ASSESSMENT OF SOME HAEMOSTATIC PARAMETERS DURING NORMAL PREGNANCY IN ILORIN.

SUBMITTED BY

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TO THE NATIONAL POSTGRADUATE MEDICAL COLLEGE OF NIGERIA IN PART FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE FINAL (PART II) FELLOWSHIP OF THE MEDICAL COLLEGE IN PATHOLOGY (FMCPATH)

NOVEMBER 2007
DECLARATION

It is hereby declared that this work is original unless otherwise acknowledge.

The work has not been presented to any other college for fellowship, nor has it been submitted elsewhere for publication.

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DR. I.A. DUROTOYE
CERTIFICATION

The study reported in this dissertation was done by the candidate under our supervision.

We have also supervised the writing of the dissertation

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DEDICATION

This work is dedicated to Almighty Allah, who made this seemingly impossible task possible, and to my beloved husband Alh. B.A. Durotoye and the children Shakirat, Aminat, Suleiman, Zainab and Abdullahi for their moral support and understanding throughout the trying period.
ACKNOWLEDGEMENTS

I am grateful to Almighty Allah for his guidance during this study. My sincere gratitude goes to my supervisors Prof. J.O. Adewuyi and Prof. P.O. Olatunji for their able supervision and guidance despite their busy schedules seeing me to a meaningful conclusion.

I thank late Dr. I.A. Adediran of Obafemi Awolowo University Teaching Hospital, Ile-ife for his immerse contributions toward this project, may his gentle soul rest in perfect peace, and may Almighty Allah grant him paradise.

My appreciation goes to my head of department Dr. H.O. Olawumi for her encouragement in the course of this study. I am grateful to Dr. A.S. Babatunde for his valuable contributions to this work. I appreciate the help and suggestions rendered by my colleagues, Dr. D.O. Olanrewaju, Dr. O.J. Adegbamigbe and Dr. M.A. Sanni. I thank the pregnant women who made the study population.

I thank all the scientists and laboratory staff of Haematology department for their kind assistance. I am highly grateful to the medical students, student nurses, and students from the school of Health Technology Offa who volunteered as controls for this project.

Finally, my special thanks go to my husband and children who steadfastly endured loneliness created by my absence while pursuing this study.
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SUMMARY

The effect of pregnancy on some haemostatic parameters i.e. prothrombin time, partial thromboplastin time with Kaolin; Fibrinogen level; Euglobulin clot lysis time and platelet count were studied among non-hypertensive pregnant women attending antenatal clinic of the University of Ilorin Teaching Hospital over a period of 6 months. A total of one hundred and eighty women with non-complicated pregnancy within the age range of 17-40 years (median 27.1) were recruited for the study. Sixty non-pregnancy; non-hypertensive apparently healthy age – matched women served as controls. The haemostatic parameters were determined using standard haematological techniques as described in Dacie and Lewis. Statistical analysis of data was done using statistical package for social sciences (SPSS) and statistical significance of data was based on P value of less than 0.05.

The gestational age of the subjects were between 6 and 42 weeks which was obtained from their last menstrual period and ultrasound estimation. The subjects were grouped into the three trimesters of pregnancy based on their gestational age.

Sixty pregnant women with gestational age between 6-13 weeks were in the first trimester, another sixty with gestational age between 14-26 weeks were in the second trimester, while the remaining sixty with gestational age 27 weeks and above were in the third trimester. Significant differences were observed between the haemostatic parameters studied among the subjects and controls except for platelet count as follows: prothrombin time (p value of 0.000); partial thromboplastin time with kaolin ([p value 0.000]; fibrinogen level (p value 0.000) Euglobulin lysis.
time [p value 0.000] and platelet count (p-value 0.057) Prothrombin time was significantly shorter (p=0.001) and fibrinogen level significantly higher (p=0.000) as pregnancy advanced. There was no statistically significant difference in the results of partial thromboplastin time with kaolin; Euglobulin clot lysis time and platelet count among the subjects in the three trimesters of pregnancy. P value of 0.726; 0.821 and 0.795 respectively.

There was a significant difference between the fibrinogen level in older subjects compared with subjects of younger age group. The mean fibrinogen level in older subjects was 5.5g/l while in the younger subjects the mean fibrinogen level was 4.0g/l (p= 0.023). There was however no statistically significant different between age of subjects and other parameters i.e. PT, PTTK, ELT and platelet count. p value of 0.268; 0.799; 0.217 and 0.678 respectively. There was no statistically significant relationship in the haemostatic parameters with parity and educational level of all the subjects, p value of 0.443; 0.182; 0.835; 0.380; and 0.515 respectively. The results of coagulation screening tests carried out in this study suggest that there is increased activation of the coagulation system in the subjects and that normal pregnancy produces a hypercoagulable state.
CHAPTER ONE

INTRODUCTION

Haemostasis can be defined as a group of integrated physiological processes by which blood is kept in the fluid state, haemorrhage is arrested and vascular patency is restored when necessary, through clot dissolution.

Normal haemostasis depends on a delicate balance and complex interaction between at least five components\(^1\). These components include:

1. The blood vessels
2. The platelets
3. Coagulation factors including their cofactors and activators.
5. The fibrinolytic system.

An essential role of the haemostatic system is a rapid reaction to injury, such that the effect of injury is confined to the area of damage. This requires control mechanisms which stimulate coagulation after trauma but limit the extent of the response to prevent further damage.

The objectives of haemostasis is the production of a haemostatic plug and substances involved in this regard normally circulate in the blood in inactive forms, and only become activated at the site of vascular injury or by some factors released into the circulation which trigger intravascular coagulation\(^2\).

Intact endothelial cells do not induce platelet adherence and blood clotting, but, following vascular injury, activation of endothelial cells results in a procoagulant phenotype that augments local clot formation\(^3\). Vascular wall has both prothrombotic (Synthesis of von Willebrand factor, release of tissue factors,
production of Plasminogen activator inhibitor) and antithrombotic (production of Prostacyclin, Nitric Oxide, Synthesis of Thrombomodulin, Heparin-like molecules and Antithrombin III) properties.

The balance between endothelial antithrombotic and prothrombotic activities critically determines whether thrombus formation, propagation or dissolution occur\textsuperscript{4,5,6}.

Platelets play a central role in normal haemostasis\textsuperscript{7}, and conditions in which their number is depleted or their function is abnormal are characterized by widespread spontaneous capillary haemorrhage\textsuperscript{2}. It is thought that in health, the platelets are constantly sealing micro defects of the vasculature, mini fibrin clots being formed and the unwanted fibrin being removed by a process of fibrinolysis. Platelets have glycoprotein receptors of the integrin family on their surfaces through which they adhere to the subendothelial matrix following vascular injury. Deficiencies of some of the surface glycoproteins (e.g. GPIlb in Bernard soupier syndrome, GPIIb-IIIa Complex in Glanzmann thromboasthenia) result in bleeding disorder\textsuperscript{8}.

Platelets also contain granules in which are stored some haemostatic constituents. Some coagulation factors like Fibrinogen, Factors V, VIII and Fibronectin are stored in alpha granules while substances like Histamine, Serotonin, Epinephrine, Adenosine diphosphate and Adenosine triphosphate are stored in the dense granules. These latter substances cause vasoconstriction and reduce blood flow to the injured area. Platelet activation leads to the surface expression of phospholipids complexes which provide critical nucleation and binding site for calcium and coagulation factors in the intrinsic clotting pathway\textsuperscript{9}. 
Following a break in the endothelial lining, there is an initial adherence of platelets to exposed connective tissue, potentiated by vWF, deficiency of which (as in von Willebrand disease) results in defective platelet adhesion and bleeding disorders.

When the vascular injury is minimal, the platelet adhesion with the formation of primary haemostatic plug is enough to limit bleeding, but in severe vascular injury, activation of coagulation cascade is required to convert the primary haemostatic plug to a firm, definitive and stable haemostatic plug.

The coagulation cascade is the third component of the haemostatic process and is a major contributor to thrombosis\(^2,10,11\). It consists of a series of enzymatic reactions converting inactive proenzymes into activated enzymes and culminating in the formation of thrombin. Thrombin then converts the soluble plasma protein fibrinogen into the insoluble protein fibrin. Each reaction in the pathway results from the assembly of a complex composed of an enzyme (activated coagulation factor) a substrate (proenzyme form of coagulation factor), and a cofactor (reaction accelerator). These components are typically assembled on a phospholipid complex provided by activated platelet and held together by calcium ions\(^10\). Although blood coagulation is traditionally divided into extrinsic and intrinsic pathway, there is no strict division between the two pathways in vivo; both pathways are activated by components of the vessel wall and both are required for normal haemostasis\(^12\). All the coagulation factors are important for haemostasis and deficiency or abnormality of any factor, can lead to severe haemorrhage into the tissues. Some factors are however more critical than others. Examples of the more critical ones are factor VIII deficiency in Haemophilia A and deficiency of factor IX in Christmas disease. Overproduction of coagulation
factors as occurs in normal pregnancy or in patients taking Estrogen containing oral contraceptives, or Estrogen replacement therapy, or in some hereditary conditions such as factor V leiden can lead to increased thrombotic tendency, (Deficiency of antithrombin III; Protein C and Protein S etc can lead to hypercoagulable state and increase risk of thrombo-embolism). Once the coagulation cascade is activated, it must be restricted to the local site of vascular injury to prevent clotting of the entire vascular tree. This is the function of the naturally occurring coagulation inhibitors. These are circulating plasma proteins and include, Antithrombins (e.g Antithrombin III) which inhibits activity of thrombin and other serine proteases like factors IXa, Xa, XIa and XIIa. Others are Protein C and Protein S which inactivate factors Va and VIIIa, while tissue factor-VIIa is inactivated by Tissue factors pathway inhibitor (TFPI).

The removal of the product of coagulation, namely fibrin from the circulation is the function of fibrinolytic system. Fibrinolysis (like coagulation) is a normal response to vascular injury. It is also a multicomponent system composed of a circulating pro-enzyme, plasminogen, which is activated by plasminogen activators in the plasma. Initiation of fibrinolytic activity depends on these plasminogen activators whose activation is in turn triggered by a number of physical and biochemical stimuli, including venous occlusion, strenuous exercise, thrombin etc.

Plasmin the proteolytic enzyme produced from plasminogen hydrolyses numerous bonds in fibrin to dissolve it into fibrin degradation products (FDPs). Plasmin also degrades fibrinogen and activated factors such as Va and VIIIa. Generalized fibrinolysis is prevented by a potent inhibitor, alpha-2 antiplasmin
and to a lesser extent by alpha-2 macroglobulin. Free plasmin in plasma is immediately inactivated by alpha-2 antiplasmin, whereas plasmin adhering to Fibrin within the local haemostatic plug is protected from the effect of alpha-2 antiplasmin and is able to digest fibrin into FDPs. Inhibition of plasminogen activators by other proteins such as plasminogen activator inhibitors type 1 and 2 play an important role in regulating fibrinolysis and limiting its effect to the site of injury.

The focus of this study is to determine the ‘normal’ haemostatic status in pregnant women in the local population through the determination of baseline values of the haemostatic screening tests including the prothrombin time (PT), Partial thromboplastin time with Kaolin (PTTK), the platelet count (Plt), plasma fibrinogen level and euglobulin clot lysis time[ELT].
CHAPTER TWO

AIM AND OBJECTIVES

The aim of this project is to document some haemostatic parameters among pregnant women and to determine if they are in hypercoagulable state.

SPECIFIC OBJECTIVES

1. To determine the PT, PTTK, Fibrinogen concentration, Euglobulin clot lysis time and platelet count in the different trimesters of normal pregnancy in women attending antenatal clinic.

2. To determine the variations in the values of the listed parameters during different trimesters of normal pregnancy.

3. To find out the effect of age, parity and educational level on haemostatic parameters.

4. To recommend the results obtained as base-line values in the management of pregnancy at UITH.
CHAPTER THREE

JUSTIFICATION FOR THE STUDY.

While several studies that were carried out on the haemostatic parameters in normal pregnancy have agreed on some pattern of change in both the coagulation and fibrinolytic systems,\textsuperscript{39,42,46,59,82,83,91,92} there are still divergent views for some parameters like the platelet counts in normal pregnancy\textsuperscript{70, 71,72,73,74,75,76}.

The majority of the studies that have been carried out on the haemostatic parameters in normal pregnancy were done amongst Caucasian women.\textsuperscript{12,34,38,93}

There is paucity of data on the haemostatic changes in normal pregnancy amongst the black population especially in Africa. This kind of study will therefore be relevant to establish the values for some coagulation parameters in normal pregnancy in this environment and see how they compare or differ from the established values in Caucasians.

Also, the normal range for the coagulation profile in pregnancy in this centre is not known. The results that will be obtained from this study can therefore serve as baseline values which can be used to monitor pregnant women in this centre. Pregnant women with results outside the established range may need to be closely monitored for early detection of pre-eclampsia and prevent the attendant complications.
CHAPTER FOUR

LITERATURE REVIEW.

HAEMOSTASIS

Haemostasis comprises of a number of protective and integrated physiological processes by which blood is kept in the fluid state, haemorrhage is arrested and vascular patency is restored through clot dissolution.

Haemostasis depends on 5 major components

- Vascular component, including the endothelium
- Normal platelet count and function
- Coagulation system with its activators and inhibitors.
- Fibrinolytic activity

A bleeding tendency occurs when there is a deficiency of clotting factors and platelets, inhibition of coagulation processes or excessive activity of the fibrinolytic system\(^1\).\(^3\)

The haemostatic process which retains the blood within the vascular system, basically consists of two steps primary haemostasis and secondary haemostasis\(^1\).\(^4\). In primary haemostasis, there is platelet adherence to the subendothelial matrix and aggregation whereas secondary haemostasis, there is activation of coagulation cascade, culminating in the activation of thrombin which then converts soluble plasma fibrinogen to fibrin resulting in local fibrin
When there is injury to the blood vessels there is a complex chain of events which includes vasoconstriction, platelet adhesion, platelet aggregation, platelet plug formation and subsequent blood coagulation and clot retraction of a haemostatic plug\textsuperscript{15}.

**THE ROLE OF BLOOD VESSELS IN HAEMOSTASIS**

The blood vessel is composed of endothelial cells which line the intima and rest on a basement membrane of subendothelial microfibrils especially in capillaries but contain increased amounts of elastin, innervated smooth muscle cells and collagen in large vessels.

Intact endothelial cells serve primarily to inhibit platelet adherence and blood clotting. Injury or activation of endothelial cells however results in a procoagulant phenotype that augments local clot formation\textsuperscript{16}.

**PROTHROMBOTIC PROPERTIES OF VASCULAR ENDOTHELIUM**

1. Production of von Willbrand factor (vWF)\textsuperscript{10}
2. Production of tissue factor\textsuperscript{11}
3. Secretion of Plasminogen activator inhibitor (PAIs)

**Endothelial cell derived coagulant inhibitors**

3. Tissue factors pathway inhibitor
4. Antithrombin III
5. Protein S
6. Thrombomodulin
7. Heparin-like molecules e.g - Heparan sulphate
   - Glycosaminoglycans

The blood vessel under normal condition prevents the leakage of blood
cells, but if damaged the collagen in the subendothelial layer becomes exposed.

The collagen attracts platelet and this involves interaction between the platelet GPIaIIa complex and α chain of collagen. Collagen also binds vWF, which serves to anchor platelet to the collagen. Following platelet adhesion, there is a change in platelet shape and release of some of its contents like adenosine diphosphate (ADP) and serotonin. These two products are potent vasoconstrictors and reduce the blood flow to the site of injury. Vasoconstriction however occurs even in micro-circulation in vessels, without smooth muscle cells.

The ADP released from the α-granule attracts more platelets and strong stimuli such as thrombin also leads to release of lysosomal enzymes. Activity of endothelial cells leads to the secretion of von Willebrand factor, prostacyclin and plasminogen activator, vWF is part of a molecular complex which also possesses factor VIII clotting activity. vWF is solely involved in the adhesion of platelet to the sub-endothelial microfibrils and the lack of this protein is responsible for the prolonged bleeding in von-Willebrand disease.

The platelet release reaction occurs concomitantly with and is dependent upon phosphatidyl inositol turnover and prostaglandin generation, both of which contribute to platelet aggregation and formation of haemostatic plug.

Prostacyclin (PGI₂) is an unstable prostaglandin first described by Moncada and colleagues in 1976. It is the principal prostanoid synthesized by blood vessels and is a powerful vasodilator and potent inhibitor of platelet aggregation. Moncada and Vane, proposed that there is a balance between the production of
prostacyclin by the vessel walls and the production of the vasoconstrictor and powerful aggregating agent thromboxane by the platelets\textsuperscript{20}.

**Platelet procoagulant activities.**

In small blood vessels, platelet aggregation, together with local vasoconstriction, is usually sufficient to achieve and maintain haemostasis, but in larger vessels, reinforcement of the platelet plug by fibrin is necessary to prevent its dislodgement by mechanical forces. Platelet-derived procoagulant activities fall into two categories

(a) **Phospholipid -Mediated platelet procoagulant activities**

Platelets contain a number of coagulation factors within the Alpha -granules (fibrinogen, factor V; vWF) or in the cytosol (factor XIIIa subunit) or tightly bound to the cell membrane (factor XI) The intact platelets have little or no intrinsic clot-promoting activity, but within seconds of onset of aggregation and adhesion, a reorientation (flip-flop) of the membrane phospholipids occur. The negatively charged phospholipid becomes exposed and a number of coagulation factors notably those that are vitamin k - dependent (factors II VII IX and X) bind avidly to these negatively charged phospholipids. These factors are protected from naturally-occurring inhibitors such as Antithrombin III and protein C.

(b) **Platelets and contact factors**

Factor XI is tightly bound to platelets and can be activated there, either by other contact factors which are attached to the negatively-charged phospholipids
on the outer membrane of activated platelets or by the trace amounts of thrombin generated via tissue factor pathway.

**BLOOD COAGULATION**

Blood coagulation involves a biological amplification system in which relatively few initiation substances are sequentially activated by proteolysis of a cascade of circulatory precursor proteins (the coagulation factor enzymes), which culminate in the generation of thrombin. This in turn converts soluble plasma fibrinogen to fibrin. Thrombin also activates factor XIII that causes the formation of cross-links between fibrin, thereby forming an insoluble clot. The fibrin enmeshes the platelet aggregates at the site of vascular injury and converts the unstable primary platelet plugs to firm, definitive and stable haemostatic plugs.

The coagulation factors are present in plasma in inactive form and they are either enzyme precursors, or co-factors except fibrinogen.

All the enzymes are serine proteases except factor XIII (A transglutaminase enzyme) and their ability to hydrolyse peptide bonds depends upon the amino acid serine at their active centre\(^{18}\).
**NOMENCLATURE OF CLOTTING FACTORS**

The International Committee for nomenclature of blood clotting factors in 1962 have assigned Roman numerals to the coagulation proteins.

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<tr>
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<tr>
<td>I</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>III</td>
<td>Thromboplastin (Tissue extract)</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin or Labile factor</td>
</tr>
<tr>
<td>VI</td>
<td>Not identified as an entity</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin (stable Factor)</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihaemophilic factor</td>
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<tr>
<td>IX</td>
<td>Christmas factor</td>
</tr>
<tr>
<td>X</td>
<td>Stuart prower factor</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin Antecedent</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor</td>
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</table>

The original enzyme cascade of the coagulation system proposed by Macfarlane has been modified as a result of the recognition of complexes which form between certain activated factors\(^1\). After the discovery of factor V clotting factor by Owren in 1947\(^2\), Macfarlane\(^3\) postulated a modern theory of coagulation. In this theory Macfarlane found that the factors leading to blood coagulation are more
complicated and showed that the coagulation mechanism, involves all the thirteen coagulation factors and of two pathways; intrinsic and extrinsic pathway which are dependent on each other.

When a blood vessel is injured, blood clotting is initiated by activation of factors XII by collagen (intrinsic mechanism) and activation of factor VII by thromboplastin release (extrinsic mechanism) from damaged tissue.

Both the intrinsic and extrinsic mechanisms are activated by components of the vessel wall and both are required for normal haemostasis. Strict division between the two pathways does not exist and interactions between activated factors in both system have been shown\textsuperscript{24}.

Morawitz (1905) postulated that when platelets come in contact with foreign bodies, such as water and wettable surface, they disintegrate and release a substance known as thromboplastin which reacts with calcium present in the blood and prothrombin formed in the liver to produce thrombin.

Thrombin in turn reacts with fibrinogen molecules in the blood to form fibrin clot\textsuperscript{25}.

\textbf{THE EXTRINSIC PATHWAY}

The extrinsic pathway starts with the activation of factor VII by Tissue Thromboplastin. The potent coagulant activity of tissue extract has been known since the time of Rauschenbach\textsuperscript{26} and Wooldridge\textsuperscript{27}. This substance was variously called Thrombokinase implying it was an enzyme or Thromboplastin\textsuperscript{25}. Tissue factor is a lipoprotein complex. It was isolated from microsomal preparations from
human placenta, brain and bovine lung.  

Jesty and Nemerson (1974) concluded that factor VII circulates in the plasma as inactive enzyme, which requires tissue factor for its action on factor X. It may be that the interaction with tissue factors exposes a binding site on factor VII for factor X.

It was suggested that the unique step in the extrinsic pathway is the formation of a complex (calcium; factor VII and tissue factor). Within the complex, factor VII is then converted into Vlla, which in turn acts enzymatically to activate factor X.

Factor X discovered in 1956 by Telfer, Denson and Wright, and Hougie, Barrow and Graham in 1957 this factor is the first factor in the common pathway of both the intrinsic and extrinsic clotting systems, and is also the substrate for Russell’s viper venom.

**THE INTRINSIC PATHWAY**

The intrinsic pathway was considered to start with activation of factor XII upon exposure to collagen and other negatively charged surfaces. In vivo, substances like skin; stearate, uric acid and homocystine can activate Hageman factor. In the invitro test, a wide range of negatively charged substance like glass, celite, kaolin, asbestos, ellagic acid, cellulose sulphate and calcium pyrophosphate can activate factor XII.
The activated factor XII acts enzymatically on the next pro-enzyme factor XI. Recent evidence suggests that the upper section of the intrinsic pathway involving kallikrein and high molecular-weight kininogen is irrelevant to coagulation in vivo and is only relevant to invitro test of the intrinsic pathway. The activated factors XI now activate factor IX. The activated factor IX in association with calcium, cofactor (factor VIII) then activate factor X on the membrane surface provided by platelet phospholipid.

Activation of factor IX is now considered to occur in vivo mainly via factor VII which is activated by tissue factor. This is the dominant pathway for blood coagulation. Factor XI in vivo is activated by thrombin and only becomes important at major sites of trauma. The activated factor X in association with factor V (co factor) on the phospholipid surface and calcium converts prothrombin to thrombin$^{34}$. The thrombin hydrolyses arginine-glycine bonds of fibrinogen, releasing fibrinopeptides A and B to form fibrin monomers. Fibrin monomers link spontaneously by hydrogen bonds to form a loose, insoluble fibrin polymer. Factor XIII activated by thrombin and calcium stabilizes the fibrin polymers with the formation of covalent bond cross- links.

The extrinsic and intrinsic systems complement each other. Factor VIIa generated by tissue factor in the extrinsic system activates factor IX in the intrinsic system.
Thrombin positively feeds back to activate the procofactors V and VIII and also activates factor XI. It also activate protein C to limit coagulation. Patients with deficiency of factors in either system or pathway may suffer from severe bleeding problems. It is obvious that both intrinsic and extrinsic systems are required for normal haemostasis.

When the endothelial cell of a blood vessel is damaged, both pathways are triggered off.

The extrinsic pathway is very relevant because only small amounts of tissue factor is needed to activate it and the enzymatic reaction occurs very rapidly\(^{34}\).

It has also been adduced that the major function of the extrinsic system is to produce small amount of thrombin which can then accelerate the activation of factor VIII and V there by amplifying the intrinsic and the common pathways \(^{35}\).
The intrinsic and Extrinsic pathways of blood coagulation.\textsuperscript{1}

The pathway of blood coagulation\textsuperscript{1}
NATURALLY-OCCURRING INHIBITORS OF BLOOD COAGULATION

Though normal blood clots readily when withdrawn from the body or when extravasated into damaged tissues, in the vessels it remains fluid. In the living body there are effective mechanisms for limiting coagulation.

A small amount of thrombin produced invivo can breakdown all of the fibrinogen in the body within a short period. Unchecked blood coagulation would lead to dangerous occlusion of blood vessels (thrombosis) if the following protective mechanisms were not in operation.

Physiological anticoagulation factors fall into two main groups; those that inhibit the serine proteases of the coagulation cascade (Antithrombins) and those that neutralize activated coagulation co factors (component of protein C system).

In addition to the specific inhibitors, other physiological processes are in place, including; rapid blood flow, clearances of activated clotting and fibrinolytic factors by the Kupfer cells of the liver and selective degradation of these activated factors by the liver. Free thrombin is also removed by its adsorption onto fibrin or fibrinogen degradation products (FDPs), which do not clot and may interfere with normal fibrin polymerization\textsuperscript{36}.

SERINE PROTEASE INHIBITORS (SERPINS)

These are single chain glycoprotein with a molecular weight of 40,000 - 60,000. Specificity is imparted by their tertiary structure which engenders high affinity for a defined substrate.
<table>
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<tr>
<th>Inhibitor</th>
<th>Major substrate</th>
<th>other substrate</th>
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<tbody>
<tr>
<td>Antithrombin</td>
<td>Ila : Xa</td>
<td>IXa; Xla; XIla</td>
</tr>
<tr>
<td>Heparin cofactor II</td>
<td>Ila</td>
<td>-</td>
</tr>
<tr>
<td>α1-antitrypsin</td>
<td>XIa; Xa</td>
<td>Plasmin</td>
</tr>
<tr>
<td>C1-esterase inhibitor</td>
<td>KK; XIa</td>
<td>XIa</td>
</tr>
<tr>
<td>α2-antiplasmin</td>
<td>plasmin</td>
<td>KK; XIIa; XIa</td>
</tr>
<tr>
<td>α2-Macroglobulin</td>
<td>KK</td>
<td>Ila</td>
</tr>
<tr>
<td>Tissue factor pathway-Inhibitor</td>
<td>TF-VIIa</td>
<td>Xa</td>
</tr>
</tbody>
</table>

**ANTITHROMBIN III**

Antithrombin III is a major inhibitor of coagulation cascade. It is a single chain glycoprotein with a molecular weight of 58,200, principally synthesized by the liver; endothelial cells also synthesize little antithrombin III. The mean concentration in plasma is approximately 150 μg/ml. It is formerly known as heparin cofactor-I.

It forms a stable 1:1 stoichiometric complex with several serine protease coagulation factors. The serine at the active centre of the protease cleaves a peptide bond involving arginine 393 near the carboxyl-terminal of antithrombin causing a configuration change, which traps the enzyme in an inactive form.

Complex formation is progressive and only with thrombin and factor Xa is it
rapid enough to be of physiological significance.

Heparin, without altering the 1:1 stoichiometry, induces a 2000 fold increase in the rate of thrombin inactivation by antithrombin\textsuperscript{36}.

Heparin also strongly enhances the speed of neutralization of factor Xa and to a lesser extent of factor IXa in the presence of calcium\textsuperscript{21}.

Recently it was shown that Antithrombin in the presence of Heparin also neutralizes Tf-VIIa\textsuperscript{36}.

**HEPARIN CO-FACTOR II**

Heparin cofactor II is a single chain glycoprotein. It is present in plasma in a concentration of 80µg/ml and appears to be a specific inhibitor of thrombin, with which it forms 1:1 stoichiometric complex. It has little or no antifactor Xa activity. The rate of thrombin neutralization by HCII is increased approximately 1000 folds by heparin, although because of its lower heparin affinity, it requires 5-10 times more heparin than does antithrombin. That HCII has some physiological significance is suggested by the fact that it falls in parallel with Antithrombin III in DIC. However, since AT III is in two fold molar excess over HCII, the latter cannot altogether compensate for a deficiency of Antithrombin III which is a well-established cause of a thrombotic tendency\textsuperscript{36}.

**Alpha 1 antitrypsin** is a single chain glycoprotein, whose primary substrates are pancreatic and leukocyte elastases. It is responsible for about 70% and 35% respectively of the factor XIa and Xa neutralizing activity in plasma. It has little
effect on thrombin inhibition and a deficiency of \( \alpha 1 \)-antitrypsin is not associated with hypercoagulability\(^{36} \).

**C1-esterase inhibitor** Is a single chain glycoprotein whose primary substrate is the activated form of the first component of complement, but it is also responsible for 90% and 50% respectively of the factor XIIa and Kallikrein inhibitory activity of the plasma and contributes in a minor way to neutralization of factor Xla and plasmin. It forms a 1:1 stoichiometric complex with the serine active center of all proteases, but its association with the contact factors may be impeded somewhat by their prior binding to high molecule weight kininogen. A deficiency of C1-esterase inhibitor, although of no haemostatic consequence, causes angioneurotic oedema.

**Alpha 2 antiplasmin** - Is a single -chain glycoprotein, the principal inhibitor of the fibrinolytic enzyme Plasmin. It also has weak activity against several coagulation proteases especially the contact factors. Its action against proteases late in the coagulation cascade factor Xa, is only apparent at a higher concentration.

**Alpha -2 macroglobulin.** Is a large glycoprotein composed of four identical chains and is unique in that its effects are not restricted to serine proteases. It binds to coagulation factors at a site away from the serine active center, the interaction involving the formation of a bond between cysteine and glutamyl residues in the inhibitor and a lysly group in the proteases. Inhibition is produced by steric hindrance rather than by inactivation and the protease retain some esterolytic and amidolytic activity particularly against small peptides. It is responsible for inhibition
of Kallikrein; thrombin and factor Xa. The possibility of thrombotic complication due to sustained low grade bioactivity of protease alpha-2 macroglobulin is unlikely, since these are rapidly removed from the circulation by the liver.

A deficiency of $\alpha_2$-MG is not associated with a thrombotic tendency. It has been suggested that the raised level of $\alpha_2$-MG which exists in children, may compensate for a low level of Antithrombin and explain why thrombotic episodes do not usually occur before puberty in congenitally antithrombin III deficient patient$^{36}$.

**TISSUE FACTOR PATHWAY INHIBITOR** is a proteinase inhibitor similar in structure to aprotinin (Trasylol) and circulates in plasma bound to lipoprotein. In contrast to the SERPINS inhibition mediated by TFPI is reversible. At high concentration it directly inhibits factor VIIa-TF complex and at lower concentration it inhibits factor FXa - FVIIa / tissue factor complex. It also directly inhibits FXa. Most of the TFPI is associated with and thought to be synthesized by endothelial cells. Platelets contain 8% of TFPI and is released following stimulation by thrombin. Heparin raises the levels of TFPI by 2-4 folds. Heparin also enhances the anti-factor Xa activity of TFPI. Part of the antithrombotic effect of heparin appears to be mediated by TFPI. The level of TFPI is modestly increased in late pregnancy$^{36}$. 
INHIBITORS OF COAGULATION COFACTORs

The activated forms of coagulation co-factors are potent procoagulants. They are subject to negative feedback mechanism to limit their catalytic activity. There are five factors involved in the Protein C system. These include protein C; Thrombomodulin; Proteins S; activated Protein C inhibitor and C4 b binding proteins.

Protein C: Is a vitamin K-dependent serine protease. It is synthesized by the liver as a two-chain molecule comprising a Glycine rich light chain, which is important for its Ca^{2+}-dependent, low affinity phospholipid binding and a heavy chain containing the serine -active center. It must first be activated before it can exert its anticoagulant effect. This is achieved by the action of thrombin, which cleaves the heavy chain, releasing a 12 residue (Gly 158-Arg 169) activation peptide and revealing the active site serine. This action of thrombin is slow in the absence of thrombomodulin. In low concentration, the light chain of factor Va also accelerates thrombin-induced cleavage of Protein C although, high levels are inhibitory. The activated Protein C, inactivates factors Va and VIIIa destroying the prothrombinase and tenase complexes^{36}.

Thrombomodulin is an integral membrane constituent of the endothelial cells. It is synthesized predominantly in the endothelial cells of virtually all the body tissues. It forms a 1:1 stoichiometric complex with thrombin, resulting in altered affinity in the protease for its various substrates. There is a 20, 000 fold increase in the rate of activation of protein C and a concomitant loss of procoagulant
properties (fibrinogen clotting, factors V, VIII and XIII activating and platelet aggregating), so that thrombin effectively becomes an anticoagulant. Enhancement of AT activity by the Chondroitin Sulphate that forms part of the thrombomodulin molecule may also contribute to some loss of thrombin’s procoagulant effects. The binding of protein C to thrombomodulin is enhanced by the recently discovered endothelial protein C receptor.

**Protein S** is a single-chain glycoprotein, a vitamin K dependent factor chiefly synthesized in the liver and the endothelial cells. About 40% is in the plasma and exists as free form while the remaining 60% is associated in a 1:1 complex with C4b-binding protein and does not enhance Protein C function. Both forms bind strongly to negatively-charged phospholipids exposed on the surface of activated platelets. Free protein S although having no direct inhibitory effect on factors Va and VIIIa, forms a calcium dependent complex with activated protein C, enhancing tenfold the latter’s phospholipid binding potential (and thereby its functional activity). Free thrombin cleaves Protein S, preventing its ability to bind to both phospholipid and Protein C, and consequently, abolishing its Protein C cofactor activity.

**The C4b-binding protein** is a large molecule that regulates the activity of the C4b component of complement system. It is an acute-phase reactant and it levels increases during inflammation. The raised level that occurs during severe inflammation can disturb the equilibrium between free and bound protein S, and the result is a fall in the free biologically active form. Free Protein S falls during
pregnancy and to a lesser extent in females receiving estrogen supplements, but it is disputed whether this results from altered reactivity with $C_4b$ binding protein, or if it contributes to hypercoagulability in pregnancy or during estrogen therapy\textsuperscript{36}.

**Activated protein C** inhibitor is also a single-chain glycoprotein, which has several features in common with (though being immunologically distinct from) antithrombin. It forms a 1:1 inhibitor: protease stoichiometric complex. It slowly but progressively blocks the action of activated protein C and to a lesser extent, thrombin and factor Xa. It’s a labile factor and so assay must be performed and interpreted with great caution\textsuperscript{36}.

**FIBRINOLYSIS**

This system is very efficient in preventing excess deposition of fibrin in the vascular bed which can culminate in occlusion of blood vessel. The excess fibrin is either prevented or rapidly removed so as not to compromise blood circulation. It is a localized surface bound phenomenon which is being catalysed by the presence of fibrin.

**The factors involved in fibrinolysis include\textsuperscript{36}:**

1. Tissue plasminogen activator (tPA)
2. Urokinase plasminogen activator (UPA)
3. Streptokinase
4. Plasminogen activator inhibitor type 1 [PA 1-1]
5. Plasminogen activator inhibitor type 2 [PA 1-2]
6. $\alpha_2$-antiplasmin
7. Histidine rich glycoprotein
8. Apoprotein
FIBRINOLYTIC SYSTEM ACCORDING TO HUTTON

**Plasminogen Activators**
- Tissue activator
  - urokinase
  - Streptokinase
  - RBC and WBC activator
  - kallikrein

**Anti-activators**
- Fast acting anti-activator
- Alpha2 macroglobulin
- Anti-urokinase
- Antistreptokinase

**Anti-plasmins**
- Alpha2 antiplasmin
- Alpha2 macroglobulin
- Alpha2 antitrypsin
- C1 esterase inhibitor
- Antithrombin III

Glu-Plasminogen

Glu-Plasmin

Lys-plasminogen

Lys-plasmin

Fibrin(ogen)

Degradation products

Activation pathway

Inhibition pathway
PLASMINOGEN AND PLASMIN

Plasminogen is a single -chain glycoprotein zymogen of the serine protease plasmin. It has a serine active site and five homologous looped structure, four of which have a lysine - binding site’ through which it interacts with lysine residues in its substrates e.g. Fibrin, tissue plasminogen activator urokinase, plasminogen activator and plasminogen activator inhibitor type 1. It has a glutamic acid residue in its native form known as glu-plasminogen. Most plasminogen activators cleave the Arginine 561 - Val 562 bond, to form glu plasmin which is a two chain molecule containing a single disulphide bridge.

The glu-plasmin, although a serine protease is functionally ineffective until its lysine - binding sites is unmasked. The glu-plasmin is converted autocatalytically to lys-plasmin before reacting with fibrin. Both glu-plasmin and lys-plasmin attack the lys 76-lys 77 bond in glu-plasminogen to form lys plasminogen. This binds to fibrin before it develops protease activity and is brought into close proximity with the physiological plasminogen activators that convert it to lys- plasmin. The conversion of plasminogen to plasmin, is enhanced by tissue plasminogen activator, and serves to limit the fibrinolytic response to the fibrin clot, where plasmin is to some extent protected from the effects of circulating antiplasmin.

Tissue plasminogen activator is a single chain glycoprotein synthesized by endothelial cells. It is found in most body fluids including plasma, saliva, milk, bile, cerebrospinal fluids and urine. The half life is approximately 2 minutes. A number of physical and biochemical stimuli, including venous occlusion, strenuous exercise, thrombin, adrenaline, and vasopressin or its analogues such as
DDAVP (1-deamino 8-D arginine vasopressin), markedly increase the rate of tissue plasminogen activator release, although its biological activity remains negligible until it becomes bound to fibrin, whereupon its affinity for and action upon plasminogen is greatly potentiated. Any free tPA in the plasma as opposed to that bound to fibrin is quickly cleared by the liver or inactivated by the fast acting plasminogen inhibitor.36

**FIBRINOLYSIS INHIBITORS**

There should be checks and balances in the action of fibrinolysis otherwise the whole fibrinogen in the body will be completely degraded within a short period leading to haemorrhage.

This is prevented by the presence of inhibitors of plasmin and plasminogen activator which are present in plasma, most of which belong to the serpin family.

**Plasminogen activator inhibitor type 1** - This is synthesized and released from endothelial cells. It is also found in the α-granules of platelets. It inhibits both tissue plasminogen activator and urokinase plasminogen activator. There is formation of 1:1 stoichiometric complex between the fibrinolytic serine proteases and the inhibitor. An elevated level of PAI-1 is associated with an increased incidence of venous and arterial thrombosis. Soluble tPA-PAI-1 complexes are rapidly removed by the liver.

**Plasminogen activator inhibitor type 2** is produced by the placenta; monocytes and epidermal cells. It is not usually found in the plasma of non-pregnant subjects. The inhibitory action of PAI -2 involves its Arg 380- Thr 381 residues. It is more effective against UPA than tPA, although for both, the potency is at least 10 folds
less than that of PAI-1

Other protease inhibitors such as $\alpha_1$ - antitrypsin; C1 - esterase inhibitor, $\alpha_2$ - anti plasmin; $\alpha_2$ - macroglobulin and protease nexin 1 also neutralize tPA, but at a rate probably too slow to be of physiologic significance. **Alpha-2 antiplasmin** is a single chain glycoprotein with molecular weight of 70,000. It shows considerable sequence homology with antithrombin III and alpha I antitrypsin. Its physiological importance is supported by the fact that a congenital deficiency is associated with a clinically significant bleeding disorder due to uncontrolled fibrinolytic activity and that levels are reduced in DIC and during thrombolytic therapy. It is synthesized in the liver and has a half life of about 60 hours and a plasma concentration of around 80 ug/ml (approximately 1umol/L). It forms a stable 1:1 stoichometric complex with plasmin, and the protease completely inactivates plasmin. Alpha2-anti plasmin can also bind to native glu-plasminogen and to fibrin, this reaction is mediated by factor XIIIa-mediated cross linking. Thus in addition to inactivating preformed plasmin; $\alpha_2$-antiplasmin retards fibrinolysis, by reducing plasminogen activation and by masking the lysine binding sites through which plasminogen interacts with fibrin$^{37}$. 


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Normal pregnancy is accompanied by major changes in the coagulation and fibrinolytic system. There are marked increase in fibrinogen and factor VIII levels, while others such as factors VII, IX, X and XII also increase though to a lesser extent. There are significant alterations in the coagulation and fibrinolytic system during pregnancy and these, together with increase in blood volume and the unique phenomenon of myometrial contraction are thought to help in minimizing the hazard of haemorrhage during and after placental separation. However these changes also carry the risk of rapid and excessive response to coagulant stimuli.

Howie in 1979, suggested that during normal pregnancy, the concentrations of many of the clotting factors rise, thereby increasing the potential to generate fibrin. There is also evidence of increased thrombin activity during normal pregnancy, which sharply increases during placental separation. Pregnancy is associated with a hypercoagulable state, which becomes more pronounced in the prenatal period. Delee reported changes in coagulation factors in pregnancy and since then, this subject has received extensive attention in the literature.

The plasma fibrinogen level increases from non-pregnant levels of about 2.5-4.0g/L to as high as 6.0g/L during late pregnancy and labour. Indeed, with increase in plasma volume, circulating fibrinogen levels towards the end of pregnancy approach twice those of non-pregnant state. The plasma fibrinogen
level increases because of its utilization in the uteroplacental circulation\textsuperscript{38}.

The plasma fibrinogen level was also reported by some authors to gradually increase from the second month of pregnancy to reach levels in the range of 3.0-6.0\text{g/L} in late pregnancy and labour. After delivery a further slight increase in fibrinogen level occurs, followed by a decline from around the fifth day of the puerperium to normal values by the 4\textsuperscript{th} week following delivery\textsuperscript{39}.

Kobayashi et al,\textsuperscript{40} in their study reported fibrinogen to be an extremely important factor in pregnancy and that fibrinogen level must be at least 60\text{mg/dl} during pregnancy, 120\text{mg/dl} during surgery and 150\text{mg/dl} during labour. If possible as high as 200\text{mg/dl} to prevent abruptio placentae. Fibrinogen concentrations were always increased in pregnancy with respect to controls\textsuperscript{41}. The increased level of fibrinogen and coagulation factors during pregnancy probably represent a compensatory response to local utilization. The resulting hypercoagulability will be advantageous to meet the sudden demand for haemostatic components at placental separation\textsuperscript{42}.

The increased quantity of fibrinogen in the plasma alters the negative surface charges on the red cells which on standing will form aggregates, this accounts for accelerated erythrocyte sedimentation rate observed during pregnancy\textsuperscript{44}.

The concentrations of high molecular weight fibrin/fibrinogen complexes in the plasma have been demonstrated during normal pregnancy\textsuperscript{45}. This was also confirmed by Mckillop et al\textsuperscript{46}. During the second gestational month, high
molecular weight fibrin/fibrinogen complexes are significantly raised and this preceded the rise in plasma fibrinogen. This result however suggests that intravascular fibrin deposition is the earliest and most persistent alteration in blood coagulation during pregnancy\textsuperscript{39}.

Weiner et al\textsuperscript{,80} reported that fibrinopeptide A increased significantly over control by the end of the first trimester from 1.3ng/ml to 2.8ng/ml and that this continued to increase until 30-32 weeks gestation and then plateaued or stabilized at 4.3-4.7ng/ml, remaining so up to the immediate post partum period. Thrombin generation as reflected by fibrinopeptide A production is increased throughout pregnancy thus confirming a hypercoagulable milieu\textsuperscript{80}.

Factor VIII (antihemophilic factor) is elevated during late pregnancy, the coagulant activity being approximately twice that in non-pregnant state. Some workers have found a parallel increase in both the biological activity of factor VIII and in the factor VIII related antigen\textsuperscript{88}, while others have found an increase in the ratio of antigenic to coagulant activity\textsuperscript{51,89}. The level of factor VIII during pregnancy may be as much as tenfold, an increase in this factor has also been observed in women taking oestrogen/progestogen contraception\textsuperscript{47}.

Walker MC (1997) found that there is a progressive increase in factor VIII level reaching its peak during the third trimester\textsuperscript{48}. In some patients markedly elevated levels of factor VIII and von Willbrand factor have been reported\textsuperscript{34,49}. The rise in factor VIII begins around the second and third month of pregnancy and continues until delivery. In most pregnant women, there is a disproportionate rise
in the level of vWF compared to factor VIII and for this reason, the use of a ratio of these factor for carrier detection of haemophilia can be misleading during pregnancy\textsuperscript{51}.

Kadir et al (1998) in their study also reported that factor VIII and von Willbrand factor antigen and activity levels increased significantly in pregnancy except in those with severe von Willebrand’s disease\textsuperscript{52}.

Eleanor\textsuperscript{33} also reported that the level of vWF increases in most patient with non-type III disease. Thus in patients with functionally normal vWF, labour and delivery usually proceed normally. However patients with type II disease may experience haemorrhagic problems and those with type IIB may experience thrombocytopenia due to the increase in plasma level of abnormal vWF. For this reason, all patients with von Willebrand Disease must be monitored for excessive bleeding particularly during the first week post partum\textsuperscript{53}.

Prothrombin (factor II) is only slightly affected by pregnancy, some investigators having noted small increases, while others have reported normal values\textsuperscript{34}. An increase in the levels of fibrinopeptide A and a thrombin like influence on factor V activity have been described. Sterling and colleagues, also found a slight rise of factor V in early pregnancy followed by a gradual decrease\textsuperscript{55}.

Elevations of factor IX (Christmas factor) during pregnancy, have been reported\textsuperscript{34}. Factor XII was found to be increased in late pregnancy, an increase in factor XII and a decrease in anetithrombin III have been described\textsuperscript{51} as well as a gradual fall in fibrin stabilizing factor reaching 50% of the normal non pregnant
value at term\textsuperscript{56}. 

In contrast to the general increase of other coagulation factors, factor XI has been shown to decrease as pregnancy advances, with average levels of between 60 and 70\% near term, \textsuperscript{57} possibly as a result of increased factor XI consumption.

The antithrombin III, the main inhibitor of thrombin and activated factor X, shows no compensatory rise during pregnancy but increase during the puerperium\textsuperscript{42}. However other studies found lower activity during pregnancy\textsuperscript{58,85,86}. It was reported by Weenik et al, that antithromobin III plasma levels are in the normal range during normal pregnancy but significant depression of antitrombin III does occur in pregnancy-induced hypertension and the decrease is proportional to the degree of both maternal and fetal morbidity\textsuperscript{60}.

The risk of thrombo-embolism in women with congenital antithrombin III deficiency is further increased during pregnancy. \textsuperscript{61,62,63} Pregnancy per se, obstetric complications and delivery may however lower antithrombin III in women without an inherited deficiency\textsuperscript{64,65,66}. Heparin has been used successfully for prophylaxis and treatment of women in pregnancy with normal antithrombin III levels, \textsuperscript{67,68,69} and prophylaxis of thrombo-embolism with heparin and other agents have also been reported in women with congenital or acquired antithrombin III deficiency\textsuperscript{61,63}.

The level of protein S is decreased during pregnancy reaching half the basal level at term. There is increased level of plasminogen activator inhibitor in pregnancy\textsuperscript{36}.

The level of $\alpha_i$ antitrypsin rises steeply from early pregnancy, antithrombin
III, (antifactors Xa) and α2-Macroglobulin either remains unchanged or showing a slight decrease.

The platelet count and function during pregnancy has generated a lot of controversy. Some authors reported no significant change compared with the normal non-pregnant state, while others reported a significant decrease in the numbers of circulating platelets as pregnancy advances. Fay et al. showed a significant fall in the platelet count during the last 8 weeks of pregnancy, although the level is still within the normal non-pregnant range. In pregnancies complicated by preeclampsia and intrauterine growth retardation, there is a significant fall in platelet count. Other workers have documented higher random platelet count in the majority of pregnant and post partum women than non-pregnant state leaving 5% of per parturient women as being thrombocytopenic. In another series, 8% of pregnant women have been reported to have gestational thrombocytopenia while some other thrombocytopenias in pregnancy result from underlying pathology other than pregnancy for example associated idiopathic thrombocytopenic purpura and immune thrombocytopenia associated with antiphospholipid antibodies. It has been suggested that the occurrence of thrombocytopenia in labour or prepartum might be related to the low grade “physiologic DIC” that might accompany normal delivery.

Also reported in normal pregnancy is reduced plasminogen activator activity leading to reduce fibrinolytic activity. Plasma fibrinolytic activity is decreased during pregnancy, remaining low during labour and delivery and returning to normal within one hour of placental delivery.

The rapid return of systemic fibrinolytic activity to normal following delivery
of the placenta together with the fact that the placenta has been shown to contain inhibitors which block fibrinolysis suggest that inhibition of fibrinolysis during pregnancy is mediated through the placenta\textsuperscript{83}.

Pregnancy, delivery and puerperium are associated with complex and still incompletely understood physiological changes involving the blood coagulation and plasma fibrinolytic system\textsuperscript{39}. Plasminogen levels increase concomitantly with fibrinogen level causing an equilibration of clotting and lysing. Clearly, coagulation and fibrinolytic systems undergo major alterations during pregnancy. Understanding of these physiologic changes is necessary to manage two of the more serious problems of pregnancy i.e. thrombo-embolism and disseminated intravascular coagulation.
TESTS OF HAEMOSTATIC FUNCTION

Tests for Assessment of haemostasis are classified according to the components of haemostatic function being tested.\textsuperscript{78}

TESTS OF VASCULAR INTEGRITY

1. Hess test or Rumple - leaden test
2. Bleeding time which also assesses platelet quantity and quality

TESTS FOR PLATELET FUNCTION

Platelet count

Bleeding time - IVYS method
- Standardized Template method

Adhesion test - Retention in a glass - bead column
(Baumgartner’s technique)

Clot retraction

Aggregation tests - Turbidiometric technique using
ADP, Collagen, Ristocetin.
Adrenaline, Thrombin; Arachidonic acid

INVESTIGATION OF GRANULAR CONTENTS AND RELEASE

Dense bodies - Electron microscopy

ADP and ATP content (Bioluminescence) serotonin release

Granules - B- thromboglobulin
- Platelet factor 4
- von Willebrand factor
PROSTAGLANDIN PATHWAY.

- Thromboxane B₂ - Radio immunoassay
- MDA assay using thiobarbituric acid
- Studies with radio active arachidonic acid

PLATELET COAGULANT ACTIVITY

3 Prothrombin consumption index.

TESTS FOR COAGULATION PROFILE

(a) Plasma Coagulation factors

4 Prothrombin time [PT]

5 Partial Thromboplastin time with Kaolin (PTTK)

6 Thrombin time (TT)

7 Factor assays

8 VIII antigen (By electro immunoassay)

9 Ristocetin co-factor (von Will brands factor)

(b) Anticoagulants

10 Antithrombin III assay using thrombin

11 Antithrombin III measurement using a chromogenic assay

12 Measurement of functional protein C by the protac method

13 Assay of Protein S by electrophoresis

14 Heparin cofactor II assay
TESTS OF FIBRINOLYTIC SYSTEM

15 Chromogenic assay for plasminogen
16 Euglobulin lysis time
17 Lysis of fibrin plates
18 Venous occlusion test
19 Tissue plasminogen activator (t-PA) (Amidolytic assay)
20 Plasminogen activator inhibitor (PA I -1) assay
21 $\alpha_2$ - Antiplasmin - Amidolytic assay
22 FDP latex agglutination, Immunoelectrophoresis
23 D - DIMER latex assay (DIMER TEST).
MATERIALS AND METHODS

The study was prospective and cross-sectional.

SUBJECT SELECTION

Pregnant women attending antenatal clinics of the University of Ilorin Teaching Hospital (Maternity wing) at different trimesters of pregnancy were selected. A total of 8 to 12 consecutive patients who fit into the inclusion criteria were selected every week. (The study duration was 6 months)

Sample Size: The sample size was determined by using this formula \(^{112}\):

\[ nf = \frac{n}{1 + \frac{n}{N}} \]

Where

- \( nf \) = The desired sample size when population is less than 10,000.
- \( n \) = The desired sample size when the population is more than
  - 10,000 \((= 400)\)
- \( N \) = The estimate of the population size

(An average of 80 patients are normally booked for antenatal every week, therefore, about 4,160 patients are booked annually.)

\[ nf = \frac{400}{1 + \frac{400}{4,160}} = \frac{400}{1.096} = 364 \]

Since the study duration was 6 months then the sample size was half of that number.

i.e minimum sample size \( = 182 \).

1) 60 healthy pregnant women with gestational period (6-13 weeks) were used
for first trimester.

2) 60 healthy pregnant women with gestational period (14-26 weeks) served for second trimester.

3) 60 healthy pregnant women with gestational period (27-39 weeks) were used for third trimester

(The abdominal ultrasound and last menstrual period were used to classify the subjects into various trimesters.)

CONTROL SUBJECTS

Sixty age-matched non-pregnant, non-hypertensive women were recruited as controls including laboratory staff, students of school of Health Technology Offa, Medical students, Student Nurses. Their pregnancy status was established by doing pregnancy test and only those who are negative were included. The controls were not on hormonal contraceptives. Exclusion criteria were the same as for the study group. Verbal and written consent was obtained in each case.

INCLUSION CRITERIA

To be included in the study, subjects were aged between 17-40 years who were pregnant with gestational period between 6-42 weeks and certified fit based on careful history taking and physical examination which were documented on a protocol form for each subject.

EXCLUSION CRITERIA

Subjects with the following conditions were excluded from the study;
- Pregnancy-induced Hypertension  
- Gestational diabetes mellitus  
- Sickle cell disease  
- History of thrombophlebitis, thromboembolic disorders or a past history or these conditions.  
- Liver disease in pregnancy  
- Varicose veins in the legs  
- Drugs that can affect haemostatic parameters such as Aspirin, Dicumarol etc.

**ETHICAL CONSIDERATION**

Subjects verbal and written consent after thorough explanation of the procedure were obtained before samples were collected. The hospital ethical and research committee approval was obtained before commencing the study.

**SPECIMEN COLLECTION**

After informed consent was obtained and after certification of the subject as being fit to participate in the study, about 7.0ml of venous blood sample was taken from the antecubital vein (between 8.00-10.00am), with adequate antiseptic skin preparation and minimal stasis.

The blood sample collected was then dispensed into two separate covered bottles as follows.  
4.5ml of blood into a bottle containing 0.5ml of 3.8% trisodium citrate (1 part of citrate to 9 parts of blood). The sample was gently mixed to avoid lysis and then centrifuged for about 10 minutes at 2000 rpm to obtain platelet poor plasma. The
plasma was carefully aspirated using a plastic pasteur pipette and used immediately for the coagulation tests, or kept on ice until analysed. The separation of the sample was done within 2 hours of collection.

2.5 ml of the remaining blood was dispensed into a bottle contain EDTA (Ethylene diamine tetra acetic acid) as anticoagulant. The sample was thoroughly mixed and used for platelet counts. The control blood samples were treated the same way.

**METHODS OF ANALYSIS.**

The following tests were carried out on all the blood samples.

(1) Prothrombin Time
(2) Partial thromboplastin time with kaolin
(3) Fibrinogen concentration estimation
(4) Euglobulin clot lysis time
(5) Platelet counts
PROTHROMBIN TIME

This was determined using commercially prepared reagents based on the one stage test of Owren\textsuperscript{77}.

PRINCIPLE

Prothrombin time is the time in seconds, required for plasma to clot at 37\textdegree{}C, when tissue thromboplastin (an external coagulation factor) and calcium are added. The test indicates the overall efficiency of the extrinsic clotting system, and assesses in addition to prothrombin other factors such as factors V, VII, X and fibrinogen.

REAGENTS

Patients and control plasma samples
Calcified-tissue thromboplastin (Plasmascann\textsuperscript{®} freeze-dried commercial preparation, product of Quimica Clinica Aplicada S.A. Amposta/Spain). This was reconstituted with de-ionised water.

METHOD

The procedure described by Dacie and Lewis was followed\textsuperscript{78}.

(1) The freeze-dried calcified-tissue thromboplastin was reconstituted with de-ionized water as directed by the manufacturer. This was gently mixed and warmed to 37\textdegree{}C in a water bath for 2 minutes. Thromboplastin from the same batch was used throughout the study.

(2) 0.1ml of the test plasma was dispensed into a 75mm x 12mm glass tube placed in a rack in the water bath at 37\textdegree{}C. This was warmed for 2 minutes.
(3) 0.2ml of the warmed reconstituted calcified-tissue thromboplastin was added to the plasma.

(4) A stop watch was started immediately the calcified thromboplastin was added and time taken for clot to form was recorded.

(5) The procedure above was carried out on the test and control plasma in duplicate.

The prothrombin time was expressed as the mean of the duplicate readings in seconds.
PARTIAL THROMBOPLASTIN TIME WITH KAOLIN [PTTK]

This was determined using commercially prepared reagents based on the method of Proctor and Rapaport (1961).\textsuperscript{79}

**PRINCIPLE**

The test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin. It indicates the overall efficiency of the intrinsic pathway. To standardize the activation of contact factors, the plasma is first pre-incubated with kaolin. Standardized phospholipids are provided to allow the clotting reaction to be performed on platelet poor plasma. The test depends not only on the contact factors and factor VIII and IX, but also on the reactions with factors X, V, prothrombin and fibrinogen. It is also sensitive to the presence of circulating anticoagulants (inhibitors) and heparin. (Dacie and Lewis 1991).\textsuperscript{78}

**REAGENTS**

Test and control plasma samples prepared as described previously

Standardized Kaolin-phospholipids reagent (commercially prepared Obtained from Quimica Clinica Aplicada S.A. Amposta/ Spain).

Calcium chloride 0.025m (Product of Dade diagnostics Inc. Agued, Puerto Rico).

**METHOD**

The procedure described by Dacie and Lewis was followed\textsuperscript{78}:

- Calcium chloride 0.025m was dispensed into a test tube and warmed for 3 minutes at 37\textdegree c in a water bath.

- 0.1ml of test plasma, and 0.1ml of the kaolin-phospholipid reagent was also
pipetted into a pre-warmed 75mmx12mm glass tube, and then incubated for 6 minutes
- 0.1ml of pre-warmed CaCl$_2$ was then mixed with the sample and a stopwatch was started simultaneously.
- After 20 seconds, the tube was lifted up from the water bath and tilted gently. This was repeated at interval of 2-3 seconds until a clot is observed and the stop watch was stopped. The time taken for clot to form was recorded. This procedure was carried out in duplicate both on patient and control samples. The results were expressed as the mean of the paired clotting time for the test and control.

C  FIBRINOGEN ESTIMATION

This estimation was done by dry clot weight method of Ingram 1951$^9$.  

PRINCIPLE

The quantity of clot formed is proportional to the fibrinogen concentration.

REAGENTS

Citrated plasma - patient and control sample

0.025M Calcium chloride (Stored at 4$^0$C)

METHOD

1. 1.0ml of plasma was pipetted into a 75x12mm glass tube and then warmed to 37$^0$C in a water bath.

2. A wooden applicator or swab stick was placed in the tube, and 1ml of CaCl$_2$ (In lieu of thrombin) was added to the tube. The reagent was mixed together
and incubated at 37°C for 30 minutes

3. The fibrin clot formed was wound onto the swab stick or applicator, and excess serum was squeezed out. The clot was carefully washed under running tap to remove any serum left that could lead to lysis of the clot so formed.

4. The clot was carefully blotted with filter paper and the fibrin clot was removed from the stick.

5. The clot was allowed to dry in a hot air over for 30 minutes. This was allowed to cool and then weighed

The fibrinogen level was expressed as g/L (i.e. the weight of fibrin obtained from 1ml of plasma x 1000)

D  EUGLOBULIN LYSIS TIME

] PRINCIPLE

When plasma is diluted and acidified, the precipitate which is forms contains plasminogen activator, plasminogen and fibrinogen. Most of the inhibitors are left in the solution. The precipitate is redissolved, the fibrinogen is clotted with calcium chloride and the time for clot lysis measured.
Reagents

- Patients and control plasma samples
- CaCl₂ (0.025M) 2.8g/l of distilled water stored at 4°C
- 1% (cv/v) acetic acid from which 13.5ml was diluted to 1000ml with distilled water to give a pH of 3.67 (Normal range 3.66-3.70)
- Borate buffer at Ph 7.8 (9g NaCl₂ + 1g Sodium Borate in 1 liter of distilled water)

Method

Test was carried out in duplicate using a clean conical centrifuge tubes. 0.5ml of plasma was added to 9.5ml of 0.01% acetic acid, the solution was mixed properly and incubated at 4°C for 30minutes to precipitate the euglobulin fraction. It was then centrifuged at 1200 rpm for 10minutes.

The supernatant was discarded and tubes were inverted over the filter paper. 0.5ml of borate buffer was added to dissolve the precipitate.

Both the 0.025M CaCl₂ and the reconstituted euglobulin fraction was warmed to 37°C in a water bath.

0.5ml of the warmed CaCl₂ solution was added to the euglobulin fraction and tubes maintained at 37°C. The timing was started on the observation of clot. ELT was the time taken for the euglobulin clot to lysed completely. Time was recorded in minutes (Average of the time for the test and control was taken). Normal value (90-240minutes).
PLATELET COUNT

The platelet count was determined by using an automated blood cell counter, Sysmex KX 21. (Product of Sysmex Corporation Kobe, Japan).

DETECTION PRINCIPLE

This instrument performs blood cell count by Direct Current (DC) detection method.

DC DETECTION METHOD

Blood sample is aspirated, measured to a predetermined volume diluted at the specified ratio, then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are electrodes between which flows direct current. As blood cells suspended in the diluted sample pass through the aperture, the direct current resistance changes between the electrodes, and each change is registered as a pulse. The magnitude of the pulse is also proportional to the size of cell passing through the aperture. A histogram of pulse sizes is produced by the counter from which the average size of blood cells can be determined. Various other parameters can also be obtained by analyzing other histograms produced by the counters.

RBC/PLT Analyzing Flow in Whole Blood Mode.

1. Blood is aspirated from the sample probe into the sample rotor valve.
2. 4.0μl of blood measured by the sample rotor valve is diluted into 1:500 with 1.996ml of diluents and brought to the mixing chamber as diluted sample (1st
Step dilution).

3. Out of the 1:500 dilution samples, 40\(\mu\)l is measured by the sample rotor valve, diluted into 1:25000 with 1.966ml of diluents, and then transferred to the RBC/PLT transducers chamber (2\textsuperscript{nd} step dilution).

4. 250\(\mu\)l of the sample in the RBC/PLT transducer chamber is aspirated through the aperture. At this time RBC and Plt are counted by the DC detection method.

   The platelet count is calculated from the particle counts between the lower discriminator and upper discriminator, the optimum position in 2-6fI and 12-30fI respectively which are automatically determined by the microcomputer.
ANALYSIS OF DATA AND RESULTS

Statistical analysis of data was done using statistical package for social sciences (SPSS) and statistical significance of differences was based on p value of less than 0.05. Result was presented in tables and figures where applicable. Comparisons was made using standard statistical methods in which categorical data was compared by Chi-square and discrete variables by T-test; 95 percent confidence level was observed. Conclusion and recommendations were based on scientific evidence from the results.
RESULTS

One hundred and eighty pregnant women were studied for haemostatic parameters i.e. prothrombin time (PT); Partial thromboplastin time with kaolin (PTTK); Fibrinogen level (FIB); Euglobulin clot lysis time (ELT) and platelet count (PLT).

The study period was between May and October of the same year. The subjects were within the age range of 18-40 years with a mean age of 27.1± 3.9 years. The mean age of subjects in the first trimester was 27.6± 3.2yrs, mean age for subjects in the second trimester was 26.3± 4.1yrs, while the mean age of subjects in the third trimester was 27.5 ± 4.8 years (Table2).

Sixty non-hypertensive, non pregnant women served as controls. The mean age of control was the same as for the subjects The exclusion criteria were similar to that of the subjects.

The gestational age for the subjects ranged between 6-42 weeks. Subjects were grouped into the three trimesters of pregnancy with sixty with gestational age between 6-13 weeks grouped as 1st trimester, another sixty pregnant women with gestational age between 14-26 weeks were grouped as being in second trimester, while the remaining sixty subjects with gestational age 27 weeks and above made up those in the 3rd trimester.

Ten of the subjects (5.6%) had no formal education, thirty-five (19.4%) had primary education, seventy-three completed secondary school (40.6%), and sixty-two subjects (34.4%) went beyond secondary school level. (Table 3).
Sixty nine subjects (38.3%) were primigravida; one hundred and one (56.1%) were multigravida; while ten subjects (5.6%) were grandmultigravida. (Table 4).
**HAEMOSTATIC PROFILE**

**Prothrombin time (PT)**

The subjects had a mean PT of $12.7 \pm 1.7$ sec while the controls had a mean of $13.6 \pm 1.3$ secs. The difference was statistically significant, p value of 0.000 (Table 5).

The prothrombin time was significantly affected by the trimester of pregnancy. The mean prothrombin time for the subjects in the first trimester was $13.2 \pm 1.9$ secs, mean for the subjects in the second trimester was $12.7 \pm 1.5$ secs while those in the third trimester had mean PT of $12.1 \pm 1.5$ secs, P value of 0.001 (Table 6).

**Partial Thromboplastin Time with Kaolin (PTTK)**

There was significant difference between the PTTK among that of the subjects and controls. The mean PTTK of subjects was $36.1 \pm 5.4$ s, while that of control was $41.6 \pm 5.9$ secs. p value of 0.000.

The PTTK among the subjects was not affected by the trimester of pregnancy. The mean PTTK of subjects in the first trimester was $35.7 \pm 6.4$ s, mean PTTK for subjects in the second trimester was $35.6 \pm 4.9$ secs while those in the third trimester had mean PTTK of $36.8 \pm 4.9$ secs p value 0.405 (table 6).

**Fibrinogen Level**

There was a significant difference between the fibrinogen level among the subjects and that of the controls.

The mean fibrinogen level in the subjects was $4.8 \pm 1.5$ g/L while in the
control it was $3.2 \pm 1.1\text{g/L}$ p value 0.000 (table 5)

The mean fibrinogen level for subjects in the first trimester was $4.1 \pm 1.5\text{g/L}$, those in the second trimester had mean fibrinogen level of $5.1 \pm 1.7\text{g/L}$ and those in the last trimester had mean fibrinogen level of $5.2 \pm 1.3\text{g/L}$.

There was significant increase in the mean fibrinogen level as pregnancy advanced. P value of 0.000 {table 6}.

**Euglobulin clot lysis time (ELT)**

There was significant difference between the ELT among the subjects and controls. The mean ELT for subject was $228.3\pm 65.9$ minute while that of control was $192.9\pm 56.4$ minutes p value of 0.000 (table 5)

There was no significant difference between the ELT among the subjects in various trimester. P values 0.770.

The mean ELT for subjects in the first trimester was $225.4\pm 64.9$ minute, the mean ELT for second trimester was $226.3\pm 73.7$ minutes, while mean ELT of $228.4\pm 65.9$ minutes was for subjects in the third trimester. {Table 6}

**Platelet Count**

There was no significant difference between the platelet count in controls and that of the subjects.

The mean platelet count in control was $167.6\pm 63.9 \times 10^9/\text{L}$ while in subject the mean was $172.3\pm 66.3 \times 10^9/\text{L}$(p values of 0.057).
The mean platelet count for subject in the first trimester was \(164.5 \pm 59.9 \times 10^9/L\), mean platelet count for subject in the second trimester was \(168.0 \pm 64.9 \times 10^9/L\), while the subject in the third trimester had a mean platelet count of \(170.4 \pm 66.9 \times 10^9/L\). There was no significant difference between the platelet count among the subjects in various trimester, \(p\) values of 0.880.

**Effect of Age on haemostatic parameters**

The fibrinogen level was found to increase with age of subjects. The mean fibrinogen level in younger subjects (18-27 years) was 4.0g/l while the mean fibrinogen level in the older subjects (28-40 years) was 5.5g/l\(p\) value=0.023). The PT,PTTK,ELT,and platelet count were not affected by the age .\(p\) value=0.268,0.799,0.217 and 0.678 respectively)

**Effect of Educational level on Haemostatic parameters**

There was no significant difference between the haemostatic parameters of the subjects at the different levels of education.

In the group of women with no formal education, the means of haemostatic parameters were – prothrombin time (12.6 \(+1.6\) sec), PTTK (33.0 + 5.2sec); Fibrinogen level (4.7 \(+1.4\) g/L); ELT [223.2 \(+64.3\) min] and platelet count of 170.8 \(+27.9\times10^9/L\)

The mean haemostatic parameters for subjects with primary school education were – prothrombin time (12.3 \(+1.5\) sec); PTTK (35.9 \(+5.8\)sec); fibrinogen (5.1 \(+
1.4g/L) ELT (245.5 ± 48.4min) and platelet count (180.4 ± 61.8 x 10^9 / L).

In the subjects with secondary education, the mean haemostatic parameters were
- PT (12.9 ± 1.7sec); PTTK (36.2 ± 5.4sec); fibrinogen (4.9 ±1.8g/L); ELT (223.0 ±74.2.min); and platelet count of (166.2±70.1/ x 109/L).

In the subjects with tertiary education, the mean haemostatic parameters were
PT (12.6 ± 1.2s); PTTK (41.7 ± 6.6s); fibrinogen level (3.0 ± 1.1.g/L); ELT (195. 9 ± 59.5 min) and platelet count (189. ± 70.1 x 10 g/L).

The differences in the mean of all the haemostatic parameters were found not to be statistically significant with the P value of 0.443; 0.182; 0.835; 0.380 and 0.515 respectively.

**Effect of Parity on Haemostatic Parameters**

There was no significant difference between the parity of the subjects and their haemostatic parameters.

In the nulliparous women the mean haemostatic parameters were
- PT 12.8 ± 1.8sec, PTTK 36.8 ± 5.2sec, Fibrinogen 4.8 ±1.8g/L, ELT 223.2± 67.9min and platelet count 157.4 ± 67.9 x 10 9/L.

In the multiparous subjects[parity 1-4] the mean haemostatic parameters are
PT 12.6 ± 1.7sec PTTK 35.6 ± 5.5sec, fibrinogen 4.7 ± 1.4g/L; ELT 231.1+ 66.0min and platelet count of 175.7± 65.0 x 10^9/L.
In the grand multiparous women [parity above 5] the mean haemostatic parameters were PT 12.6 ± 1.1 sec; PTTK 36.0 ± 6.5 sec, fibrinogen 5.1 ± 1.3 g/L; EL T 236.8 ± 52.6 min and platelet count of 167.6 ± 63.7 x 10^9/L.

The differences in the mean of all the haemostatic parameters in the parity group were found not to be statistically significant with the p – value of 0.820; 0.350; 0.750; 0.696 and 0.161 respectively. [table 8]
### TABLE 1  THE MEAN AGE OF SUBJECTS AND CONTROLS

<table>
<thead>
<tr>
<th>STATUS</th>
<th>MEAN</th>
<th>N</th>
<th>STD.DEVIATION (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.8</td>
<td>60</td>
<td>5.4</td>
</tr>
<tr>
<td>Subjects</td>
<td>27.1</td>
<td>180</td>
<td>3.9</td>
</tr>
<tr>
<td>Total</td>
<td>26.9</td>
<td>240</td>
<td>4.6</td>
</tr>
</tbody>
</table>

N - number of patients

P-value 0.187
## TABLE 2

THE MEAN AGE OF SUBJECTS ACCORDING TO THE TRIMESTER

<table>
<thead>
<tr>
<th>GAGR</th>
<th>MEAN</th>
<th>NUMBER</th>
<th>STD.DEVIATION (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>27.6</td>
<td>60</td>
<td>3.2</td>
</tr>
<tr>
<td>B</td>
<td>26.3</td>
<td>60</td>
<td>4.1</td>
</tr>
<tr>
<td>C</td>
<td>27.5</td>
<td>60</td>
<td>4.2</td>
</tr>
<tr>
<td>Total</td>
<td>27.1</td>
<td>180</td>
<td>3.7</td>
</tr>
</tbody>
</table>

P – value 0.180;  
GAGR: gestational age group

A - First trimester
B - Second trimester
C - Third trimester
TABLE 3

EDUCATIONAL LEVEL OF SUBJECTS

<table>
<thead>
<tr>
<th>Level of education</th>
<th>Number</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10</td>
<td>5.6</td>
</tr>
<tr>
<td>Primary</td>
<td>35</td>
<td>19.4</td>
</tr>
<tr>
<td>Secondary</td>
<td>73</td>
<td>40.6</td>
</tr>
<tr>
<td>Tertiary</td>
<td>62</td>
<td>33.4</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>100%</td>
</tr>
</tbody>
</table>
TABLE 4

PARITY DISTRIBUTION OF SUBJECTS

<table>
<thead>
<tr>
<th>Parity</th>
<th>Number</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>69</td>
<td>38.3</td>
</tr>
<tr>
<td>1-4</td>
<td>101</td>
<td>56.1</td>
</tr>
<tr>
<td>5 and above</td>
<td>10</td>
<td>5.6</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>100%</td>
</tr>
</tbody>
</table>
**TABLE 5**

**THE MEAN HAEMOSTATIC PARAMETERS AMONG SUBJECTS AND CONTROLS**

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Subjects</th>
<th>Number</th>
<th>Control</th>
<th>Number</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Prothrombin time sec (PT)</td>
<td>12.7±1.7</td>
<td>180</td>
<td>13.6±1.3</td>
<td>60</td>
<td>0.000</td>
</tr>
<tr>
<td>2. Partial thromboplastin time with kaolin (PTTK) s</td>
<td>36.1±5.4</td>
<td>180</td>
<td>41.6±5.9</td>
<td>60</td>
<td>0.000</td>
</tr>
<tr>
<td>3. Fibrinogen level g/L</td>
<td>4.8±1.5</td>
<td>180</td>
<td>3.2±1.1</td>
<td>60</td>
<td>0.000</td>
</tr>
<tr>
<td>4. Euglobulin clot lysis time (min)</td>
<td>228.4±65.9</td>
<td>180</td>
<td>192.9±56.5</td>
<td>60</td>
<td>0.000</td>
</tr>
<tr>
<td>5. Platelet count x 10^9/L</td>
<td>167.6±63.7</td>
<td>180</td>
<td>172.3±66.3</td>
<td>60</td>
<td>0.057</td>
</tr>
</tbody>
</table>
### TABLE 6

MEAN HAEMOSTATIC PARAMETERS OF SUBJECTS IN RELATION TO TRIMESTERS.

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; trimester</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; trimester</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; trimester</th>
<th>Sign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time (s)</td>
<td>13.2±1.9</td>
<td>12.7±1.5</td>
<td>12.1±1.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Partial thromboplastin time with kaolin (s)</td>
<td>35.7±6.4</td>
<td>35.6±4.8</td>
<td>36.8±4.9</td>
<td>0.405</td>
</tr>
<tr>
<td>Fibrinogen level g/L</td>
<td>4.1 ±1.5</td>
<td>5.1±1.7</td>
<td>5.2±1.3</td>
<td>0.000</td>
</tr>
<tr>
<td>Euglobulin clot lysis time (min)</td>
<td>225.4±64.9</td>
<td>226.3±73.8</td>
<td>228.4±65.9</td>
<td>0.770</td>
</tr>
<tr>
<td>Platelet x 10&lt;sup&gt;9&lt;/sup&gt;/L</td>
<td>164.5±59.9</td>
<td>168.0±64.9</td>
<td>170.3±66.9</td>
<td>0.880</td>
</tr>
</tbody>
</table>
### TABLE 7

**EFFECT OF EDUCATIONAL LEVEL ON HAEMOSTATIC PARAMETERS**

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>No formal education</th>
<th>Primary</th>
<th>Secondary</th>
<th>Tertiary</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Prothrombin time (sec)</td>
<td>12.6±1.6</td>
<td>12.3±1.5</td>
<td>12.9±1.7</td>
<td>12.7±1.8</td>
<td>0.432</td>
</tr>
<tr>
<td>2. Partial thromboplastin time with Kaolin (sec)</td>
<td>33.0±5.2</td>
<td>35.9±5.8</td>
<td>36.2±5.4</td>
<td>36.5±5.4</td>
<td>0.307</td>
</tr>
<tr>
<td>3. Fibrinogen level g/L</td>
<td>4.7±1.4</td>
<td>5.1±1.4</td>
<td>4.9±1.8</td>
<td>4.5±1.4</td>
<td>0.243</td>
</tr>
<tr>
<td>4. Euglobulin lysis time (min)</td>
<td>223.7±64.3</td>
<td>245.5±48.4</td>
<td>223.0±74.2</td>
<td>225.8±64.3</td>
<td>0.380</td>
</tr>
<tr>
<td>5. Platelet x 10^9/L</td>
<td>170.8±27.9</td>
<td>180.4±61.8</td>
<td>166.2±70.1</td>
<td>171.3±56.8</td>
<td>0.394</td>
</tr>
</tbody>
</table>
### TABLE 8

**EFFECT OF PARITY ON HAEMOSTATIC PARAMETERS**

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Nulliparous</th>
<th>Multiparous</th>
<th>Grandmultiparous</th>
<th>P value</th>
</tr>
</thead>
</table>
| 1. Prothrombin time (sec) | 12.8±1.8  
 | 12.6±1.7  
 | 12.6±1.1  
 | 0.820 |
| 2. Partial thromplastin time Kaolin (sec) | 36.8±5.2  
 | 35.6±5.5  
 | 36.0±6.5  
 | 0.350 |
| 3. Fibrinogen level g/L | 4.8±1.8  
 | 4.7±1.4  
 | 5.1±1.3  
 | 0.750 |
| 4. Eugloulin lysis time (min) | 223.2 ± 67.9  
 | 231.1 ± 66.0  
 | 236.0 ± 52.6  
 | 0.696 |
| 5. Platelet count x 10⁹/L | 157.4±59.6  
 | 175.6±65.0  
 | 167±63.7  
 | 0.161 |
DISCUSSION

During normal pregnancy, the haemostatic balance changes in the direction of hypercoagulability thus decreasing bleeding complications in connection with delivery.  

The changes in the coagulation system during normal pregnancy are consistent with a continuing low-grade process of coagulant activity.

The hormones which are necessary for maintenance of pregnancy i.e. estrogen and progesterone increase many folds and these especially estrogen stimulate hepatocytes thereby increasing the production of virtually all coagulation factors. The progesterone also increase decidual tissue factor and increase synthesis of plasminogen activator inhibitor type 1.

Elevation of the levels of certain coagulation factors and the fibrinolytic inhibitors occur in practically all healthy pregnant women which is most likely the result of small amounts of procoagulant factors such as tissue thromboplastin which could cause direct and slow systemic activation of the coagulation cascade.

In this study, the results of coagulation screening tests that were carried out were in support of a hypercoagulable state in pregnancy.

The prothrombin time, which assesses the factors in the extrinsic pathway i.e. factor II VII IX and X, was reduced when compared to the value in non-pregnant controls. This result is consistent with increase in coagulation factors that are reported to occur in pregnancy.

The findings in the study are similar to those of Hellgren in 2003, who
observed increase in prothrombin complex level (prothrombin time) expressed as international normalized ratio (INR) of less than 0.9. Similarly, Uchikova et al, 2005, reported prothrombin time as being statistically significantly shortened in pregnancy compared with control. By contrast however, the work of Adediran et al, 1999 in Ile-Ife showed prolongation of prothrombin time in the face of what was otherwise a hypercoagulable state.

In this study, the mean prothrombin times in the subjects in the 1st, 2nd and 3rd trimesters of pregnancy showed that production of these coagulation factors progresses as pregnancy advanced, as there was statistically significant reduction in prothrombin time from the 1st to the 3rd trimesters of pregnancy (P value 0.001).

The progressive shortening of prothrombin time as pregnancy advanced may also be due to the coagulant promoting effect of estrogen which is produced in many folds in maintenance of pregnancy. Exogenous estrogen as found in oral contraceptive pills has been shown to accumulate in the liver and to stimulate the synthesis of clotting factors, the synthesis of fibrinogen, factors II, VII and X being more greatly increased than that of factors VIII and IX. 102,103,106.

There was no correlation between the prothrombin time and the age, parity and educational level of subjects in this study.

Significant difference was also noticed in the partial thromboplastin time with Kaolin (PTTK) among the subjects and controls, which shows that levels of factors in the intrinsic pathway are also increased in normal pregnancy.

The PTTK was found to be reduced in pregnant women, a finding which is
similar to that of Hellgren 2003\textsuperscript{95} who reported slightly decreased activated partial thromboplastin time and increased prothrombin complex level. There was no statistically significant difference in the result of PTTK in various trimesters of pregnancy. This may probably be due to the fact that the estrogen – induced stimulation of factors VIII and IX production is much less than for the extrinsic pathway factors. Furthermore estrogen has less effect in stimulating endothelial cells and macrophages, which are also sites of production and storage of F VIII.

Absence of significant difference in PTTK as pregnancy advanced may also be due to the technique of the test. In the PTTK test, there are more activating steps than in the prothrombin time test, and the longer the time it takes to perform, the less sensitive that test is due to extraneous interferences.

There was no statistically significant difference between the PTTK among subjects with formal education and those without and PTTK was not affected by the age and parity of the subject.

The fibrinogen level was significantly elevated in normal pregnancy and the level increased as pregnancy advanced. This finding is similar to that of Adediran et al \textsuperscript{91}, who found significantly higher value for fibrinogen in normal pregnancy.

Similar findings were reported by Hellgren\textsuperscript{95} and Kobayashi\textsuperscript{40} who concluded that increased fibrinogen was an important factor in pregnancy. As pregnancy advanced, increased synthesis of fibrinogen leads to deposition of fibrin in the intervillous space of the placenta and the elastic lamina and smooth muscle of these spiral arteries are replaced by a matrix containing fibrin which allows an
expansion of the vascular lumen to accommodate an increasing blood flow and reduces the pressure in arterial blood flowing to the placenta\textsuperscript{38}. At placental separation, rapid closure of the terminal part of the spiral arteries by the unique mechanism of myometrial contraction will be facilitated by the structural changes in those arteries and placental site will be rapidly covered by a fibrin mesh to prevent post partum haemorrhage. 5-10\% of the total circulatory fibrinogen is deposited in the placental site. The increased levels of fibrinogen and coagulation factors during pregnancy represent a compensatory response to local utilization and the resulting hypercoagulability will be advantageous to meet the sudden demand for haemostatic components at placental separation\textsuperscript{42}.

There was statistically significant difference in the fibrinogen level in the older age group compared with subjects of younger age group. P value 0.023.

The increase in fibrinogen observed with age is well supported by various studies in a number of populations\textsuperscript{107, 108}. The increase in fibrinogen with age is thought to be mainly attributable to a slower rate of disposal rather than increased synthesis\textsuperscript{97, 110}. Fibrinogen is an acute-phase protein and a central component of coagulation cascade, as well as being an important determinant of blood viscosity, which affects blood flow. Fibrinogen level is also affected by external factors including gender, age, body mass index, physical exercise, smoking, stress and alcohol consumption\textsuperscript{109}. There was no relationship between the level of fibrinogen and the parity of the subjects in this present study. However Almagor et al 2003\textsuperscript{105} reported decreased level of fibrinogen in very high parity women when compared
with age-matched women with lower parity.

Educational level, which is a reflection of socio economic status of an individual, did not affect fibrinogen level in this study. Marmot et al 1978\textsuperscript{97} reported that high fibrinogen level might account in part for the higher rate of coronary disease experienced by people of lower socio-economic status, which cannot be explained by the classical determinants of coronary risk. Higher fibrinogen level was also noticed in lower socio-economic status and also found to be higher among those in rented rather than owned accommodation\textsuperscript{97}. Balance diet with respect to fats, carbohydrate, proteins, necessary vitamins, minerals and microelements are effective in restoring and preserving the function of coagulation system\textsuperscript{96}.

From this study, the euglobulin clot lysis time, which is one of the tests of fibrinolysis, was significantly increased in normal pregnancy when compared to non-pregnant control. This finding is similar to that of Adediran et al\textsuperscript{91} who found significant increase in euglobulin clot lysis time in normal pregnancy.

Wright et al in 1988\textsuperscript{31} also confirmed a reduction of the fibrinolytic activity of the plasma euglobin fraction from the second trimester and a parallel reduction in tissue plasminogen activator and increase in tissue plasminogen activator inhibitor activity with rapid return to non-pregnant level post partum\textsuperscript{101,104}. Plasminogen activator inhibitor type–2 which is undetected in non-pregnant control plasma was measurable in the first trimester, increased through pregnancy and remained at a higher concentration post partum\textsuperscript{31,100}.

There was no statistically significant difference between the euglobulin lysis
times in various trimesters of pregnancy. Therefore the significant difference seen between subjects and control shows that plasminogen activator inhibitor type 1 and 2 are increased right from early pregnancy.

The euglobin lysis time was not affected by the age, parity and educational level of the subjects. This suggests that plasminogen activator is not affected by these factors. The increased euglobin lysis time is an indication of a reduced fibrinolytic activity in normal pregnancy, which allows strengthening of the fibrin produced in the placental site thereby decreasing bleeding following parturition.\(^{38}\)

There was no significant difference between the platelet count in controls and that of the subjects; this finding is similar to that of Adediran et al.\(^ {91}\), who reported no difference in the mean platelet count in the normal pregnant women and non-pregnant controls.

Bonnar and Syneny both reported reduction in platelet count in pregnancy.\(^ {71, 72}\) Other workers however, have documented higher random platelet count in some pregnant and post partum women than in the non-pregnant state with 5% only of periparturient women being thrombocytopenic.\(^ {74}\) Platelet count may vary from person to person but during pregnancy, platelet count may fall slightly, and up to 8% of pregnant women are reported to have gestational thrombocytopenia which may be due to increased fluid retention which dilutes the count.\(^ {73, 74, 75}\) It has also been suggested that the occurrence of thrombocytopenia in labour or prepartum might be related to the low grade “physiologic DIC” that might accompany normal delivery.\(^ {15, 34}\) The hypothesis is that small amounts of activated procoagulant
substances may be released into the circulation, which may activate platelets and result in thrombocytopenia.

There was no significant difference between the platelet count in various trimesters of pregnancy \((p = 0.880)\) unlike in the findings of Hellegren 2005\(^9\), who reported lower platelet count and more severe benign gestational thrombocytopenia during the third trimester.

The platelet count was not affected by the age, parity and educational levels of the subjects.

According to Eichinger et al 1999\(^{11}\), the levels of coagulation and fibrinolytic indices in healthy pregnant women in the third trimester are similar to or higher than those found in the patient following a deep vein thrombosis or pulmonary embolism yet none of the women in their study developed clinical symptom of deep vein thrombosis. So, although the various physiological changes, which occur in pregnancy, make pregnancy a hypercoagulable state, there is a balance between coagulation and fibrinolytic activities which limits the likelihood of actual thrombosis. Furthermore, changes in the haemostatic systems during pregnancy may be more marked in the uteroplacental than in the systemic circulation\(^{34}\). Thus the pregnant women may be relatively well protected against the thrombotic effects of a hypercoagulable state.

**PROBLEMS AND LIMITATIONS OF THE STUDY**

1. Ideally a longitudinal study of the haemostatic parameters should be
carried out in which the same subjects will be followed up during pregnancy and after the puerperium so that the subjects will serve as their own controls. The presence or absence of thrombotic complications may also be better studied during such a longitudinal study.

2. Euglobulin lysis time was delayed for up to 48 hours due to the cumbersome nature of that test, although plasma was kept frozen and subjects and controls samples were analysed the same time.

3. Factor assay would have been carried out, but due to difficulty in getting deficient plasma, this test was abandon.

4. Plasminogen activator and plasminogen activator inhibitor, although Labile factors and measurement of fibrinogen degradation products (FDP) using D-Dimer kit which are more representative of fibrinolytic activity rather than ELT were not carried out due to lack of reagents.

5. Bleeding time which could be used for assessing vascular integrity was not done since this method also assesses the platelet function and platelet count was already studied.
CONCLUSION

From this study, we have confirmed, as in other reports worldwide the development of a transient hypercoagulable state in normal pregnant women in Nigeria. The evidence for this is found in the significant shortening, compared to the non-pregnant state, of the prothrombin time and the partial thromboplastin time with Kaolin, the increase in plasma fibrinogen and the reduction of fibrinolytic activity as shown by a prolonged euglobulin lysis time. The results obtained from this study have given us a better interpretation of the coagulation screening tests in pregnancy and pregnant women with results outside the ‘normal range’ can be closely monitored for any attendant complications in pregnancy.
PROTOCOL FORM

Hospital No…………………………………… Serial No…………

Name:……………………………………………………………………

Age:……………………………………………………

Marital Status:……………………………. Married/ Single

Occupation:………………………………………………………………

Address:………………………………………………………………

Education Level:........None…… Primary/Secondary.....................

                      Higher Institution.........................

Reproductive History

Date of last menstrual period…………………………………..

Gestational age…………………………………………………

Parity:………………………………………………………….

No of miscarriages / still birth / Abortion……………………………………

Previous complications in the past pregnancy…………………………

Previous contraceptive use  i. Oral .................................

                        ii.  IUCD........................................

                        iii. Injectable ................................

                        iv. Barrier methods.............................


**Medical Examination**

Weight (kg)…………………… Height (CM) ..............................

Blood pressure……………… Fundal Height…………………………

Varicose Veins Yes/No

**Laboratory Results**

1.  Prothrombin Time (Seconds)

    Test .................

    Control..............

    Normal Range (10-14 seconds)

2.  Activated Partial Thromboplastin Time (In Seconds)

    Test .................

    Control..............

    Normal Range (35-45 seconds)

3.  Fibrinogen Level (G/L)

    Test .................

    Control..............

    Normal Range (1.5-4.0 g/L)

4.  Euglobulin Lysis Time minutes (NR 90-240minutes)

5.  Platelet Count (N x 10^9 /L)

    Test .................

    Control..............

    Normal Range (100-300 x 10^9/L)

6.  Pregnancy Test  (Positive or Negative).........................
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