بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Estimation of Fibrinogen level in Pregnant Women at 3rd Trimester in Different Ages in Khartoum State

قياس مستوى الفيبرينوجين لدى النساء الحوامل في الثلث الثالث في مختلف الفئات العمرية في ولاية الخرطوم

A Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of M.Sc in Haematology and Immunohematology.

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2014

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(قل لو كان البعر مداداً الحلمات ربي لنغد البعر قبل أن تنفد كلمات ربي ولو جئنا بمثله مدداً)

قال تعالى:

حدق الله العظيم

سوره الكهف - 109

Dedication

To our dear fathers for..... Their continuous guidance and support in each and every step.

> To our lovely mother for..... Their love, care and sense, By which we have always being supported.

To our grand mother and aunts for.....

Provide me a suitable environment and continoues encouragement through our live.

To our teachers who.....

Always ready to help me.

To our lovely brothers, sisters, friends and everyone who supported me.

Acknowledgment

Praise to God how gave me the health, strength and patience to conduct this study. I wish to express my great thankful to my supervisor Dr. Mubarak Alkarsani for his continuous supervision. Also we would like to thank the head of Haematology department in college of Medical Maboratory Sciences Dr. Mudather and all staff member of the department for useful advices and encouragement. And I would like to express my special thanks to the staff of Khartoum teaching Hospital and Medical Corps Hospital for their help in sample collection and very special thank for the patients who allowed me to take blood sample for this study. And finally I would like to thank all people who have contributed to my knowledge.

Abstract

In Pregnancy many clotting factors increase and anticoagulation factors decrease causing augmented coagulation and decrease fibrinolysis . Haemostasis abnormalities have been associated with various complications of pregnancy. Pregnancy is a hypercoagulable state with changes in procoagulant, anticoagulant, and fibrinolytic systems.

This study was aimed to look at the changes in plasma fibrinogen level among different age groups in pregnant Sudanese ladies at third trimester. This study was carried out at Khartoum Teaching Hospital and Medical Corps Hospital from March to June 2014.This study included 100 ladies at third trimester of pregnancy and 20 non-pregnant ladies as control group at different ages between 15 and 45 years.

All ladies gave their consent before entering the study blood samples were taken from both groups for measurement of fibrinogen. The one sample T-test and correlation coefficient study were used for statistical tests.

The Results show There was significant difference in mean plasma fibrinogen between pregnant ladies at 3^{rd} trimester (4.245±0.72) and control group (2.330±0.45). (P value <0.0001), and no correlation between fibrinogen level in pregnant ladies at 3^{rd} trimester and age group and insignificant p.value (0.911)

There is a significant increase in plasma fibrinogen level in pregnant ladies at 3^{rd} trimester in compare to control group and there is no significantly difference in fibrinogen level between different age group in pregnant ladies at 3^{rd} trimester.

المستخلص

في فترة الحمل تلاحظ زيادة العديد من عوامل التخثر و وكذلك نقصان العوامل التي تعمل كمضادات لتخثر الدم مما يتسبب في زيادة حالات تجلط الدم وتقليل انحلال الفيبرين .وقد ارتبطت شذوذ تخثر الدم مع مضاعفات مختلفة من اثناء فترات الحمل المختلفة.يعتبر الحمل في حد ذاته من المراحل التي تزداد فيها العوامل التي تؤدي إلى تجلط الدم و نقصان العوامل التي تمنع التجلط وكذلك العوامل الحالة للفبرين.

تهدف الدر اسة إلى كشف التغير ات الفيسيولوجية في فبرينوجين البلاز ما بين مختلف الفئات العمرية في السيدات السودانيات اللاتي في الربع الثالث من الحمل .

أجريت هذه الدراسة على السيدات الحوامل في كل من مستشفى الخرطوم التعليمي ومستشفى السلاح الطبي في الفترة من مارس إلى يونيو سنة 2014 .وشملت الدراسة حوالي 100 سيدة في الثلث الثالث من الحمل وكذلك 20 سيدة من غير الحوامل كمجموعة ضبط تتراوح اعمار هن ما بين 15 و 45 عاما .

أعطت كل السيدات موافقتهم قبل جمع العينات وأخذت عينات الدم للدراسة من المجمو عتين لقياس مستوى الفيبرينوجين, كما تم استخدام اختبار T المستقل ومعامل الارتباط في دراسة اختبارات التحليل الإحصائي.

كان هناك فرق كبير في متوسط مستوى فيبرينوجين البلازما بين السيدات الحوامل في الثلث الثالث من الحمل وكان المتوسط (4.245g/l) مقارنة بمجموعة الضبط حيث كان متوسط مستوى الفرينوجين (2.330g/l) وكانت قيمة (P <0.0001) ، وكذلك اظهر عدم وجود ارتباط بين مستوى الفيبرينوجين في السيدات الحوامل في الثلث الثالث من الحمل والفئات العمرية المختلفة وكانت قيمة p.value تساوى (0.911).

نستنتج ان هناك زيادة كبيرة في مستوى فيبرينوجين البلازما في السيدات الحوامل في الثلث الثالث من الحمل مقارنة بغير الحوامل. وأظهرت الدراسة أيضا ان ليس هناك فرق كبير في مستوى الفيبرينوجين بين مختلف الفئات العمرية في السيدات الحوامل في الثلث الثالث من الحمل.

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Abbreviation

Abbreviation	Term	
APTT	Activated Partial Thromboplastin Time	
APC	Activated Protein C	
ADP	Adenosine diphosphate	
AT-III	Antithrombin III	
ВК	Bradykinin	
DIC	Disseminated Intravascular Coagulation	
ECs	Endothelial cells	
EPCR	Endothelial protein C receptor	
FDB	Fibrin Degradation Product	
HDL	High Density Lipoprotein	
HMWK	High molecular weight kininogen	
HUK	Human urinary kallikrein	
IP3	Insitol triphosphate Pathway3	
ICAMs	Intercellular adhesion molecules	
INR	International Normalized Ratio	
LNMP	last normal menstrual period	
NSAIDs	Non-steroidal antiinflammatory drugs	
OAT	Oral anticoagulant therapy	
PIP2	Phosphatidylinositol Pathway2	
PLC	Phospholipase C	
PAI	Plasmenogen Activator Inhibitor	
PECAMs	Platelet endothelial cell adhesion	
	molecules	
PG1	Prostaglandin I	
РТ	Prothrombin Time	
SPSS	Statistical Package for Social Science	
ТМ	Thrombomodulin	
TF	Tissue Factor	
TFPI	Tissue Factor Pathway Inhibitor	
ТРА	Tissue Plasminogen Activator	
VWF	Von Willebrand factor	

Chapter One

Introduction and Literature Review

1-Introduction and literature review:

1.1Introduction:

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 proteins may lead to bleeding. Laboratory tests can monitor the hemostatic status of individuals including the prothrombin time, which monitors the extrinsic pathways and the activated partial thromboplastin time, which monitors the intrinsic pathway. (Harmening,1997)

1.2 literature review:

1.2.1Normal Haemostasis:

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. (Rodak,2002)

It consists of a complex regulated system which is dependent on a delicate balance among several systems. The systems involved in the hemostatic process include the vascular system, coagulation system, fibrinolytic system, platelets, kinin system, serine protease inhibitors, and the complement system.(Hoffmeister,2001)

The systems work together when the blood vessel endothelial lining is 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 hypercoagulation or hypocoagulation, respectively. (Hoffmeister,2001)

Hemostasis is categorized as either a primary or secondary process. Primary hemostasis involves the response of the vascular system and platelets to vessel injury.(Stiene-Martin,1989)

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 the subendothelium 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.(Rodak,2002)

Secondary hemostasis involves the response of the coagulation system to vessel injury.(Stiene-Martin, 1989)

It is required to control bleeding from large wounds and is a continuation of the primary hemostatic mechanisms. Whereas the outcome of primary hemostasis is the formation of the platelet plug, the outcome of secondary hemostasis is the formation of a thrombus.(Stiene-Martin, 1989)

1.2.1.1 The 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 plasma proteins. The ECs are supported by a collagen- rich basement membrane and surrounding layers of connective tissues. (Rodak,2002)

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. (Harmening,1997)

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). (Rodak,2002)

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. (Bombeli,1997)

1.2.1.2 Platelets:

Platelets are anuclear fragments derived from the bone marrow megakaryocytes. They have a complex internal structure, which reflects their hemostatic functions. The two 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. (Esmon,2001)

When platelets aggregate, they expend their stored energy sources, lose their membrane integrity, and form an unstructured mass called a syncytium. In addition to the plug formation, platelet aggregates release micro-platelet membrane particles rich in phospholipids and various coagulation proteins which provide localized environment that support plasma coagulation. (Rodak,2002)

Platelets and ECs have biochemical pathways involving the metabolism of arachidonic acid (AA), which is released from membrane phospholipids by phospholipase A2. Subsequently, cyclooxygenase converts AA to cyclic endoperoxides. The endoperoxides are then converted by thromboxane synthetase to thromboxane A2. Thromboxane A2 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. (Triplett,2000)

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, the activation 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. (Triplett,2000)

1.2.1.2.1 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 dysfunctionmay 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 of hemostasis such as activated partial thromboplastin time (APTT) and prothrombin time (PT) are normal in most circumstances but not all. 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). (Platelet Dysfunction, 2002)

1.2.1.2.2 Platelet Aggregation:

Following platelet adhesion to proteins exposed in the subendothelial matrix at sites of vascular injury, intraplatelet signal transduction pathways are initiated resulting in platelet activation and the ability of the platelets to aggregate. These platelet-platelet interactions are mediated through platelet bridging via fibrinogen. The integrin α IIb β 3, expressed in a non-binding conformation on the surface of the resting platelet undergoes conformational changes exposing the binding site for fibrinogen in the extracellular domain of the receptor to yield a platelet/fibrinogen interaction following platelet activation. By crosslinking α IIb β 3molecules on adjacent platelets, fibrinogen induces platelet aggregate formationFibrinogen cross-links platelets via a unique mechanism. The strong binding of fibrinogen to platelet α IIb β 3 is Ca²⁺dependent (Hawiger ,1992) Human fibrinogen is composed of three pairs of non-identical chains (α , β , and V). Regions in the C-terminal segment of the V chain and the α chain are involved in fibrinogen interaction with α IIb β 3, One molecule of fibrinogen is able to bridge two platelets together to subsequently result in aggregation (Kloczewiak, et al., 1983).

1.2.1.3 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 fibrin. The 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. (Harmening,1997)

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. (Harmening,1997)

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. (Harmening, 1997)

The coagulation factors may also be categorized into 3 groups on the basis 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. (Harmening,1997)

1.2.1.3.1 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 also a 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 microvessel permeability. (Colman,1997) 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. It is a multifunctional protein, a parent protein of bradykinin, and serves as a cofactor for FXI and PK assembly on biologic membranes. The docking of HMWK to platelet and EC membranes requires its binding by regions on both its heavy and light chains. (Maier,1983)

1.2.1.3.2 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. (Esmon,2001)

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. (Esmon,2001)

Therefore, anticoagulants that inhibit any of these factors would be 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 the generation of the inflammatory response. (Esmon,2001)

Serine proteases (such as thrombin, FXa, 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. (Rodak,2002)

More than 90% of the antithrombin activity of normal plasma is derived from AT-III. (Harmening,1997)

Antithrombin-III has been shown to exert marked anti-inflammatory properties and proven to be efficacious in experimental models of sepsis, septic shock, and disseminated intravascular coagulation (DIC). Antithrombin- III also inhibits factors XIIa, XIa, IXa, protein S, protein C, plasmin, and kallikrein. (Oelschlager,2002)

1.2.1.3.3 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. (Molina,2002)

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 an opsonin that causes immune adherence. (Harmening,1997)

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 inhibits assembly of the large MACs by inhibiting the insertion of additional C9 molecules into the C5b-9 complex. (Farkas,2002)

1.2.1.3.4 Protein C and Protein S System:

The protein C system provides important control of blood coagulation by regulating the activities of factor VIIIa (FVIIIa) and factor Va (FVa), cofactors in the activation of factor X and prothrombin, respectively. The system comprises membrane-bound and circulating proteins that assemble into multi-molecular complexes on cell surfaces. Vitamin K-dependent protein C, the key component of the system, circulates in blood as zymogen to an anticoagulant serine protease. It is efficiently activated on the surface of endothelial cells by thrombin bound to the membrane

protein: thrombomodulin. The endothelial protein C receptor (EPCR; or thrombomodulin) further stimulates the protein activation. С Thrombomodulin binds and sequesters circulating thrombin, therebyinducing thrombin coagulation deactivation. The bound thrombin may then activate protein C. Activated protein C (APC) together with its cofactor protein S inhibits coagulation by degrading FVIIIa and FVa on the surface of negatively charged phospholipid membranes (Muszbek, et al ,1999).

1.2.1.3.5 Antithrombin III :

Antithrombin is a small protein molecule that inactivates several enzymes of the coagulation system. Antithrombin is a glycoprotein produced by the liver and consists of 432 amino acids. It contains three disulfide bonds and a total of four possible glycosylation sites. α -Antithrombin is the dominant form of antithrombin found in blood plasma and has an oligosaccharide occupyingeach of its four glycosylation sites. A single glycosylation site remains consistently un-occupied in the minor form of antithrombin. Its activity is increased manyfold by the anticoagulant drug heparin, which enhances the binding of antithrombin to factor II and factor X. (Bjork, 1997).

1.2.1.3.6 Fibrinogen:

Fibrinogen is a soluble plasma glycoprotein protein produced in the liver by hepatocytes. It is a large molecule, made up of two identical halves, each half composed of three protein chains (α alpha, β beta, and γ gamma). Thrombin cleaves fibrinogen with the release of fibrinopeptides A and B, producing fibrin monomer which then polymerizes and is stabilized by the action of factor XIII, Fibrin is then cross linked by factor XIII to form a clot. In its natural form, fibrinogen can form bridges between platelets, by binding to their GpIIb/IIIa surface membrane proteins. Thrombin is the essential enzyme product of the blood coagulation enzymatic cascade. Thrombin is a "trypsin-like" serine protease protein that in humans is encoded by the F2 gene (Degen and Davie, 1987). Prothrombin (coagulation factor II) is proteolytically cleaved to form thrombin in the first step of the coagulation cascade, which ultimately results in the stemming of blood loss. Thrombin in turn acts as a serine protease that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalyzing many other coagulation-related

reactions. In the blood coagulation pathway, thrombin acts to convert factor XI to XIa, VIII to VIIIa, V to Va, and fibrinogen to fibrin.

1.2.1.3.7 Screening tests of blood coagulation:

Screening tests provide an assessment of the extrinsic and intrinsic systems of blood Coagulation and also the central conversion of fibrinogen to fibrin. The prothrombin time (PT) measures factors VII, X, V, prothrombin and fibrinogen. Tissue thromboplastin (a brain extract) and calcium are added to citrated plasma. The normal time for clotting is 10-14 sec. It may be expressed as the international normalized ratio (INR). Among the most common causes of prolonged PT are liver diseases, warfarin therapy, and disseminated intravascular coagulopathy (DIC). The activated partial thromboplastin time (APTT) measures factors VIII, IX, XI and XII in addition to factors X ,V, prothrombin and fibrinogen. Three substances-phospholipid, a surface activator (e.g. kaolin) and calcium-are added to citrated plasma. The normal time for clotting is approximately 30-40 sec (Furie, 2005).

1.2.1.3.7.1 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. (Stiene-Martin, 1998)

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. (Turgeon, 1999)

1.2.1.3.7.2 Prothrombin Time:

Prothrombin time is the routine test used to screen for deficiencies of factorsI, 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. (Stiene-Martin, 1998)

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).

(Kagawa,2002)

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. (Turgeon, 1999)

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 the thromboplastin reagent. The problems associated with the INR are that the concept and reasons for use are poorly understood and the value is generally misused. (Turgeon, 1999).

1.2.1.3.8 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 screening tests 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. 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. (Disseminated intravascular coagulation, 2002)

1.2.1.4 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 may have 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. (Turgeon,1999).

Recent evidence suggest that the renin-angiotensin-aldosterone system (RAAS) may participate in the regulation of fibrinolytic function. (Lottermoser, 2000)

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, possibly by interacting with Ang II. (Lottermoser, 2000)

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 α 2- plasmin inhibitor, tissue plasminogen activator

inhibitor, and plasminogen activator inhibitor-1 (PAI-1). (Turgeon, 1999)

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. (Vaughan,2002)

1.2.1.4.1 Fibrinogen and Fibrinogen Degradation Products:

Fibrinogen levels are useful in detecting deficiencies of fibrinogen and alterations 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. An increased concentration of fibrinogen degradation

products (FDPs) is commonly used in conjunction with other hemostatic test abnormalities to identify patients with DIC. (Lozier, 1999)

1.2.2 Pregnancy:

Pregnancy is the development of one or more offspring, known as an embryo or fetus, in a woman's uterus. It is the common name for gestation in humans. A multiple pregnancy involves more than one embryo or fetus in a single pregnancy, such as with twins. Childbirth usually occurs about 38 weeks after conception; in women who have a menstrual cycle length of four weeks, this is approximately 40 weeks from the start of the last normal menstrual period (LNMP). Human pregnancy is the most studied of all mammalian pregnancies. Conception can be achieved through sexual intercourse or assisted reproductive technology.An embryo is the developing offspring during the first 8 weeks following conception, and subsequently the term *fetus* is used until birth. (MedicineNet,2011)

In many societies' medical or legal definitions, human pregnancy is somewhat arbitrarily divided into three trimester periods, as a means to simplify reference to the different stages of prenatal development. The first trimester carries the highest risk of miscarriage (natural death of embryo or fetus). During the second trimester, the development of the fetus can be more easily monitored and diagnosed. The third trimester is marked by further growth of the fetus and the development of fetal fat stores. (MedicineNet,2011)

1.2.2.1 Physiology:

The most commonly used event to mark the initiation of pregnancy is the first day of the woman's last normal menstrual period, and the resulting fetal age is called the gestational age. This choice is a result of a lack of a convenient way to discern the point in time when the actual creation of the baby naturally happens. In case of in vitro fertilisation, gestational age is calculated by days from oocyte retrieval + 14 days. (Tunón *et al*,2000)

1.2.2.1.1 Maternal physiological changes in pregnancy:

During pregnancy, the woman undergoes many physiological changes, which are entirely normal, including cardiovascular, hematologic, metabolic, renal and respiratory changes that become very important in the event of complications. The body must change its physiological and homeostatic mechanisms in pregnancy to ensure the fetus is provided for. Increases in blood sugar, breathing and cardiac output are all required. Levels of progesterone and oestrogens rise continually throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle. The fetus inside a pregnant woman may be viewed as an unusually successful allograft, since it genetically differs from the woman. (Clark *et al*, 1986)

1.2.2.1.2 First trimester:

The uterus as it changes in size over the duration of the trimesters. Minute ventilation is increased by 40% in the first trimester. The womb will grow to the size of a lemon by eight weeks. Many symptoms and discomforts of pregnancy (further described in later sections) appear in the first trimester. (Campbell, 2001)

1.2.2.1.3 Second trimester:

By the end of the second trimester, the expanding uterus has created a visible "baby bump". Although the breasts have been developing internally since the beginning of the pregnancy, most of the visible changes appear after this point. Weeks 13 to 28 of the pregnancy are called the second trimester. Most women feel more energized in this period, and begin to put on weight as the symptoms of morning sickness subside and eventually fade away. The uterus, the muscular organ that holds the developing fetus, can expand up to 20 times its normal size during pregnancy. Although the fetus begins to move and takes a recognizable human shape during the first trimester, it is not until the second trimester that movement of the fetus, often referred to as "quickening", can be felt. This typically happens in the fourth month, more specifically in the 20th to 21st week, or by the 19th week if the woman has been pregnant before. It is common for some women not to feel the fetus move until much later. During the second trimester, most women begin to wear maternity clothes. (Campbell, 2001)

1.2.2.1.4 Third trimester:

The uterus expands making up a larger and larger portion of the woman's abdomen. At left anterior view with months labeled, at right lateral view

labeling the last 4 weeks. During the final stages of gestation before childbirth the fetus and uterus will drop to a lower position. Final weight gain takes place, which is the most weight gain throughout the pregnancy. The woman's abdomen will transform in shape as it drops due to the fetus turning in a downward position ready for birth. During the second trimester, the woman's abdomen would have been very upright, whereas in the third trimester it will drop down quite low, and the woman will be able to lift her abdomen up and down. The fetus begins to move regularly, and is felt by the woman. Fetal movement can become quite strong and be disruptive to the woman. The woman's navel will sometimes become convex, "popping" out, due to her expanding abdomen. Head engagement, where the fetal head descends into cephalic presentation, relieves pressure on the upper abdomen with renewed ease in breathing. It also severely reduces bladder capacity, and increases pressure on the pelvic floor and the rectum. It is also during the third trimester that maternal activity and sleep positions may affect fetal development due to restricted blood flow. For instance, the enlarged uterus may impede blood flow by compressing the lower pressured vena cava, with the left lateral positions appearing to providing better oxygenation to the infant. (Stacey *et al*,2011)

1.2.2.2 Body Adaptation To Pregnancy:

In pregnancy, physiological changes affect coagulation and fibrinolytic system. These physiological changes are seen in blood volumes, blood pressure, gastrointestinal system, metabolism, renal physiology, the endocrine system, haematological parameters, liver enzymes (Jamjute *et al.*, 2009). Many clotting factors increase and anticoagulation factors decrease causing augmented coagulation and decrease fibrinolysis (Dahlman *et al.*, 1999; Thorton and Dauglas, 2010). Haemostasis abnormalities have been associated with various complications of pregnancy (Awodu and Enosolease, 2003). These changes result in a state of hypercoagulability and are likely due to hormonal changes and increase the risk of thromboembolism. These changes in haemostatic system are considered to be in preparation for the maintenance of the placental function which occurs during pregnancy. These substances stimulate clot formation to stop maternal blood loss. As placental blood flow is up to 700ml/min considerable haemorrhage can occur if clotting

fails (Dahlman *et al.*, 1999). Increase in the blood level of these coagulation factors is maximal in the 3rd trimester of pregnancy.

1.2.3 Correlation between fibrinogen and pregnancy:

There are significant increases in the production of several procoagulant factors and a reduction in plasma fibrinolytic activity. There is a marked increase in plasma fibrinogen concentration. The need for relative hypercoagulability is particularly apparent at the time of placental separation. At term, about 500-700 ml of blood flows through the placental bed per minute. Without effective and rapid haemostasis , a woman could die from exsanguinations within a few minutes. Fibrin begins to be deposited over the placental site and ultimately between 5 and 10 per cent of all the fibrinogen in the circulation is used up for this purpose . Factors that impede this haemostatic process , such as inadequate uterine contraction or incomplete placental separation , can therefore rapidly lead to depletion of fibrinogen reserve.(Philip,2006)

The disadvantage of the potentially life-saving physiological adjustment of hypercoagulation is the substantially increased risk of thromboembolism . Venous thromboembolism remains the largest cause of direct maternal death in the Sudan and other countries .

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1.3 Rationale:

Pregnancy is a hypercoaguble state in itself.

The determination of the level of plasma fibrinogen in pregnant women is very important in diagnosis of disease and follow up of pregnancy.

Because of the importance of fibrinogen in the process of clotting, and in the maintainance of the fluid state of blood in vessels this study was carried out to asses fibrinogen levels during pregnancy.

1.4 Objectives:

1.4.1 General objective:

-To determine fibrinogen level among different age groups in pregnant Sudanese ladies at third trimester.

1.4.2 Specific objectives:

-To evaluate fibrinogen level among pregnant Sudanese ladies at third trimester.

-To compare the fibrinogen level in different age groups among pregnant ladies at third trimester.

Chapter Two

Materials and Methods

2- Materials and Methods

2.1 Materials:

2.1.1 Study design:

This is case-control study carried between March to June 2014 in Khartoum State. .

2.1.2 Study population:

Referred to Khartoum Teaching Hospital and Medical Corps Hospital pregnant ladies at third trimester without any complication ,and her ages between 15-45 year.

2.1.2.1 Sample size:

The size is 120 samples, 100 case and 20 as control.

2.1.2.2 Sampling:

In this study 2.5ml venous blood samples were collected from each patient using standard venipuncture technique. Blood specimens were collected in 3.2% trisodium citrate container, plasma was obtained after centrifugation at 1500 rpm for 15 minutes. plasma specimens were stored freeze to 5 days until analysis.

2.1.2.3 Inclusion criteria:

-Healthy Sudanese pregnant ladies at 3rd trimester.

- Healthy non-pregnant ladies (as control)

2.1.2.4 Exclusion criteria:

-Presence of any diagnostic disease like (DIC, history related to high or low fibrinogen.

2.1.3 Data collection:

Data was collected by random selected of pregnant ladies at third trimester and Data collected by questionnaire and the samples were analyzed and determined fibrinogen level by using coagulometer device. Also the pregnant ladies data like age, number of pregnancy and using contraceptive drugs were collected.

2.1.4 Data analysis:

The collected data was analyzed to obtain the correlation between fibrinogen level and age at different age groups and its frequencies by using statistical package for social sciences (SPSS) computer program 15.0 evaluation version.

2.1.5 Ethical consideration:

All information obtained from ladies were confidential data and specimens or results were not permitted. The participants were provided with information about the study and any risk that may rise especially when the collection technique was applied.

2.2 Methods

2.2.1 Fibrinogen Clauss

The Clauss method measures the rate of conversion of fibrinogen into

fibrin in a diluted plasma in the presence of an excess of thrombin.

The measured clotting time is inversely proportional to fibrinogen

concentration.

2.2.2 Principle of the method:

The Clauss method measures the rate of conversion of fibrinogen into fibrin in a diluted plasma in the presence of an excess of thrombin. The measured clotting time is inversely proportional to fibrinogen concentration (Clauss,1957).

2.2.3 Reagent

A. Reagent: Highly purified human alpha thrombin in buffered medium with calcium and stabilizer.

B. Reagent: Imidazol buffer solution with stabilizer.

2.2.4 Specimen collection and handling:

-Collect blood specimen and separate the plasma.

-Specimen were frozen at -4° C for a month.

2.2.5 Sample:

Venous blood collected by clean venipuncture. Mix nine parts of blood with one part of 0.109 mol/L trisodium citrate dihydrate. Mix the blood carefully and centrifuge at 1500 x g during 15 minutes to obtain the plasma.

2.2.6 Procedure:

1. Reagent A was incubated at room temperature.

2. 100 μ L of the diluted patient sample or control was pipetted to the test tube.

3. samples incubated at 37°C for 2 minutes.

4. 50 μ L of reagent A was pipetted and simultaneously started the stopwatch and determined the coagulation time.

This procedure refered to manual or semi-automatic coagulometers. In case of determination by an automatic coagulometer.

2.2.7 Calibration:

The citrated platelet poor plasma of control pool plasma was used and the following dilutions in buffer were made: Dilution: 1:5 1:10 1:20

1. In plastic plane tube 90ul of buffer + 10ul of control plasma was added to obtain a 1:5 dilution.

2. In each of a second and a third plastic plain tubes 50 ul of buffer was added.

3. In the second tube 50 ul from the first tube was added to 50 ul buffer to obtain a dilution of 1:10.

4. In the third tube 50 ul from the second tube was added to the 50 ul buffer to obtain a dilution of 1:20.

Alternatively, a calibration curve which was provided with each kit. The same calibration curve could be used when using the same lot of reagent and performing a daily quality control.

2.2.8 Calculation:

The Coagulation Calibrator concentrations was plotted (in g/L) of the diluted samples versus the clotting time (in seconds). The concentration of fibrinogen present in the sample was calculated by interpolating its clotting time in the calibration curve and multiplyed by 10 (sample dilution).

Reference values : 2.0 - 4.0 g/L

Chapter Three

Results

3.Result

This is analytical case control based study was conducted from (March to June 2014).

100 blood samples were taken from healthy Sudanese pregnant ladies at 3rd trimester and 20 blood samples from healthy Non pregnant women as control group.

Table (3.1) Shows the mean of fibrinogen level in pregnant ladies at 3^{rd} trimester (4.245) and the control group(2.33), results show significant difference in fibrinogen level between cases and control group (P.value < 0.05).

parameter	Group	Ν	Mean	Std.	
				Deviation	<i>P</i> .value
Fibrinogen	case	100	4.245	.7270	
g/dl	control	20	2.330	.4532	0.000





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Figure (3.1) Comparison of Fibrinogen level between pregnant ladies at 3rd trimester and control group.

Table (3.2) Show no correlation and insignificant p.value between fibrinogen level in pregnant ladies at 3rd trimester and age group.

correlations					
		fibrinogen	age		
		g/L			
fibrinogen	Pearson	1	.010		
g/L	Correlation				
	Sig. (2-tailed)		.911		
	Ν	120	120		
age	Pearson	.010	1		
_	Correlation				
	Sig. (2-tailed)	.911			
	N	120	120		

Table (3.2): Correlation between fibrinogen level in pregnant ladies at 3rd trimester and age group.



Figure (3.2) Comparison of Fibrinogen level in pregnant ladies at 3rd trimester at different age groups.

Chapter Four

Discussion, Conclusion and Recommendation

4. Discussion, Conclusion and Recommendation

4.1 Discussion

In the current study, there is a significant elevated value of fibrinogen concentration in pregnancy when compared with the non pregnant control subject. The mean of fibrinogen level in pregnant ladies at 3rd trimester was 4.245 g/l, 2.33g/l in control group (P-Value=.000) almost two fold of the control group which indicate that pregnant ladies have higher fibrinogen level than non-pregnant ladies. Fibrinogen level result that obtained from pregnant ladies samples was significantly higher than that obtained from the control group. This was also observed by (Ekaterine and Ilija, 2005), the slightly elevated fibrinogen level was as a result of increased fibrinogen generation in pregnancy which also accounts for the hypercoagulability state in pregnancy,(Dacie and Lewis, 2002).

The result agreed also with the previous studies of (Duperray *et al*,1997) who was reported a significant increase in fibrinogen concentration which was mainly due to the inflammatory state of pregnancy and also fibrinogen being an acute-phase protein. Duperray *et al.* (1997), (Romero *et al*,2007) Show a significant elevated fibrinogen in pregnancy; this elevation may be as a result of its involvement in both cell–cell interaction and the interaction of cell and extracellular matrix like collagen.

This highly elevated fibrinogen concentration was markedly seen in the third trimester. In this study we reported there was no any significant difference in fibrinogen level between pregnant ladies at 3^{rd} trimester at different age groups (P-Value = 0.911), also there was no correlation between plasma fibrinogen level in pregnant ladies and different age groups, There was no previous study work at same subject, but in our study the only result of control group agreed with the previous studies of (Krobot *et al*,1992) which find significant positive correlation between plasma fibrinogen level in different stages of age at normal non-pregnant ladies.

4.2 Conclusion

- There is a significant increase in plasma fibrinogen level in pregnant ladies at 3rd trimester in compare to control group.
- ➤ The results of this work conclude that there is no significantly difference in fibrinogen level between different age group in pregnant ladies at 3rd trimester.
- Pregnancy causes hypercoagubility which was markedly seen at the third trimester of pregnancy.

4.3 Recommendation

This study Recommended:

- Further investigation should be done to investigate the mechanism involve the changes of fibrinogen level which occur in pregnancy exactly at third trimester of pregnancy.
- Clinical and medical strategists should be adopted to follow up haemostatic changes at third trimester of pregnancy.
- Measurement of fibrinogen level should be added as cardiovascular risk factor profile of pregnant ladies.
- More studies should be conducted on a larger number of pregnant ladies.

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Appendix

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Medical Laboratory Sciences

Department of Hematology

Questionnaire

-Name		••••	••••	••••	••••••	•••
-Age	•••••	••••	••••••	••••	••••••	••••
-Residece	• • • • • • • • • • • • • •		• • • • • • • • • •	••••		••••
-Tribe	••••••	••••		••••		••••
-Number of previous preg	gnancy	••••	• • • • • • • • • •	••••	• • • • • • • • • • • • •	••••
-History of coagulopathy	diseases ?	?	Yes()	No()
-Drugs use?	Yes()			No()
-If yes,						
-What type of drugs?	••••••			•••••		
	•••••	••••		••••	•••••	•••••
-Contraceptive use?	Yes()			No()
-If yes:						
-What is it?		•••••		•••••		•
-When		••••	••••••	••••		•••
-Why				••••		••



Figure (3.3): Correlation of fibrinogen level in pregnant ladies at 3rd trimester with age group.



Figure (3.4) Comparison between Fibrinogen level among pregnant ladies at 3rd trimester and control group at different age groups .



COD 61002	COD 61003
STORE	AT 2-8°C
Reagents for measu	rement of Fibrinogen

PRINCIPLE OF THE METHOD

The Clauss method measures the rate of conversion of fibrinogen into fibrin in a diluted plasma in the presence of an excess of thrombin. The measured clotting time is inversely proportional to fibrinogen concentration¹.

CONTENTS

	COD 61002	COD 61003
A. Reagent	4 x 2 mL	-
B. Reagent	-	4 x 15 mL

COMPOSITION

A. Reagent: Highly purified human alpha thrombin in buffered medium with calcium and stabilizer. Lyophilized.

B. Reagent: Imidazol buffer solution with stabilizer

STORAGE

Store at 2-8°C.

Reagents are stable until the expiry date shown on the latel when stored tightly closed and if contaminations are prevented during their use.

AUXILIARY REAGENTS

Coagulation Control Level I (BioSystems Cod. 61007), Coagulation Control Level II (BioSystems Cod. 61008)

REAGENT PREPARATION

Reagent A: add the amount of distilled water stated in the label. Mix gently (Note 1) and keep at 18-25°C for 30 minutes. Stability after reconstitution in the original vial: three days at 22°C, five days at 15°C, and seven days at 2-8°C.

Do not freeze Reagent B is ready to use.

ADDITIONAL EQUIPMENT

Coagulometer.

SAMPLES

Venous blood collected by clean venipuncture². Mix nine parts of blood with one part of $0.109\ \text{mol/L}$ trisodium citrate dihydrate. Mix the blood carefully and centrifuge at 1500 x g during 15 minutes to obtain the plasma3.

Uncentrifuged or centrifuged samples without separating plasma from cellular components may be stored for up to 4 hours at noom temperature³. Separated plasma (without cells) may be stored for up to fifteen days at -20°C for or up to six months at -70°C. Thaw frozen plasma at 37°C just prior to use3

Dilute samples 1/10 with Reagent B

PROCEDURE

1. Bring the Reagent A to room temperature.

2. Pipette 100 µL of the diluted patient sample or control to the test tube

3. Incubate samples at 37°C for 2 minutes.

4. Pipette 50 µL of Reagent A and simultaneously start stopwatch

5. Determine the coagulation time.

This procedure refers to manual or semi-automatic coagulometers. In case of determination by an automatic coagulometer, please refer to the manual for specific instructions.

CALIBRATION

Use Cosquiation Calibrator (BioSystems Cod. 61006) to prepare a calibration curve starting from 1/1 and followed by 1/5, 1/10, 1/20 and 1/40 dilutions with Reagent B.

Alternatively, a calibration curve is provided with each kit. The same calibration curve can be used when using the same lot of reagent and performing a daily quality control.

CALCULATIONS

M61002i-01

Plot the Coagulation Calibrator concentrations (in g/L) of the diluted samples versus the clotting time (in seconds). The concentration of fibrinogen present in the sample is calculated by interpolating its clotting time in the calibration curve and multiplying by 10 (sample dilution)

FIBRINOGEN CLAUSS



REFERENCE VALUES

20-40 g/

When the fibrinogen concentration is <1 g/L, samples should be diluted 1/5 with Reagent B

and rea This range is given for orientation only. Each laboratory should establish its own reference range.

QUALITY CONTROL

It is recommended the use of Coagulation Control Level I (BioSystems Cod. 61007) and Coagulation Control Level II (BioSystems Cod. 61008) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not meet the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Repeatibility (within run):

Mean fibrinogen (g/L)	CV %	n
2.92	2.36	10
1.42	2.54	10

- Reproducibility (run to run):

Mean fibrinogen (g/L)	CV %	n
3.11	2.72	10
1.55	5.04	10

- Measuring range: 0 - 3 g/L.

 Trueness: the results obtained with these reagents do not show significant systematic differences compared to reference reagents. Details of this comparative study are available upon request.

Interferences: Some substances and drugs may interfere4 These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Fibrinogen is a 340kD plasma soluble glycoprotein synthesized in the liver. It's major role is as a precursor of fibrin through the action of thrombin. It is also known as the coagutation Factor I and it's concentration in plasma ranges between 2 and 4 g/L. The fibrinogen concentration is increased in infections, tissue necrosis, estrogen ingestion, diabetes, obesity and pregnancy. High levels of fibrinogen are also a significant independent risk factor for coronary artery disorders and cerebrovascular diseases. Low concentrations of fibrinogen in plasma are associated with level diseases (crmosis, jaundice) or with fibrinolysis and disseminated intravascular coagulation (DIC):

NOTES

Do not shake the vial (swirling is preferable) and avoid the formation of foam. A stirring bar can be used if necessary.

BIBLIOGRAPHY

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FIRRINOGEN CLAUSS METHOD

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