintain blood flow and the integrity of the vasculature, The vascular system prevents bleeding through vessel contraction, diversion of blood flow from damaged vessels, initiation of contact activation of platelets with aggregation, and contact activation of the coagulation system[8].Platelets are activated by collagen located in the basement membrane, the ECs secrete vWF, which is needed for platelet adhesion to exposed subendothelial collagen in the arterioles, The ECs produce a variety of other adhesion molecules, which include P-selectin, intercellular adhesion molecules *(ICAMs),* and platelet endothelial cell adhesion molecules *(PECAMs)*, The smooth muscle and fibroblast release tissue factor *(TF)* which activates factor VII *(FVII).*[7]

The vascular system provides potent anticoagulant properties, which prevents the initiation and propagation of the coagulation process, Coagulation is inhibited through the expression of thrombomodulin *(TM)*which promotes activation of protein C and heparan sulfate *(HS),* which activates antithrombin III *(AT-III)* to accelerate thrombin inhibition. Endothelial cells also release tissue factor pathway inhibitor *(TFPI),* which blocks activated factor VIIa*(FVIIa)*-TF/factor Xa*(FXa)* complex and annexin V, which prevents binding of coagulation factors [11].

2.1.2-Coagulation System

The coagulation system is where coagulation factors interact to form a fibrin clot. The coagulation system is involved in the conversion of soluble fibrinogen, a major component of the acute inflammatory exudates into fibrinthe fibrin clot reinforces the platelet plug formed during primary hemostasis Various protein factors present in the inactive state in the blood participate in the coagulation

system, The protein factors are designated by Roman numerals according to their sequence of discovery and not by their point of interaction in the coagulation cascade[8].

Some of the coagulation factors such as fibrinogen and prothrombin are referred to by their common names, whereas others such as factors VIII and XI are referred to by their Roman numeral nomenclatures, Activation of a factor is indicated by the addition of low case "a next to the Roman numeral in the coagulation cascade such as VIIa, Xa, XIIa[8].

Some of the common names were derived from the original patients in whom symptoms leading to the determination of the factor deficiency were found, Examples are the Christmas factor and Hageman factor,the coagulation factors may be categorized into substrates, cofactors, and enzymes, Fibrinogen is the main substrate. The cofactors accelerate the activities of the enzymes, which are involved in the coagulation cascade, Examples of cofactors include tissue factor, factor V factor VIII, and Fitzgerald factor, With the exception of factor XIII, all the enzymes are serine proteases when activated[8].

The coagulation factors may also be categorized into 3 groups on the basis of their physical properties. These of their physical properties, These groups are the contact proteins comprising of factors XII, XI, prekallikrein*(PK),* and high molecular weight kininogen *(HMWK),*the prothrombin proteins comprising of factors II, VII, IX, and X and the fibrinogen or thrombin sensitive proteins comprising of factors I, V, VIII and XIII.[8]

2.1.3-Fibrinolytic System

Fibrinolysis is the physiological process that removes insoluble fibrin

clots through enzymatic digestion of the cross-linked fibrin polymers plasmin is responsible for the lysis of fibrin into fibrin degradation products, which mayhave local effects on vascular permeability, Plasmin digests fibrin and fibrinogen through hydrolysis to produce smaller fragments,the gradual process occurs at the same time that healing is occurring،and eventually cells of the mononuclear phagocytic system phagocytize the particulate products of the hydrolytic digestion.[12]

Recent evidence suggest that therenin-angiotensin-aldosterone system

RAAS) may participate in the regulation of fibrinolytic function[13].(

Angiotensin II *(Ang II)* is the primary candidate to mediate this interrelationship, since this peptide is capable of stimulating plasminogen activator inhibitor-1 *(PAI-1)* in vitro and in vivo It has been suggested that aldosterone may also modulate fibrinolysis, possiblyby interacting with *Ang II*.[13]

Fibrinolysis is controlled by the plasminogen activator system,the proteolytic activity of this system is mediated by plasmin, which is generated from plasminogen by 1 of 2 plasminogen activators, Inactive plasminogen circulates in plasma until such a time that an injury occurs, Then, plasminogen is activated by means of a number of proteolytic enzymes known as plasminogen activators, These activators are present at various sites such as the vascular endothelium. Some of the activators include tissue-type plasminogen activator urokinase, streptokinase, and acyl-plasminogen streptokinase activator complex Inhibitors of

fibrinolysis include α2- plasmin inhibitor, tissue plasminogen activator inhibitor, and plasminogen activator inhibitor-1 *(PAI-1).*[12]

Individuals with reduced fibrinolytic activity are at increased risk for ischemic cardiovascular events and reduced fibrinolysis may underlie some of the pathological consequences of reduced nitric oxide *(NO)* availability.[14]

2.1.4-Platelets

Platelets are anuclear fragments derived from the bone marrow megakaryocytes,they have a complex internal structure, which reflects their hemostatic functions, The 2 major intracellular granules present in the platelets are the α-granules and the dense bodies, The α-granules contain platelet thrombospondin, fibrinogen,fibronectin, platelet factor 4, *vWF* platelet derived growth factor, β-thromboglobulin, and coagulation factors V and VIII, The dense granules contain *ADP,*adenosine triphosphate *(ATP)*and serotonin, When stimulated platelets release both the α-granules and the dense bodies through the open canalicular system.[15]

When platelets aggregate, they expend their stored energy sources, lose their membrane integrity, and form an unstructured mass called a syncytium, In addition to theplug formation, platelet aggregates release micro-platelet membrane particles rich in phospholipids and various coagulation proteins which provide localizedenvironment that support plasma coagulation. [7]

Platelets and ECs have biochemical pathways involving the metabolism of arachidonic acid *(AA),* which is releasedfrom membrane phospholipids by phospholipase A_2 Subsequently, cyclooxygenase converts AA to cyclic

endoperoxides, The end peroxides are then converted by thromboxane synthetase to thromboxane $A_{2,}$

Thromboxane A_2 is a potent agonist that induces platelet aggregation, Endothelial cells also contain AA and preferentially convert cyclic endoperoxides to prostacyclin which is a potent inhibitor of platelet aggregation.[16]

During primary hemostasis platelets interact with elements of the damaged vessel wall leading to the initial formation of the platelet plug, The platelet/injured vessel wall interaction involves a series of events that include platelet adhesion to components of the subendothelium, activation, shape change release of platelet granules, formation of fibrin stabilized fibrin platelet aggregates and clot retraction, In this process, theactivation of platelets with exposure of negatively charged phospholipids facilitates the assembly of coagulation factors on the activated platelet membrane, leading to the generation of thrombin and subsequent fibrin deposition[16]

2.1.4.1-Platelet Function

In some disorders, platelets may be normal in number, yet hemostatic plugs do not form normally, and therefore bleeding time will be long. Platelet dysfunction may stem from an intrinsic platelet defect or from an extrinsic factor that alters the function of otherwise normal platelets, defects may be hereditary or acquired, Tests of coagulation phase ofhemostasis such as activated partial thromboplastin time *(APTT)* and prothrombin time *(PT)* are normal in most circumstances but not all.[17]

When a patient's childhood history reveals easy bruising and bleeding after tooth extraction, tonsillectomy, or other surgical procedures, the finding of normal platelet count but a prolonged bleeding time suggests a hereditary disorder

affecting platelet function,the cause is either VWD (which is the most common cause of hereditary hemorrhagic disease) or a hereditary intrinsic platelet disorder whatever the cause of platelet dysfunction, drugs that may further impair platelet function should be avoided such as aspirin and other non-steroidal antiinflammatory drugs *(NSAIDs).*[18]

2.1.4.2-Thrombocytopenia

Thrombocytopenia may be the consequence of failed platelet production splenic sequestration of platelets increased platelet destruction, or dilution of platelets, Regardless of the cause, severe thrombocytopenia usually results in a typical pattern of bleeding such as multiple petechiae in the skin, scattered small ecchymoses at the sites of minor trauma mucosal bleeding, and excessive bleeding after surgery, Heavy gastrointestinal *(GI)* bleeding and bleeding into the central nervous system *(CNS)* may be life threatening, However, thrombocytopenia does not cause massive bleeding into tissues which is characteristic of bleeding secondary to coagulation disorders, Adultidiopathic thrombocytopenic puerperal*(ITP)* usually results from development of an antibody directed against a structuralplatelet antigen, In childhood ITP, viral antigen is thought to trigger synthesis of an antibody that may react with viral antigen associated with the platelet surface Platelet count is usually maintained in arange of 150,000 to 400,000/µL and counts of 100,000 to 150,000/µL are regarded as borderline for thrombocytopenia while counts that are less than100,000 µL are considered abnormal.[18]

Symptoms do not usually develop until the platelet count is less than50,000 at which time easy bruising may be evident and petechiae may appear on the skin, Surgeons usually do not perform routine surgery on patients whose platelet counts

are <50,000/µL because the risk of prolonged bleeding after dental procedures or childbirth will be increased, When the platelet count reaches 10,000 to20,000 µL, the risk of spontaneous and serious bleeding rises.[18]

This includes strokes, GI bleeding, and prolonged nose bleeds, When these conditions develop platelet transfusions are often used to stop the bleeding, Unfortunately, transfused platelets are short-lived and cannot be used indefinitely as antibodies may develop against the platelets,Platelet transfusions are most appropriate when the cause of thrombocytopenia is a temporary lack of production such as after intensivechemotherapy.[19]

2.2-Kinin System

The kinins are peptides of 9 to 11 amino acids of which the most important vascular permeability factor is bradykinin*(BK),*The kinin system is activated bycoagulation factor XII,Bradykinin is alsoa chemical mediator of pain, which is a cardinal feature of acute inflammation Therefore, bradykinin is capable of reproducing many of the characteristics of an inflammatory state, such as changes in local blood pressure, edema, and pain resulting in vasodilation and increased micro vessel permeability.[19]

Human HMWK, a single-chain protein with a molecular weight of 120,000 daltons, is cleaved by human urinary kallikrein*(HUK)* to release kinin from within a disulfide loop and form a 2-chain protein that retains all the procoagulant activity of the native molecule.[20]

It is a multifunctional protein, a parent protein ofbradykinin, and serves as a cofactor for FXI and PK assembly on biologic membranes, the docking of HMWK

to platelet and EC membranes requires it's binding by regions on both its heavy and light chains. [15]

2.3-Serine Protease Inhibitors

It is becoming increasingly clear that coagulation augments inflammation and that anticoagulants particularly natural anticoagulants, can limit the coagulation induced increases in the inflammatory response, The latter control mechanisms appear to involve not only the inhibition of the coagulation proteases but interactions with the cells that either generate anti-inflammatory substances or limit cell activation Recent studies have demonstrated a variety of mechanisms by which coagulation, particularly the generation of thrombin, FXa, and the TF/FVIIa complex, can augment acute inflammatory responses. [15]

Many of these responses are due to the activation of 1 or more of the protease activated receptors, Activation of these receptors on endothelium can lead to the expression of adhesion molecules and platelet activating factor thereby facilitating leukocyte activation.[15]

Therefore, anticoagulants that inhibit any of these factors wouldbe expected to dampen the inflammatory response, The 3 major natural anticoagulant mechanisms seem to exert a further inhibition of these processes by impacting cellular responses Antithrombin has been shown in vitro to increase prostacyclin responses and activated protein C has been shown to inhibit a variety of cellular responses including endotoxin induced calcium fluxes in monocytes, a key step in thegeneration of the inflammatory response.[15]

Serine proteases (such as thrombinFXa, elastase, trypsin) are implicated in many clinical disorders such as emphysema, arthritis, and cardiovascular diseases,

Naturally occurring serine protease inhibitors (such as antithrombin) which are involved in thrombin inhibition regulate these enzymes in normal physiological conditions, Serine protease inhibitors attach to various enzymes and inactivate them, Antithrombin was the first of the plasma coagulation regulatory protein to be identified and the first to be assayed routinely in the clinical laboratory Other members of the serine protease inhibitor family are heparin cofactor II, α_1-antitrypsin and α_2- macroglobulin. [7]

More than 90% of the antithrombin activity of normal plasma is derived from AT-III.[8]

Antithrombin-III has been shown to exert marked anti-inflammatory properties and proven to be efficacious in experimental models ofsepsis , septic shock, and disseminated intravascular coagulation *(DIC).*[21]

Antithrombin-III also inhibits factors XIIa, XiaIXa, protein S, protein C, plasmin, and,kallikrein. [22]

2.4-Complement System

Complement has an important role in inflammation and in the normal function of the immune system, Activated complement fragments have the capacity to bind and damage self-tissues, On their surfaces, cells express regulators of complement activation that protect the cell from the deleterious effects of cell-bound complement fragments, Abnormalities in these regulators may participate in the pathogenesis of autoimmune diseases and inflammatory disorders.[22]

The complement system consists of approximately 22 serum proteins, which together with antibodies and clotting factors perform an essential role as mediators of both immune and allergic reactions, Complement

protein are involved in reactions which lead to the lysis of cells, This is due to the production of the membrane attack complex *(MAC),*The activation of complement may follow the classical pathway or the alternative pathway Complement is activated by plasmin through the cleavage of C3 into C3a and C3b, C3a is an anaphylotoxin that causes increased vascular permeability via degranulation of mast cells leading to the release of histamine, C3b is anopsonin that causes immune adherence.[8]

During reperfusion, complement may be activated by exposure to intracellular components such as mitochondrial membranes or intermediate filaments, In order to protect themselves from the complement attack, cells express several regulatory molecules including the terminal complex regulator CD59 that inhibitseassembly of the large *MACs* by inhibiting the insertion of additional C9 molecules into the C5b-9 complex.[23]

2.5- Activated Partial Thromboplastin Time

Activated partial thromboplastin time was developed from the observation that hemophiliacs have prolonged clotting time,However, when tissue thromboplastin is added, the plasma clots as normal plasma does.[10]

Thromboplastins are lipoproteins, They may be classified as either complete or partial, which means that they consist of only phospholipids Addition of negatively charged activators to the system results in significantly shorter clotting times, It is the most widely used test for screening for factor deficiencies in the intrinsic and common pathways. The *APTT* reflects the activity of *PK,HMWK,* and factors XII, XI, VIII X, V, II, and I, The APTT may be prolonged due to either a factor decrease or the presence of circulating anticoagulants the normal *APTT* is less than 35 seconds.[12]

2.6-Prothrombin Time

Prothrombin time is the routine test used to screen for deficiencies of factors,I, II, V,VII, and X, It is the test of choice for monitoring anticoagulant therapy by vitamin K antagonists, Three of the 5 factors measured by *PT* (II, VII, X) are sensitive to and depressed by these anticoagulants. [10]

Prothrombin time is widely utilized for evaluation of diseases with single or multiple coagulation factors disorders, such as severe liver dysfunction and DIC, However, its standardization of reagents and method is not established yet for universal purpose except International Normalized Ratio*(INR)* for control of oral anticoagulant therapy*(OAT).*[24]

Oral anticoagulants have been widely employed to decrease thrombotic risk by reducing the levels of vitamin K-dependent clotting factors The use of oral anticoagulants also decreases the levels of natural anticoagulants such as protein C and protein S The PT test investigates the production of thrombin and the formation of fibrin via the extrinsic and common pathway In the presence of calcium ions, tissue thromboplastin complexes with and activates FVII, This provides surfaces for the attachment and activation of factors X, V, and II, Normal values for PT range from 10 to 13 seconds.[12]

Values for the INR are preferable to the PT because different thromboplastin reagents have different sensitivities to warfarin-induced changes in levels of clotting factors, The INR corrects for most but not all of the reagent differences expressed as international sensitivity index *(ISI)*, which is a correction factor assigned by the manufacturer of thethromboplastin reagent,the problems associated with the INR are that the concept and reasons for use are poorly understood and the value is generally misused.[12]

2.7- Fibrinogen and FibrinogenDegradation Products

Fibrinogen levels are useful in detecting deficiencies of fibrinogen andalterations in the conversion of fibrinogen to fibrin,The normal value for fibrinogen ranges from 200 to 400 mg/dL this may be decreased in liver disease or the consumption of fibrinogen due to accelerated intravascular coagulation.[25]

An increased concentration of fibrinogen degradation products *(FDPs)* is commonly used in conjunction with other hemostatic test abnormalities to identify patients with *DIC.*[26]

2.8- Investigation of Disseminated Intravascular Coagulation

A variety of tests are used to detect *DIC,* The most sensitive tests are markers of endogenous thrombin generation in the practical management of patients cruder measures of *DIC* are often used Some of these tests include screeningtests such as *PT* and *APTT,* which may be prolonged reflecting consumption of many coagulation proteins

Plasma concentrations of coagulation proteins consumed in *DIC,* such as fibrinogen, FV and FVIII, all show decreases in concentration.[26]Fibrinogen/*FDPs* or D-dimers (a fragment from fibrin alone) are both increased in concentration and fibrin monomer may be present,the thrombin clotting time may be prolonged reflecting hypofibrinogenemia and the presence of *FDPs.*[26]

2.2- Hemostasis in normal pregnancy:-

Thrombosis prevention and management has become the major focus for hematologists with an interest in women's health, normal pregnancy is associated with increasing hypercoagulability as gestation progresses in addition, the pregnant woman experiences increasing lower limb venous stasis due to compression of the venous flow by the gravid uterus and inevitably suffers endothelial damage due to the vascular trauma associated with delivery, particularly operative delivery.(27)

Thrombophilia's may play a role in the etiology of not only venous thromboembolism *(VTE)* but a range of other vascular complications of pregnancy, and much debate has centered on the possibility of intervention to reduce the burden of these adverse pregnancyoutcomes.(27)

Advances in artificial reproductive technology andin the management of women with serious medical disorders, including valvular heart disease, have meant that increasingly women who would have been denied pregnancy in the past now have the opportunity to have a child of their own, but these women inevitably need specialist care, often involving a hematologist.(27)

The risk of VTE associated with the use of female hormones for contraception or for estrogen replacement is now widely recognized, but much work remains to identify products that are as safe and effective

as possible.(27)

Normal pregnancy is associated with major changes in all aspects of hemostasis, increasing concentrationsof most clotting factors, including fibrinogen and factors VII, VIII, IX, X and XII, decreasing levels of some of the natural anticoagulants, such as protein S, increased resistance to activated protein C, and reducing

fibrinolytic activity , As a result, as pregnancy progresses, and during the puerperium, theoverall hemostatic balance is shifted toward hypercoagulability. [27]

2.3- Thrombophilia in Recurrent Pregnancy Loss:-

Recurrent spontaneous miscarriage affects 1–3 % of women of reproductive age.[28]at least, one-third recurrent miscarriage is unexplained, and the rest have a persistent underlying cause for their pregnancy losses Identifiable causes can be found in only about 30–50 % of these women. [29]

RPL (recurrent pregnancy loss) could also include pregnancy losses up to gestational week 28, The most common causes of recurrent miscarriage are uterine anomalies, endocrine disorders, parental chromosomal abnormalities, and immunological factors, including those associated with the *APS* (antiphospholipid antibody syndrome) and infections, Even after a thorough evaluation, however, the potential cause remains unexplained in about one third of cases.[30-31]

A number of studies have reported an increased risk of RPL in women with inherited thrombophilia.[32-33]

In the European prospective cohort study on thrombophilia *(EPCOT),* a significant association between thrombophilia and miscarriage was reported.[34]

The term thrombophilia is generally used to describe a laboratory abnormality (most often in the coagulation system) that increases the tendency to venous thromboembolism *(VTE)* in any site or pulmonary embolism Thrombophilic abnormalities can be acquired or hereditary, Hereditary thrombophilia comprise a number of conditions, such as antithrombin *(AT)* III deficiency, protein S *(PS)* and protein C *(PC)* deficiencies, factor V Leiden, prothrombin 20210A mutation,

elevated factor VIII level, and mutation of gene encoding the enzymemethylenetetrahydrofolatereductase*(MTHFR)*the evidence for pregnancy loss having a thrombophilic mechanism rests on three pillars increased prevalence of thrombophilia in *RPL*, a higher incidence ofpregnancy loss in the presence of thrombophilia and the demonstration of thrombosis in decidual vessels, however, it is still uncertain if heritable thrombophilia causes recurrent miscarriage, and routine testing in women with recurrent miscarriage is preferable but not currently advocated.[35]

The principal acquired thrombophilic states include APS and hyperhomocysteinemia.[36]

Pregnancy is an acquired hypercoagulable state and women with a prior tendency to thrombosis may develop clinical symptoms of placental vascular complications such as preeclampsia, intrauterine growth restriction, and fetal death for unknown causes, that impact the maternal-fetal morbidity and mortality.[37-38]

Recurrent pregnancy loss is an important obstetric complication with a prevalence of 1–5%.[39]

Inherited thrombophilia has been postulated as a cause of recurrent pregnancy loss, although the association between inherited thrombophilia and recurrent pregnancy loss has not been conclusively established. Some studies have demonstrated an association between recurrent pregnancy loss and prothrombotic states rendered by some genetic single nucleotide polymorphisms *(SNPs)*, such as factor V Leiden G1691A *(FV Leiden)*,

prothrombin G20210A *(FII G20210A)*, and

methylenetetrahy drofolatereductase C677T *(MTHFR C677T),* and activated protein C resistance *(APC resistance).*[40-41]In addition, a retrospective cohort study showed that women with deficiencies of antithrombin *(AT),* protein C *(PC),* or protein S *(PS)* have an eightfold increased relative risk of thrombosis during pregnancy compared to controls.[42]

Most of the studies on thrombophilia and recurrent pregnancy loss have been conducted in Caucasian populations.[43-44]

Therefore, the association between these thrombophilias and recurrent pregnancy loss is almost unknown in triethnic populations such as the Colombian population whose genetic mixture is approximately 70% Caucasian, 15% Amerindian, and 15% African.[45-46]

Recurrent pregnancy loss or miscarriage can be defined as the loss of three or more successive pregnancies before viability and includes all pregnancy losses from the time of conception until 24 weeks of gestation.[47-48]

Pregnancy loss is divided into biochemical and clinical loss, the biochemical loss is a transient positive pregnancy test without ultrasonic visualization of the pregnancy.[49]

The term clinical miscarriage is used when ultrasound examination or histological evidence has confirmed that an intrauterine pregnancy has existed. Clinical miscarriages can be subdivided into early clinical pregnancy losses (which is most common) that occur before the twelfth week of gestation, and late clinical pregnancy losses (which constitute small proportion of pregnancy losses) that occur in the twelfth week to twenty first week of gestation.[50-51]

Despite a wide range of investigations the cause of recurrent miscarriage remains unknown (idiopathic) in more than 50% of cases, [52-53]but several hypotheses have been proposed.[54]

including the following genetic disorder, uterine anatomic malformation, immunological risk factors, infection, endocrine disorders and thrombophilia, Thrombophilia is a term which describes the increased tendency of excessive blood clotting due to inherited or acquired causes, It may occur during pregnancy, where there is an increase in most clotting factors and decreased levels of anticoagulant factors with reduced fibrinolytic activity. This can result in placental insufficiency and abortion.[55-56]

Factors associated with thrombophilia include Factor V Leiden *(FVL)* mutation associated activated Protein C Resistance *(APCR)*, prothrombin G20210A gene mutation, anti-thrombin III deficiency, protein S deficiency, protein C deficiency and hyperhomocysteinaemia (methylene-tetrahydrofolatereductase mutation, *MTHFR)* stasis and endothelial cell dysfunction.[57]

2.4- Protein C:-

Protein C*(PC)* is a vitamin K-dependent glycoprotein activated bythrombin-thrombomodulin complex on the surface of endothelial cells.[58] Protein C is a precursor of the serine protease, activated protein C (APC). [59]

In the presence of protein S which is a cofactor for APCphospholipidsand calcium, APC inactivates membrane boundFVa and FVIIIa so results in attenuation of thrombin generation which leads to inhibit clot formation.[60] Protein C deficiency is generally subdivided into two types: type I (quantitative deficiency) decreased levels of protein C, and type II (qualitative deficiency) decreased

functional activity of protein C, Most patients with PC deficiency have type I deficiency, while Type II deficiency is observed in 10- 15% of the cases.[61-62]

Protein Cdeficiency is inherited as autosomal dominant disorders and, in most cases, derived from heterozygous mutations.[63] Acquired PC deficiency can develop with vitamin K deficiency, liver disease, treatment with vitamin K antagonists, severe and chronic inflammation, autoimmune syndromes, nephritic syndrome, ordisseminated intravascular coagulation *(DIC)*.[64]

Arecentstudy stated anobservation of a higher rate of late fetal loss in patients with protein C deficiency compared to non-deficient patients.[65]

There are two main types of assays, activity assay (qualitative) which is either clotting time-based assay or chromogenic by spectrophotometer.[66]

Quantitative assay for protein C antigen which are immunoassays generally done by using *ELISA*, it is considered to measure the quantity of protein C irrespective of its function.[64]

Genetic testing by DNA sequencing is indicated if the results of functional and antigenic assays do not approve the diagnosis clearly.[67]

2.4.1-Components of the Protein C Pathway

The protein C pathway comprises multiple proteins involved at different points of the pathway, eg, those affecting the protein C activation, those modulating the proteolytic activity of APC, and those that inhibit the activity of APC ,Thus, the activation of protein C is efficiently catalyzed on the endothelial cell surface by thrombin *(T)* bound to thrombomodulin *(TM)*.[68]

TM functions as a cofactor to thrombin, the high affinity binding of thrombin to TM resulting in >1000-fold amplification of the rate of protein C activation, The endothelial protein C receptor *(EPCR)*, which binds protein C, provides a further ≈20-fold stimulation of the T-TM-mediated activation of protein C in vivo.[69]

The anticoagulant activity of APC is enhanced by 2 cofactors, the vitamin K-dependent protein S and the intact form of FV, protein S being sufficient for inactivation of FVa, whereas regulation of FVIIIa in the tenase complex requires the synergistic *APC* cofactor activities of both protein S and *FV*.[70]

Protein S in human plasma is not only an important component of the protein C pathway but also takes part in the regulation of the complement system as it forms a high-affinity complex with C4b-binding protein *(C4BP)*, a regulator of the classical complement pathway. In human plasma, 30% to 40% of the protein S circulates as free protein, the remaining being bound to *C4BP*, Only free protein S has the ability to function as a cofactor to *APC*.[71-72]

Although protease inhibitors such as the protein C inhibitor, α1-antitrypsin, and α2-macroglobulin inhibit *APC*, the half-life of *APC* in the circulation is relatively long (≈20 minutes).[71]

2.4.2 Activation of Protein C on the Surface of Endothelial Cells Protein C is composed of a γ-carboxyglutamic acid residue *(Gla)*-rich domain, 2 epidermal growth factor *(EGF)*-like domains, a short activation peptide, and the serine protease domain *(SP)*.[71]

The *Gla* residues are formed as the result of a vitamin K-dependent post-translational carboxylation of glutamic acid residues in the *Gla* domain, The *Gla* residues bind calcium and are important for the proper folding of the domain.[73]

The *Gla*domain of protein C/APC binds negatively charged phospholipid membranes and also *EPCR*, both interactions being important for the physiological function of protein C.[68-69]

During the activation of protein C by the *T-TM-EPCR* complex, the activation peptide of protein C is released and the serine protease domain is converted to its active conformation All vascular endothelium contain *TM*, the concentration being particularly high in the capillaries where the ratio between the endothelial cell surface and blood volume reaches its peak, The high concentration of *TM* in the capillary circulation ensures that thrombin binds to TM (Kd ≈0.5 nmol) and activates protein C.[68-74-75]

Chapter three

Material and Methodology

3.1 Study design:-

Across sectional descriptive study, conducted in shendi town in the period between February to august-2018.

3.2 Study area:-

The study was conducted in Shendi town which is located 172km north to capital Khartoum , southern part of river nile state, and convering area about 30km.

There are several general centers for different services and purposes; also there is Shendi University with various faculties like faculty of medicine and health sciences

Shendi has three big hospital and military hospital all of them have different department which provide good health services from the population.

3.3 Study Population and Sampling:-

33 of venous blood samples were obtained from placental complicated pregnancy, such as miscarriage, pre-term labor, still birth; which diagnosed clinically by the obstetrition and 18 venous blood samples were obtained from normal pregnancy as control group, the sample we take in tri sodium citrate with ratio 1:9[(appendix 2)].

3.4 material and instrument:-

Cotton.-

Syring.-

Centerfuge.-

Automatic pipettes.-

Spectrophotometer.-

Tri sodium citrate .-

Disttilled water.-

20% acetic acid.-

Substrate PCa-2.-

Protac.-

Protein c buffer.-

3 Standard PC (1,2,3). -

3.5 Principle of protein c estimation:-

Protein c $\xrightarrow{\text{(protac)}}$ protein ca

Pad-pro-Arg-PNA AcOH $\xrightarrow{\text{(protein ca)}}$ pad-pro-Arg-OH AcOH + P-nitroaniline. (appendix 2)

3.6 Procedure of protein c estimation:-

Endpoint method

Dilute the substrate PCa-2 1:5with PC buffer(1 part substrate +4 parts buffer).

Blank: 0.20 ml from acetic acid 20% mix and add 0.60ml pc buffer+37c mix and add 0.05 from sample.

Sample: 0.05ml from sample and add 0.10 ml from protac mix and incubate for exactly 5 minute +37c , and add 0.50 ml from substrate buffer mix(1+4) 37c mix and incubate for exactly 3-5 minute at 37c,and add 0.20 ml from acetic acid 20%.

Read the absorbance of blank against filter 405nm.(appendix 2)

3.7 Data analysis:-

The data was analyzed by with spss 20 (statistical package of social science), T test was used for calculating, degree of variation , p.value <0.05 considered significant variation.

Chapter four

The Results

4-1 Mean of protein C between case and control:-

This study was conducted to know the level of protein c in ladies with placental complicated pregnancy. Our results show there was a significant variation between the test group and the control (P value 0.042) as shown in table no 4-1

Table (4-1):- Show result of analysis.

	Group	Number	Mean	Std deviation	P.value
Protein C	case	33	44.0	36.5	0.042
	control	18	72.0	59.6	

4–2 placental complicated pregnancy:-

When we look for the types of placental complicated pregnancy we found still birth, pre-term labor (PTL) and miscarriage, have a 15.8%, 10.5% and 60.5% respectively, and when we compared them with protein c level, we found strong association with pre-term labor (PTL) with (p value <0.05) and insignificant association with miscarriage and still birth with p value (0.419) as shown in table no 4-2

Table 4-2: show types of placental complicated pregnancy

Types	number	Mean	P value
Miscarriage	23	49.3	0.161
PTL	4	36.3	0.037
Still birth	6	50.5	0.419

4–3 Mean of protein C among age group:-

In our study group, the age from 36-45 years, which consist of 25% that have a significant value with low protein c levels as shown in table no 4-3

Table 4-3: show comparison between age and protein c level

Age	number	Mean	P value
15-25	10	47.3	0.265
26-35	12	46.3	0.207
36-45	8	31.3	0.028
46-55	2	141.0	0.795

4–4 Relation of other disease with protein c level:-

When we look for the relation of protein c level in placental complicated pregnancy with history of other disease; thrombosis and diabetes mellitus which both consist of 6.5% that have a significant variation when compared with the control group as shown in table no 4-4.

Table 4-4 show relation of other disease with protein c level

Disease	number	Mean	P value
Thrombosis	2	35.5	0.031
Diabetic mellitus	2	40.0	0.039
Hypertension	2	47.5	0.372
No	27	48.1	0.125

4–5 Relation between number of abortion and protein c level:-

In this study we correlate number of abortions with protein c level; the ladies without abortions have a low mean of protein c as in table no 4-5.

Table 4-5 show relation between number of abortion and protein c level

Number of abortion	Mean of PC	percentage
1-5	51.4	59.4%
6-10	35.3	9.4%
No abortion	31.8	31.3%

Chapter five

5.1Discussion

After statistical analysis, the data result of study show that the mean of protein C level is 44.0 while it is 77.0 in controls that indicate decrease level of protein C level in ladies with placental complicated pregnancy. The data in our study in recurrent pregnancy loss was disagree with the data published in the previous study that done in Babylon [76]

The current study found strong association with pre-term labor (PTL) with (p value <0.05) and insignificant association with miscarriage and still birth with p value (0.419), while Hansda and Roychowd revealed that protein C deficiency was present in 15.09 % of patients with RPL. Protein S deficiency was present in 50.94 % of patients with RPL (p=0.000). Similar statistically significant defect was observed in case of elevated factor VIII level (p=0.02). The strongest association of thrombophilia with RPL was observed with protein S deficiency. [77]

Also the study reveal association of protein c level in placental complicated pregnancy with history of other disease; thrombosis and diabetes mellitus which both consist of 6.5% that have a significant variation. Many published data that done at a family studies from the Netherlands and the US have shown that family members who are PC deficient are at an8-10 fold increased risk of venous thrombosis, and, by age 40, 50% or more will have experienced a thrombotic event [78,79].The initial episode of venous thromboembolism in patients with protein C deficiency is apparently spontaneous in approximately 70 percent of cases. The remainders of the cases suggest that other genetic or acquired factors are involved in the presentation of thrombotic events in this population. Further studies in the Netherlands showed that most patients are asymptomatic until their early twenties,

with increasing numbers experiencing thrombotic events as they reach the age of 50[80].

The study looks for association of number of abortions with protein c level; the ladies with six to ten abortions have a deficiency of protein C; while published data found about 15% of all pregnancies will terminate in miscarriage. Recurrent miscarriage (RM) is a condition defined as three consecutive miscarriages and affects 1% - 2% of women of reproductive age. Up to 5% have >.2 recurrent losses.[81]

In our study group, the age from 36-45 years, which comprise of 25% that have a significant value with low protein c levels and Salwa Khan and Joseph Dickerman found that the median age at onset for a thrombotic event and the risk of thrombosis is similar in both protein C deficiency Approximately 60 percent of affected individuals develop recurrent venous thrombosis and about 40 percent have signs of pulmonary embolism.[82]

5.2 Conclusion

On the bases of finding in the study we conclude the following:

- Protein C deficiency is major role in Placental complicated pregnancy.
- Age group (36-45) is more affected with placental complicated pregnancy in Shendi town.
- Severe protein C deficiency found in Pre-term labor.

5.3 Recommendation

By the end of this study I recommended:

1-Health education program for local obstetrics and gynecology clinics visitors about thrombophilia.

2-Intensive study should be conducted with increase sample size.

3-Recommended protein C routinely in obstetrics and gynecology clinics, and should be funded because the price is too expensive .

Chapter six

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Appendix

Questionnaire

University of Shendi
Faculty of post –Graduate studies
Faculty of medical Laboratory sciences
ROLE OF PROTEIN C IN HEREDITARY PLACENTAL COMPLICATED PREGNANCY

S. Number…….
Hospital…….
Lab no……..….
Age……...
BMI……..
Parity……
Gestational age…….
Race tribe…….
Region……..
Family history of pregnancy complication…….. ……
Relation degree……

Diagnosis

Stillbirth
Pre-eclampsiaI
IUGR
PTL
Placental abruption
Chronionamnionitis
Miscarriage

History of other disease

Thrombosis……...
Abortion……
Beeding…….
DM……
Hypertension……
Any drugs intake……...
Others……...

Results

PC……