

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

**Measurement of Plasma Von Willebrand Factor Antigen
Level among pregnant Sudanese Ladies in Third
Trimester**

قياس مستوى مستضد عامل البلازما فون فيلي براند بين النساء السودانيات في
الثالث الثالث من الحمل

**A thesis Submitted in Partial Fulfillment for the Degree of M.Sc in
Hematology and Immunohematology**

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2018

الآية

بسم الله الرحمن الرحيم

قال تعالى :

(لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إَصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَارْحَمْنَا أَنْتَ مَوْلَانَا فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ (286))

صدق الله العظيم

سورة البقرة الآية (286)

Dedication

To all who gave my life meaning?

My parents

My family

My friends

My princess Rahaf

To all who helped me even with words?

Acknowledgment

Thanks, praise to almighty **god** who give me the strength and health to achieve this work and for every things.

My supervisor **Dr.Ibrahim Khider Ibrahim** has been the ideal thesis supervisor for her sage advice, and patient encouragement aided the writing of this thesis in innumerable ways.

I would also like to thank all **hematology teaching staff** and **lab assistants** in Sudan University of Sciences and Technology.

My thanks go to the department and all laboratory staff of **Military Hospital** .

There are a number of people without whom this thesis might not have been written, and to whom I greatly indebted.

Finally my deepest go to **my family** and for their great helped and understanding.

Abstract:

Von Willebrand factor (vWF) is a large sialoglycoprotein, that circulates in normal plasma as a series of heterogeneous multimers and plays a critical role in primary hemostasis by mediating platelet adhesion to exposed collagen at sites of vascular injury. Von Willebrand factor is blood clotting protein and one of several components that work together and in sequence to stop bleeding by forming a blood clot. High molecular weight multimers of Von Willebrand factor demonstrate enhanced binding affinities to both collagen and platelets and are therefore more efficient in mediating platelet recruitment. This study aims to evaluate the vWF level among Sudanese pregnant women's. A total of 40 samples were collected (from age 18 to 41 and different trimester) from pregnant women. The blood was collected from each subject by clean venous puncture into sample container; containing 0.5 ml of 3.8 Trisodium citrate for the estimation of vWF : Ag and gently mixed and then separated immediately at 4500 rpm for 15 min after which platelet poor plasma (PPP) was collected in plain container and stored at -20 °C until analysis and control also take 40 samples from normal ladies non pregnant. The vWF level was measured using immune assay ELISA. Data were analyzed by statistical package for social science (SPSS). The result showed that there is significant association between pregnancy and plasma vWF level (p-value=0.039) but the result shows no significant association between vwf and age (P-value=0.658) and Weight (P-value=0.723). Von Willebrand factor testing measures the quantity and function of Von Willebrand factor.

The study concluded that, the vWF is increased in third trimester pregnant women which can lead to many problems and disorders.

المستخلص :

عامل الفون فيلي براند هو بروتين سياليوغلي بروتين كبير ، يدور في البلازما العادية كسلسلة من المتعددات غير المتجانسة ويلعب دورا حاسما في التخثر الأولي عن طريق التوسط في التصاق الصفائح الدموية بالكولاجين المكشوف في مواقع الإصابة الوعائية. عامل الفون فيلي براند هو بروتين تجلط الدم وأحد المكونات العديدة التي تعمل معاً وفي التسلسل لوقف النزف بتكوين جلطة دموية. تظهر المثيلات عالية الوزن الجزيئي من عامل فون فيلي براند صلات ملزمة محسنة لكل من الكولاجين والصفائح الدموية وبالتالي فهي أكثر كفاءة في التوسط للصفائح الدموية التعيين.

تهدف هذه الدراسة إلى تقييم مستوى vWF بين النساء الحوامل السودانيات. تم جمع ما مجموعه 40 عينة (من سن 18 إلى 41 و الأوزان المختلفة) من النساء الحوامل. تم جمع الدم من كل موضوع بواسطة ثقب وريدي نظيف في إناء العينة. تحتوي على 0.5 ml من 3.8 % سترات الصوديوم لتقدير AgvWF : ثم يتم مزجها بلطف ثم فصلها فورا عند 4500 دورة في الدقيقة لمدة 15 دقيقة بعد تجميع البلازما الضعيفة (ppp) في وعاء عادي وتخزينها في درجة حرارة -20 درجة مئوية حتى التحليل.

وللمراقبة ايضا تم اخذ 40 عينة من نساء طبيعيات غير حوامل .

تم قياس مستوى vWF باستخدام فحص المناعة ELISA. تم تحليل البيانات من خلال الحزمة الإحصائية للعلوم الاجتماعية (SPSS). أظهرت النتائج وجود ارتباط معنوي بين مستوى الحمل والبلازما ($P\text{-value} = 0.039$) ولكن النتيجة لم تظهر

ارتباطاً معنوياً بين vwf والعمر (P-value = 0.658) و الوزن (P- Value =)

0.723). يقيس اختبار عامل فون فيلي براند كمية ووظيفة عامل فون ويلبراند.

توصلت الدراسة الى أن هناك زيادة في عامل الفون فيلي براند في الثلث الثالث من

الحمل مما يؤدي الى الامراض والمشاكل .

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Abbreviation

ADAMTS :adisintegrin and metalloprotenase with thrombo spondin motifs

BMI: body mass index

DAH: docosa hexaenaic acid

DDAVP : desmopressin acetate de amino 8 D arginine vasopressin

ELISA :enzyme linked immuno sorbent assay

FDA: food and drug administration

Gb: glycoprotein

HUS : hemolytic uremic syndrome

IVI-G :intra venous immunoglobulin

LMP: last menstrual period

MGUS: monoclonal gammopathy of undetermined significance

MRI: magnetic resonance imaging

PDGF: platelet derived growth factor

PPP : platelet poor plasma

RGD: arginyl glycy l asprtic acid

SPSS : statistical package for Social sciences

TFR : total fertility rate

TGF : transforming growth factor

βHCG : beta human chorionic gonadotropin

VWF :Von Willebrand Factor

Chapter One

Introduction and literature review

1.1 Introduction:

During pregnancy, significant changes occur in the haemostatic system and in the plasma levels for several plasma proteins, especially towards term. In this study changes occurring during normal pregnancy and immediately postpartum were investigated to establish adequate reference intervals for important haemostatic parameters. Blood samples were collected during pregnancy weeks 33, 36, 39 and 1-3 h after delivery from 153 healthy pregnant women with at least one previous normal pregnancy. The plasma samples were analyzed for ant thrombin, von Will brand factor (vWf), free protein S and fibronectin. Fibronectin and vWf are contact-promoting proteins responsible for adhesion and aggregation during primary homeostasis, but are also released from thrombocytes during activation of the coagulation process. Ant thrombin is the most important primary physiological inhibitor of activated serine proteases related to the coagulation cascade. (Wickström K, *et al* , 2004). Protein S is a co-factor to protein C and in cooperation is also an important inhibitor of the coagulation cascade. During third-trimester pregnancy, vWf was higher than in non-pregnant women, and continued to increase postpartum. The fibronectin plasma level was mostly unchanged in comparison with non-pregnant values. Within this reference interval it gradually increased during the third trimester, but fell slightly postpartum. Ant thrombin decreased slightly during the third trimester and even further, postpartum. Free protein S decreased markedly but to a stable level from week 33 to 39, decreasing even more postpartum. The present results are concordant with clinical knowledge of increased risk

of thrombosis during pregnancy and early puerperium, with increased levels of vWf and fibronectin and decreased levels of ant thrombin and free protein S. Clearly, current reference values based on healthy non-pregnant subjects are not usable during late pregnancy and immediately postpartum. (Wickström K, *et al* , 2004).

The multimeric glycoprotein Von Willebrand factor (vWF), is produced by vascular endothelium and platelet. If some constitutional deficiencies leading to hemorrhagic syndrome have been explored in the literature, increased production of vWF observed during cellular distress and pregnancy have not been explored in our milieu. The aim of this study was to determine vWF changes during pregnancy in a group of Cameroonian women and find out the possibility of using it as a marker of fetal distress. Serum was collected from 46 women in the second and third trimesters of pregnancy. The determination of vWF concentration was performed using the Asserachrom vWF reagent: Ag (Diagnostica Stago, France). The average concentrations of vWF in the second and third trimester samples were respectively $215.47 \pm 9.38\%$ (UI/dL) and $264.09 \pm 11.58\%$ (UI/dL). The difference between (i) concentrations of vWF during the second and third trimester of pregnancy was statistically significant (P-value<0.0001); (ii) concentrations of vWF according to mother's ages was not significant; (iii) concentrations of vWF in blood group O and B women was statistically significant (P-value<0.05). We observed a difference between vWF values in women whose babies had Apgar score 4 to 6 compared to those with Apgar score between 7 and 10. The difference was not statistically significant probably due to low effective. In conclusion, vWF production during the pregnancy varies with gestational age and maternal blood group. It may increase during fetal distress (Wickström K, *et al* , 2004).

1.2 Literature Review

1.2.1 Von Willebrand Factor

1.2.1.1 VWF gene

The VWF gene provides instructions for making a blood clotting protein called von Willebrand factor. This protein contains regions that attach (bind) to specific cells and proteins during the formation of a blood clot. After an injury, clots protect the body by sealing off damaged blood vessels and preventing further blood loss.

Von Willebrand factor is made within endothelial cells, which line the inside surface of blood vessels, and bone marrow cells. The factor is made of several identical subunits. To facilitate binding to various cells and proteins, these subunits are cut into smaller pieces by an enzyme called ADAMTS13. Von Willebrand factor helps platelets stick together and adhere to the walls of blood vessels at the site of a wound. These groups of platelets form temporary clots, plugging holes in blood vessel walls to help stop bleeding. Von Willebrand factor also carries another blood clotting protein, coagulation factor VIII, to the area of clot formation (Franchini and Lippi 2007).

1.2.1.2 Health condition related to genetic change

More than 300 mutations in the VWF gene have been found to cause von Willebrand disease. Mutations in the VWF gene that reduce the amount of von Willebrand factor cause type 1 von Willebrand disease. People with type 1 von Willebrand disease have von Willebrand factor in their bloodstream, but at reduced amounts. Mutations that disrupt the function of the von Willebrand factor cause the four subtypes of type 2 von Willebrand disease. These mutations usually change one of the protein

building blocks (amino acids) used to make von Willebrand factor or problems with its function slows the formation of blood clots, which causes the factor, which can disrupt the factor's ability to bind to various cells and proteins needed to form a blood clot. Mutations that result in an abnormally short, nonfunctional von Willebrand factor generally cause the more severe type 3 von Willebrand disease. A reduction in the amount of von Willebrand prolonged bleeding episodes seen in von Willebrand disease.(Franchini and Lippi 2007).

1.2.1.3 Other name for this gene

coagulation factor VIII VWF ,F8VWF and VWD

1.2.1.4 Chromosomal location

Cytogenetic Location: 12p13.31, which is the short (p) arm of chromosome 12 at position 13.31

Molecular Location: base pairs 5,948,874 to 6,124,675 on chromosome 12 (Homo sapiens Annotation Release 108, GRCh38.p7) (franchini and Lippi 2007) see figure(1.1).

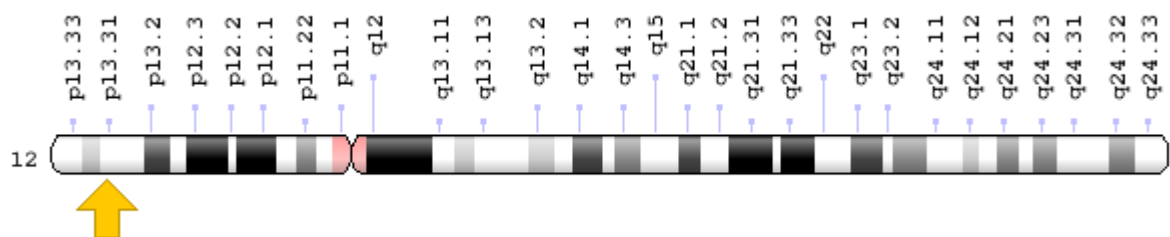


Figure (1.1)Chromosomal location

1.2.1.5 Definition

Von Willebrand factor (vWF) is a blood glycoprotein involved in hemostasis. It is deficient or defective in von Willebrand disease and is

involved in a large number of other diseases, including thrombotic thrombocytopenic purpura, Heyde's syndrome, and possibly hemolytic-uremic syndrome. Increased plasma levels in a large number of cardiovascular, neoplastic, and connective tissue diseases are presumed to arise from adverse changes to the endothelium, and may contribute to an increased risk of thrombosis (Sadler 1998).

1.2.1.6 Synthesis

vWF is a large multimeric glycoprotein present in blood plasma and produced constitutively as ultra-large vWF in endothelium (in the Weibel-Palade bodies), megakaryocytes (α -granules of platelets), and sub endothelial connective tissue (Sadler 1998).

1.2.1.7 Structure

The basic VWF monomer is a 2050-amino acid protein. Every monomer contains a number of specific domains with a specific function; elements of note are (Sadler JE 1998) the D'/D3 domain, which binds to factor VIII, (Von Willebrand factor type D domain) the A1 domain, which binds to: platelet GPIb-receptor, heparin, possibly collagen the A2 domain, which must partially unfold to expose the buried cleavage site for the specific ADAMTS13 protease that inactivates VWF by making much smaller multimers. The partial unfolding is affected by shear flow in the blood, by calcium binding, and by the lump of a sequence-adjacent "vicinal disulfide" at the A2-domain C-terminus (Jakobi *et al* 2011), the A3 domain, which binds to collagen (Von Willebrand factor type A domain)the C1 domain, in which the RGD motif binds to platelet integrin $\alpha_{IIb}\beta_3$ when this is activated (Von Willebrand factor type C domain) the "cysteine knot" domain (at the C-terminal end of the protein), which vWF shares with platelet-derived growth factor (PDGF), transforming growth

factor- β (TGF β) and β -human chorionic gonadotropin (β HCG, of pregnancy test fame). (Von Willebrand factor type C domain) Monomers are subsequently N-glycosylated, arranged into dimers in the endoplasmic reticulum and into multimers in the Golgi apparatus by crosslinking of cysteine residues via disulfide bonds. With respect to the glycosylation, vWF is one of only a few proteins that carry ABO blood group system antigens.(Sadler1998) Multimers of vWF can be extremely large, >20,000 kDa, and consist of over 80 subunits of 250 kDa each. Only the large multimers are functional. Some cleavage products that result from vWF production are also secreted but probably serve no function (Sadler 1998) for structure see (figure 1.2).

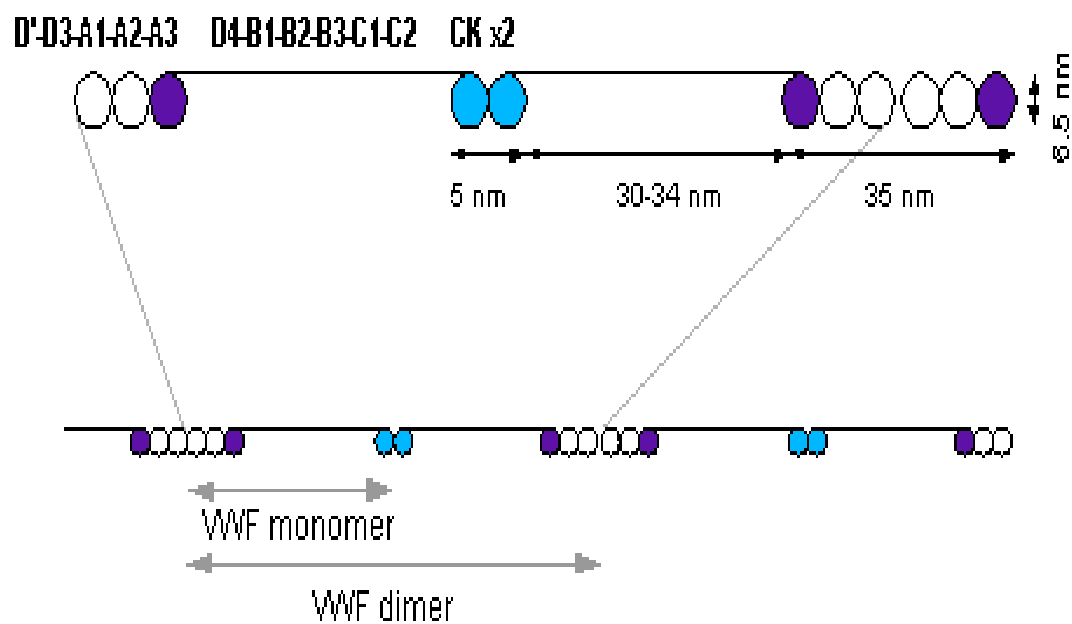


Figure (1.2) :Structure of VWF monomer and multimers

1.2.1.8 Function

Von Willebrand factor's primary function is binding to other proteins, in particular factor VIII, and it is important in platelet adhesion to wound

sites.(Sadler 1998) It is not an enzyme and, thus, has no catalytic activity. vWF binds to a number of cells and molecules. The most important ones are (Sadler 1998) Factor VIII is bound to vWF while inactive in circulation; factor VIII degrades rapidly when not bound to vWF. Factor VIII is released from vWF by the action of thrombin. vWF binds to collagen, e.g., when it is exposed in endothelial cells due to damage occurring to the blood vessel. Endothelium also releases vWF which forms additional links between the platelets' glycoprotein Ib/IX/V and the collagen fibrils vWF binds to platelet gpIb when it forms a complex with gpIX and gpV; this binding occurs under all circumstances, but is most efficient under high shear stress (i.e., rapid blood flow in narrow blood vessels, vWF binds to other platelet receptors when they are activated, e.g., by thrombin (i.e., when coagulation has been stimulated). vWF plays a major role in blood coagulation. Therefore, vWF deficiency or dysfunction (von Willebrand disease) leads to a bleeding tendency, which is most apparent in tissues having high blood flow shear in narrow vessels. From studies it appears that vWF uncoils under these circumstances, decelerating passing platelets (Sadler1998) Recent research also suggests that von Willebrand factor is involved in the formation of blood vessels themselves, which would explain why some people with von Willebrand disease develop vascular malformations (predominantly in the digestive tract) that can bleed excessively.(Randi and Laffan 2017).

1.2.1.9 Catabolism

The biological breakdown (catabolism) of vWF is largely mediated by the enzyme ADAMTS13 (acronym of "*adis*integrin-like and metalloprotease with thrombospondin type 1 motif no. 13"). It is a metalloproteinase that cleaves vWF between tyrosine at position 842 and

methionine at position 843 (or 1605–1606 of the gene) in the A2 domain. This breaks down the multimers into smaller units, which are degraded by other peptidases(Levy *et al* 2005).

1.2.1.10 Role in disease

Hereditary or acquired defects of vWF lead to von Willebrand disease (vWD), a bleeding diathesis of the skin and mucous membranes, causing nosebleeds, menorrhagia, and gastrointestinal bleeding. The point at which the mutation occurs determines the severity of the bleeding diathesis. There are three types (I, II and III), and type II is further divided in several subtypes. Treatment depends on the nature of the abnormality and the severity of the symptoms(Sadler *et al* 2006) Most cases of vWD are hereditary, but abnormalities of vWF may be acquired; aortic valve stenosis, for instance, has been linked to vWD type IIA, causing gastrointestinal bleeding - an association known as Heydes syndrome. In thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS), ADAMTS13 either is deficient or has been inhibited by antibodies directed at the enzyme. This leads to decreased breakdown of the ultra-large multimers of vWF and microangiopathic hemolytic anemia with deposition of fibrin and platelets in small vessels, and capillary necrosis. In TTP, the organ most obviously affected is the brain; in HUS, the kidney (Moake 2004). Higher levels of vWF are more common among people that have had ischemic stroke (from blood-clotting) for the first time. Occurrence is not affected by ADAMTS13, and the only significant genetic factor is the person's blood group. High plasma vWF levels were found to be an independent predictor of major bleeding in anticoagulated a trial fibrillation patients.(Rolden *et al* 2011).

1.2.1.11 Von Willebrand Disease

Von Willebrand disease (VWD) is genetic disorder caused by missing or defective von Willebrand factor clotting protein. VWF binds factor VIII, a key clotting protein, and platelets in blood vessel walls, which help form a platelet plug during the clotting process. The condition is named after Finnish physician Erik von Willebrand, a who first described it in the 1920s. VWD is the most common bleeding disorder, affecting up to 1% of the US population. It is carried on chromosome 12 and occurs equally in men and women (V.Rochester 2015) .

1.2.1.11.1 Inheritance of VWD gene

Von Willebrand disease can have different inheritance patterns. Most cases of type 1 and type 2 von Willebrand disease are inherited in an autosomal dominant pattern see (figure 1.3), which means one copy of the altered gene in each cell is sufficient to cause the disorder. Type 3, some cases of type 2, and a small number of type 1 cases of von Willebrand disease are inherited in an autosomal recessive pattern (figure 1.4), which means both copies of the gene in each cell have mutations. Most often, the parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, but they do not show signs and symptoms of the condition (V.Rochester 2015).

1.2.1.11.2 Symptoms

People with VWD experience frequent nosebleeds, easy bruising and excessive bleeding during and after invasive procedures, such as tooth extractions and surgery. Women often experience menorrhagia, heavy menstrual periods that last longer than average, and hemorrhaging after childbirth (Francois 2017)..

1.2.1.11.3 Types of von Willebrand disease

1.2.1.11.3.1 Type 1 VWD

Type 1 VWD is the most common form, accounting for 75% of all cases of VWD. In Type 1 VWD, the von Willebrand factor (VWF) works normally, but there is not enough of it. Many people with Type 1 VWD have no symptoms at all until they experience a bad injury or an operation.

1.2.1.11.3.2 Type 2 von Willebrand disease

Type 2 VWD is less common than Type 1. It represents 20-25% of all cases.

In Type 2 VWD, the amount of VWF in people's blood is often normal. The problem is that the VWF does not work properly. There are several sub-types of Type 2 VWD. It is important to get an exact diagnosis because the sub-types are treated differently. Type 2A VWD is the most common sub-type. It represents 15-20% of all cases of VWD. In Type 2A VWD, the amount of VWF is often normal. However, because of a defect in the VWF protein, the platelets do not bind together well. The VWF does not act as a glue to hold the platelets in place to plug a hole in a blood vessel.

Type 2B VWD is the next most common. It represents about 5% of all cases of VWD. In Type 2B VWD, the VWF binds to platelets in the bloodstream, instead of binding at the site of the injury to the blood vessel. Then, the body removes these large bundles of platelets from circulation. This causes a shortage of platelets.

Type 2N VWD is much rarer. (The “N” stands for Normandy, France where the sub-type was first identified.) This is what doctors know about it.

- In Type 2N the VWF works normally with platelets. As a result, the grouping of platelets around the injury happens as it should.
- VWF also helps to carry around factor VIII in the blood and stabilize it so it can take part in the formation of a solid clot. In Type 2N the VWF does not transport factor VIII. As a result, factor VIII levels are low.
- Sometimes, because of the low factor VIII levels, Type 2N is mistaken for factor VIII deficiency hemophilia.
- In order for a child to get Type 2N, both parents must pass on the defective gene.

There are several other extremely rare sub-types of Type 2 VWD, including Type 2M. The 'M' stands for 'Multimer', a part of the structure of the VWF molecule. In Type 2M, binding of the VWF to platelets is impaired.(Francois Laroche 2017).

1.2.1.11.3.3 Type 3 von Willebrand disease

Type 3 VWD is very rare. It affects about 1 in 500,000 people. However, it is the most severe type of VWD. People with Type 3 VWD have very little VWF in their blood. Because VWF transports factor VIII, they also have very low levels of factor VIII. As a result, bleeding can happen often and, if untreated, can be serious.

Usually, in order for a baby to get Type 3 VWD, both parents must pass on the defective VWD gene. However, in some cases, the disease can result from a combination of one parent passing on the defective gene and a mutation in the child's gene inherited from the other parent.(Francois 2017).

1.2.1.11.3.4 Acquired VWD

This type of VWD in adults results after a diagnosis of an autoimmune disease, such as lupus, or from heart disease or some types of cancer. It can also occur after taking certain medications. Acquired von Willebrand's disease is a rare bleeding disorder that might be caused by other medical problems or medicines. It prevents blood from clotting properly. It is rarer than the inherited form of von Willebrand's disease.(Hillman *et al* 2011).

Table (1.1) ;Differential diagnosis between AVWS and VWD

Aspect	In favor of AVWS	In favor of VWD
Personal history	Late onset of bleeding Un eventful surgery before onset of bleeding	Early onset of bleeding No un eventful surgery or no previous high risk situation
Family history	Negative	Positive
AVWS associated disorder	Present	Absent
Laboratory evaluation	Presence of inhibitor or vwf antibody	Vwf mutation
Treatment response	Remission after treatment of underlying disorder Response to IVIG(in igG MGUS associate AVWS)	Normal recovery and half life of vwf containing sustained response to desmopression

VWD: von willebrand disease **VWF:** von willebrand disease **MGUS:** monoclonal gammopathy of undetermined significance **IVIG:** intra venous immunoglobulin (Hillman *et al* 2011).

1.2.1.11.4 Diagnosis of VWD

Because many people with von Willebrand disease have mild signs and symptoms, the condition can be difficult to diagnose. If you have any indication of a bleeding disorder, The doctor may refer to a blood disorders specialist (hematologist) .To evaluate the von Willebrand disease, the doctor will likely ask detailed questions about your medical history and check for bruises or other signs of recent bleeding. The doctor will also likely recommend the following blood tests: (V.Rochester 2015).

Von Willebrand factor antigen. This test determines the level of von Willebrand factor in your blood by measuring a particular protein.

Ristocetin cofactor activity. This test measures how well the von Willebrand factor works in clotting process. Ristocetin, which is an antibiotic, is used in this laboratory testing.

Factor VIII clotting activity. This test shows whether have abnormally low levels and activity of factor VIII.

Von Willebrand factor multimers. This test evaluates the specific structure of von Willebrand factor in blood, its protein complexes (multimers) and how its molecules break down. This information helps identify the type of von Willebrand disease (V.Rochester 2015).

1.2.1.11.5 Treatment-

Even though von Willebrand disease is a lifelong condition with no cure, treatment can help prevent or stop bleeding episodes. The doctor may suggest one or more of the following treatments to increase your von Willebrand factor, strengthen blood clots or, in women, control heavy menstrual bleeding:

Desmopressin This medication is available as an injection (DDAVP) or nasal spray (Stimate). It's a synthetic hormone, similar to the natural hormone vasopressin. It controls bleeding by stimulating The body to release more von Willebrand factor already stored in the lining of your blood vessels. DDAVP is usually effective in people with type 1 and some subtypes of type 2 disease. Many doctors consider DDAVP the first treatment to use in the management of von Willebrand disease. Some women use the nasal spray (Stimate) at the beginning of their menstrual periods to control excessive bleeding. It can also be effective when used before a minor surgical procedure.

Replacement therapies. These include infusions of prepared doses of concentrated blood-clotting factors containing von Willebrand factor and factor VIII (Humate-P, others). These therapies can be useful in all disease types. The doctor may recommend them if DDAVP isn't an option for or was ineffective. Another replacement therapy approved by the FDA for treating adults 18 and older is a genetically engineered (recombinant) von Willebrand factor product Because recombinant factor is made without plasma, it may reduce the risk of a viral infection or allergic reaction.

Contraceptives. For women, these can be useful for controlling heavy bleeding during menstrual periods. The estrogen hormones present in

birth control pills can boost levels of von Willebrand factor and factor VIII activity. This effect is likely available with birth control patches, though further study is needed to confirm it.

Clot-stabilizing medications. These anti-fibrinolytic medications — such as aminocaproic acid (Amicar) and tranexamic acid — can help stop bleeding by slowing the breakdown of blood clots. Doctors often prescribe these drugs before or after a surgical procedure or tooth extraction. If your condition is mild, the doctor might recommend treatment only when you're undergoing surgery or dental work or when you've experienced trauma (V.Rochester 2015).

1.2.1.11.6 Risk factors

The main risk factor for von Willebrand disease is having a family history of it. A parent can pass the abnormal gene for the disease to his or her child. Most cases are "autosomal dominant inherited" disorders, which means only need an abnormal gene from one parent to be affected. If you have the gene for von Willebrand disease, you have a 50 percent chance of transmitting this gene to your offspring. The most severe form of the condition (type 3) is "autosomal recessive," which means both of your parents have to pass an abnormal gene to you (V.Rochester 2015).

1.2.1.11.7 Complications

Complications of von Willebrand disease may include:

Anemia. Women who experience heavy menstrual bleeding can develop iron deficiency anemia.

Swelling and pain. If abnormal bleeding occurs in the joints or soft tissue, swelling and severe pain can result.

Death from bleeding. Rarely, someone with von Willebrand disease may experience uncontrolled bleeding that can be life-threatening and needs emergency medical attention (V.Rochester 2015).

1.2.1.11.8 Prognosis

For most affected individuals, vWD is a mild, manageable bleeding disorder in which clinically severe hemorrhage manifests only in the face of trauma or invasive procedures. However, significant variability of symptomatology exists among family members. In individuals with vWD types II and III, bleeding episodes may be severe and potentially life threatening. Individuals with type III disease who have correspondingly low FVIII levels may develop arthropathies, as more commonly seen in hemophilia A patients with comparably decreased FVIII levels. Levels of vWF normally increase with age. However, Sanders and colleagues found that although vWF levels increased with aging in patients with type I vWD, elderly patients with type I reported no change in their pattern of bleeding did not change. In patients with type II vWD, vWF levels did not increase with aging, and elderly patients reported significantly more bleeding symptoms.(Sander *et al* 2014).

1.2.2 Pregnancy:

, also known as **gestation**, is the time during which one or more offspring develops inside a woman. A multiple pregnancy involves more than one offspring, such as with twins. (Wylie and Linda 2005) Pregnancy can occur by sexual intercourse or assisted reproductive technology (Shehan, and Constance L 2016).

Childbirth typically occurs around 40 weeks from the last menstrual period (LMP), (Abman and Steven H 2011).

This is just over nine months, where each month averages 29½ days. When measured from conception it is about 38 weeks (Abman and Steven H 2011). An embryo is the developing offspring during the first eight weeks following conception, after which, the term *fetus* is used until birth. Symptoms of early pregnancy may include missed periods, tender breasts, nausea and vomiting, hunger, and frequent urination. Pregnancy may be confirmed with a pregnancy test.

Pregnancy is typically divided into three trimesters. The first trimester is from week one through 12 and includes conception.(Diana W and Bianchi 2015) Conception is when the sperm fertilizes the egg. The fertilized egg then travels down the fallopian tube and attaches to the inside of the uterus, where it begins to form the embryo and placenta. The first trimester carries the highest risk of miscarriage (natural death of embryo or fetus). (Lippincott Williams and Wilkins 2012) The second trimester is from week 13 through 28. (Diana W and Bianchi 2015) Around the middle of the second trimester, movement of the fetus may be felt. At 28 weeks, more than 90% of babies can survive outside of the uterus if provided with high-quality medical care. The third trimester is from 29 weeks through 40 weeks.(Diana W and Bianchi 2015)

1.2.2.1 Signs and symptoms

The symptoms and discomforts of pregnancy are those presentations and conditions that result from pregnancy but do not significantly interfere with activities of daily living or pose a threat to the health of the mother or baby.

This is in contrast to pregnancy complications. Sometimes a symptom that is considered a discomfort can be considered a complication when it

is more severe. For example, nausea (morning sickness) can be a discomfort, but if, in combination with significant vomiting it causes a water-electrolyte imbalance, it is a complication known as hyperemesis gravidarum. Common symptoms and discomforts of pregnancy include:

- Tiredness.
- Constipation
- Pelvic girdle pain
- Back pain
- Braxton Hicks contractions. Occasional, irregular, and often painless contractions that occur several times per day.
- Edema (swelling). Common complaint in advancing pregnancy. Caused by compression of the inferior vena cava and pelvic veins by the uterus leads to increased hydrostatic pressure in lower extremities.
- Increased urinary frequency. A common complaint, caused by increased intravascular volume, elevated glomerular filtration rate, and compression of the bladder by the expanding uterus.
- Urinary tract infection (Merck 2011).
- Varicose veins. Common complaint caused by relaxation of the venous smooth muscle and increased intravascular pressure.
- Haemorrhoids (piles). Swollen veins at or inside the anal area. Caused by impaired venous return, straining associated with constipation, or increased intra-abdominal pressure in later pregnancy.(Vazquez , JC 2010)
- Regurgitation, heartburn, and nausea.
- Stretch marks

- Breast tenderness is common during the first trimester, and is more common in women who are pregnant at a young age.(David C. Dugdale *et al* 2012)

In addition, pregnancy may result in pregnancy complication such as deep vein thrombosis or worsening of an intercurrent disease in pregnancy.

1.2.2.2 Chronology:

The chronology of pregnancy is, unless otherwise specified, generally given as gestational age, where the starting point is the woman's last normal menstrual period (LMP), or the corresponding age of the gestation as estimated by a more accurate method if available. Sometimes, timing may also use the fertilization age which is the age of the embryo.

1.2.2.2.1 Start of gestational age:

According to American Congress of Obstetricians and Gynecologists, the main methods to calculate gestational age are:

- Directly calculating the days since the beginning of the last menstrual period.
- Early obstetric ultrasound, comparing the size of an embryo or fetus to that of a reference group of pregnancies of known gestational age (such as calculated from last menstrual periods), and using the mean gestational age of other embryos or fetuses of the same size. If the gestational age as calculated from an early ultrasound is contradictory to the one calculated directly from the last menstrual period, it is still the one from the early ultrasound that is used for the rest of the pregnancy.

- In case of in vitro fertilization, calculating days since oocyte retrieval or co-incubation and adding 14 days.(Tunon, K et al 2000)

1.2.2.2.2 Estimation of due date:

Distribution of gestational age at childbirth among singleton live births, given both when gestational age is estimated by first trimester ultrasound and directly by last menstrual period.(Hoffman *et al* 2008) Roughly 80% of births occur between 37 and 41 weeks of gestational age. Due date estimation basically follows two steps:

- Determination of which time point is to be used as origin for gestational age, as described in section above.
- Adding the estimated gestational age at childbirth to the above time point. Childbirth on average occurs at a gestational age of 280 days (40 weeks), which is therefore often used as a standard estimation for individual pregnancies. However, alternative durations as well as more individualized methods have also been suggested.

Naegele's rule is a standard way of calculating the due date for a pregnancy when assuming a gestational age of 280 days at childbirth. The rule estimates the expected date of delivery (EDD) by adding a year, subtracting three months, and adding seven days to the origin of gestational age. Alternatively there are mobile apps, which essentially always give consistent estimations compared to each other and correct for leap year, while pregnancy wheels made of paper can differ from each other by 7 days and generally do not correct for leap year. (Chambliss LR and Clark SL 2014).

Furthermore, actual childbirth has only a certain probability of occurring within the limits of the estimated due date. A study of singleton live births came to the result that childbirth has a standard deviation of 14 days when gestational age is estimated by first trimester ultrasound, and 16 days when estimated directly by last menstrual period.(Hoffman *et al* 2008)

1.2.2.3 Physiology:

1.2.2.3.1 Initiation:

Fertilization and implantation in humans Through an interplay of hormones that includes follicle stimulating hormone that stimulates folliculogenesis and oogenesis creates a mature egg cell, the female gamete. Fertilization is the event where the egg cell fuses with the male gamete, spermatozoon. After the point of fertilization, the fused product of the female and male gamete is referred to as a zygote or fertilized egg. The fusion of male and female gametes usually occurs following the act of sexual intercourse. Fertilization can also occur by assisted reproductive technology such as artificial insemination and in vitro fertilisation.

Fertilization (conception) is sometimes used as the initiation of pregnancy, with the derived age being termed fertilization age. Fertilization usually occurs about two weeks before the *next* expected menstrual period.

A third point in time is also considered by some people to be the true beginning of a pregnancy: This is time of implantation, when the future fetus attaches to the lining of the uterus. This is about a week to ten days after fertilization. (Weschler and Toni 2002) In this model, during the

time between conception and implantation, the future fetus exists, but the woman is not considered pregnant.

1.2.2.3.2 Development of embryo and fetus:

The sperm and the egg cell, which has been released from one of the female's two ovaries, unite in one of the two fallopian tubes. The fertilized egg, known as a zygote, then moves toward the uterus, a journey that can take up to a week to complete. Cell division begins approximately 24 to 36 hours after the male and female cells unite. Cell division continues at a rapid rate and the cells then develop into what is known as a blastocyst. The blastocyst arrives at the uterus and attaches to the uterine wall, a process known as implantation.

The development of the mass of cells that will become the infant is called embryogenesis during the first approximately ten weeks of gestation. During this time, cells begin to differentiate into the various body systems. The basic outlines of the organ, body, and nervous systems are established. By the end of the embryonic stage, the beginnings of features such as fingers, eyes, mouth, and ears become visible. Also during this time, there is development of structures important to the support of the embryo, including the placenta and umbilical cord. The placenta connects the developing embryo to the uterine wall to allow nutrient uptake, waste elimination, and gas exchange via the mother's blood supply. The umbilical cord is the connecting cord from the embryo or fetus to the placenta.

After about ten weeks of gestational age, the embryo becomes known as a fetus. At the beginning of the fetal stage, the risk of miscarriage decreases sharply.^[31] At this stage, a fetus is about 30 mm (1.2 inches) in length, the

heartbeat is seen via ultrasound, and the fetus makes involuntary motions (Lennart Nilsson 1990) During continued fetal development, the early body systems, and structures that were established in the embryonic stage continue to develop. Sex organs begin to appear during the third month of gestation. The fetus continues to grow in both weight and length, although the majority of the physical growth occurs in the last weeks of pregnancy.

Electrical brain activity is first detected between the fifth and sixth week of gestation. It is considered primitive neural activity rather than the beginning of conscious thought. Synapses begin forming at 17 weeks, and begin to multiply quickly at week 28 until 3 to 4 months after birth.(Kalverboer et al 2015)

- .Embryo at 4 weeks after fertilization. (Image from gestational age of 6 weeks). Retrieved 2007-08-28.
- Fetus at 8 weeks after fertilization. (Image from gestational age of 10 weeks). Retrieved 2007-08-28.
- Fetus at 18 weeks after fertilization. (Image from gestational age of 20 weeks). Retrieved 2007-08-28.
- Fetus at 38 weeks after fertilization. (Image from gestational age of 40 weeks). Retrieved 2007-08-28.
- Relative size in 1st month (simplified illustration)
- Relative size in 3rd month (simplified illustration)
- Relative size in 5th month (simplified illustration)
- Relative size in 9th month (simplified illustration)

1.2.2.3.3 Maternal changes:

Breast changes as seen during pregnancy. The areolae are larger and darker.

During pregnancy, the woman undergoes many physiological changes, which are entirely normal, including cardiovascular, hematologic, metabolic, renal, and respiratory changes. Increases in blood sugar, breathing, and cardiac output are all required. Levels of progesterone and oestrogens rise continually throughout pregnancy, suppressing the hypothalamic axis and therefore also the menstrual cycle.

The fetus is genetically different from the woman and can be viewed as an unusually successful allograft. The main reason for this success is increased immune tolerance during pregnancy. Immune tolerance is the concept that the body is able to not mount an immune system response against certain triggers.(Illes, ed 2008)

Pregnancy is typically broken into three periods, or trimesters, each of about three months.(Williams, Zev 2012) Each trimester is defined as 14 weeks, for a total duration of 42 weeks, although the average duration of pregnancy is 40 weeks. While there are no hard and fast rules, these distinctions are useful in describing the changes that take place over time.

1.2.2.3.3.1 First trimester:

The uterus as it changes in size over the duration of the trimesters Minute ventilation increases by 40% in the first trimester. (Cunningham, *et al* 2010) The womb will grow to the size of a lemon by eight weeks. Many symptoms and discomforts of pregnancy like nausea and tender breasts appear in the first trimester. (Campbell LA and Klocke RA 2001)

1.2.2.3.3.2 Second trimester:

By the end of the second trimester, the expanding uterus has created a visible "baby bump". Although the breasts have been developing internally since the beginning of the pregnancy, most of the visible changes appear after this point.

Weeks 13 to 28 of the pregnancy are called the second trimester. Most women feel more energized in this period, and begin to put on weight as the symptoms of morning sickness subside and eventually fade away. The uterus, the muscular organ that holds the developing fetus, can expand up to 20 times its normal size during pregnancy.

Although the fetus begins to move during the first trimester, it is not until the second trimester that movement, often referred to as "quickening", can be felt. This typically happens in the fourth month, more specifically in the 20th to 21st week, or by the 19th week if the woman has been pregnant before. It is common for some women not to feel the fetus move until much later. During the second trimester, most women begin to wear maternity clothes.

1.2.2.3.3.3 Third trimester

The uterus expands making up a larger and larger portion of the woman's abdomen. At left anterior view with months labeled, at right lateral view labeling the last 4 weeks. During the final stages of gestation before childbirth the fetus and uterus will drop to a lower position.

Final weight gain takes place, which is the most weight gain throughout the pregnancy. The woman's abdomen will transform in shape as it drops due to the fetus turning in a downward position ready for birth. During

the second trimester, the woman's abdomen would have been upright, whereas in the third trimester it will drop down low. The fetus moves regularly, and is felt by the woman. Fetal movement can become strong and be disruptive to the woman. The woman's navel will sometimes become convex, "popping" out, due to the expanding abdomen.

Head engagement, where the fetal head descends into cephalic presentation, relieves pressure on the upper abdomen with renewed ease in breathing. It also severely reduces bladder capacity, and increases pressure on the pelvic floor and the rectum.

It is also during the third trimester that maternal activity and sleep positions may affect fetal development due to restricted blood flow. For instance, the enlarged uterus may impede blood flow by compressing the hvena cava when lying flat, which is relieved by lying on the left side.

1.2.2.4 Diagnosis

The beginning of pregnancy may be detected either based on symptoms by the woman herself, or by using pregnancy tests. However, an important condition with serious health implications that is quite common is the denial of pregnancy by the pregnant woman. About one in 475 denials will last until around the 20th week of pregnancy. The proportion of cases of denial, persisting until delivery is about 1 in 2500. Conversely, some non-pregnant women have a very strong belief that they are pregnant along with some of the physical changes. This condition is known as a false pregnancy. (Jenkins A *et al* 2011)

1.2.2.4.1 Physical signs

Most pregnant women experience a number of symptoms, (Gabbe, Steven 2010) which can signify pregnancy. A number of early medical signs are associated with pregnancy. These signs include:

- the presence of human chorionic gonadotropin (hCG) in the blood and urine
- missed menstrual period
- implantation bleeding that occurs at implantation of the embryo in the uterus during the third or fourth week after last menstrual period
- increased basal body temperature sustained for over 2 weeks after ovulation
- Chadwick's sign (darkening of the cervix, vagina, and vulva)
- Goodell's sign (softening of the vaginal portion of the cervix)
- Hegar's sign (softening of the uterus isthmus)
- Pigmentation of the linea alba – linea nigra, (darkening of the skin in a midline of the abdomen, caused by hyperpigmentation resulting from hormonal changes, usually appearing around the middle of pregnancy).
- Darkening of the nipples and areolas due to an increase in hormones.

1.2.2.4.2 Biomarkers:

Pregnancy detection can be accomplished using one or more various pregnancy tests, which detect hormones generated by the newly formed placenta, serving as biomarkers of pregnancy. Blood and urine tests can detect pregnancy 12 days after implantation. (Cole, Laurence A.; Butler

and Stephen A 2015) Blood pregnancy tests are more sensitive than urine tests (giving fewer false negatives). Home pregnancy tests are urine tests, and normally detect a pregnancy 12 to 15 days after fertilization. A quantitative blood test can determine approximately the date the embryo was conceived because HCG doubles every 36 to 48 hours. (Stacey T *et al* 2011) A single test of progesterone levels can also help determine how likely a fetus will survive in those with a threatened miscarriage (bleeding in early pregnancy). (Cole , Laurence A *et al* 2004)

1.2.2.4.3 Ultrasound:

Obstetric ultrasonography can detect fetal abnormalities, detect multiple pregnancies, and improve gestational dating at 24 weeks.(Verhaegen J *et al* 2012) The resultant estimated gestational age and due date of the fetus are slightly more accurate than methods based on last menstrual period. Ultrasound is used to measure the nuchal fold in order to screen for Downs syndrome. (Nguyen TH , *et al* 1999).

1.2.2.5 Management:

1.2.2.5.1 Prenatal care:

Pre-conception counseling is care that is provided to a woman and/ or couple to discuss conception, pregnancy, current health issues and recommendations for the period before pregnancy. (Water, Thomas R *et al* 2013)

Prenatal medical care is the medical and nursing care recommended for women during pregnancy, time intervals and exact goals of each visit differ by country. Women who are high risk have better outcomes if they are seen regularly and frequently by a medical professional than women

who are low risk. A woman can be labeled as high risk for different reasons including previous complications in pregnancy, complications in the current pregnancy, current medical diseases, or social issues. (Dowswell, *et al* 2015)

The aim of good prenatal care is prevention, early identification, and treatment of any medical complications (Hurt K and Joseph, ed 2011) A basic prenatal visit consists of measurement of blood pressure, fundal height, weight and fetal heart rate, checking for symptoms of labor, and guidance for what to expect next. (Water, Thomas R *et al* 2013)

1.2.2.5.2 Nutrition:

Nutrition during pregnancy is important to ensure healthy growth of the fetus. Nutrition during pregnancy is different from the non-pregnant state. (Mc Cormick , *et al* 1999).

There are increased energy requirements and specific micronutrient requirements. Women benefit from education to encourage a balanced energy and protein intake during pregnancy. Some women may need professional medical advice if their diet is affected by medical conditions, food allergies, or specific religious/ ethical beliefs. (Ota, E *et al* 2015)

Adequate periconceptional (time before and right after conception) folic acid (also called folate or Vitamin B₉) intake has been shown to decrease the risk of fetal neural tube defects, such as spine bifida. The neural tube develops during the first 28 days of pregnancy, a urine pregnancy test is not usually positive until 14 days post-conception, explaining the necessity to guarantee adequate folate intake before conception. (Klusmann A , *et al* 2005) Folate is abundant in green leafy vegetables,

legumes, and citrus.(Stevenson RE *et al* 2000) In the United States and Canada, most wheat products (flour, noodles) are fortified with folic acid.

DHA omega-3 is a major structural fatty acid in the brain and retina, and is naturally found in breast milk.It is important for the woman to consume adequate amounts of DHA during pregnancy and while nursing to support her well-being and the health of her infant. Developing infants cannot produce DHA efficiently, and must receive this vital nutrient from the woman through the placenta during pregnancy and in breast milk after birth. (Guesnet , *et al* 2011)

Several micronutrients are important for the health of the developing fetus, especially in areas of the world where insufficient nutrition is common. Women living in low and middle income countries are suggested to take multiple micronutrient supplements containing iron and folic acid.

These supplements have been shown to improve birth outcomes in developing countries, but do not have an effect on perinatal mortality. Many developed countries suggest that all women who may become pregnant or who are pregnant take a daily multivitamin containing folic acid and iron.(Salem N *et al* 2001).

In developed areas, such as Western Europe and the United States, certain nutrients such as Vitamin D and calcium, required for bone development, may also require supplementation. (Theobald HE 2007) Vitamin E supplementation has not been shown to improve birth outcomes. (Kuoppala T, *et al* 1986) Zinc supplementation has been associated with a decrease in preterm birth, but it is unclear whether it is causative. (Rumbold, *et al* 2015) Daily iron supplementation reduces the risk of

maternal anemia.(Ota, Erika 2015) Studies of routine daily iron supplementation for all pregnant women in developed countries found improvement in blood iron levels, without a clear clinical benefit. (Pena-Rosas *et al* 2015).

Women are counseled to avoid certain foods, because of the possibility of contamination with bacteria or parasites that can cause illness.(Mc Donagh, M *et al* 2015) Careful washing of fruits and raw vegetables may remove these pathogens, as may thoroughly cooking leftovers, meat, or processed meat.

Unpasteurized dairy and deli meats may contain *Listeria*, which can cause neonatal meningitis, stillbirth and miscarriage. Pregnant women are also more prone to *Salmonella* infections, can be in eggs and poultry, which should be thoroughly cooked. Cat feces and undercooked meats may contain the parasite *Toxoplasma gondii* and can cause toxoplasmosis. Practicing good hygiene in the kitchen can reduce these risks. Women are also counseled to eat seafood in moderation and to eliminate seafood known to be high in mercury because of the risk of birth defects.

Pregnant women are counseled to consume caffeine in moderation, because large amounts of caffeine are associated with miscarriage. However, the relationship between caffeine, birth weight, and preterm birth is unclear.(Tam, Carolyn 2010) .

1.2.2.5.3 Weight gain:

The amount of healthy weight gain during a pregnancy varies. .(Tarlow ,MJ 1994) . Weight gain is related to the weight of the baby, the placenta,

extra circulatory fluid, larger tissues, and fat and protein stores. Most needed weight gain occurs later in pregnancy. (Jahanfar ,*et al* 2015)

The Institute of Medicine recommends an overall pregnancy weight gain for those of normal weight (body mass index of 18.5–24.9), of 11.3–15.9 kg (25–35 pounds) having a singleton pregnancy.^[103] Women who are underweight (BMI of less than 18.5), should gain between 12.7–18 kg (28–40 lbs), while those who are overweight (BMI of 25–29.9) are advised to gain between 6.8–11.3 kg (15–25 lbs) and those who are obese (BMI>30) should gain between 5–9 kg (11–20 lbs). These values reference the expectations for a term pregnancy.

The Friedmann- Balayla Model provides a more accurate calculation of weight gain by gestational age.

During pregnancy, insufficient or excessive weight gain can compromise the health of the mother and fetus. The most effective intervention for weight gain in underweight women is not clear. (Jahanfar ,*et al* 2015) Being or becoming overweight in pregnancy increases the risk of complications for mother and fetus, including cesarean section, gestational hypertension, pre-eclampsia, macrosomia and shoulder dystocia. Excessive weight gain can make losing weight after the pregnancy difficult. .(Tarlow ,MJ 1994)

Around 50% of women of childbearing age in developed countries like the United Kingdom are overweight or obese before pregnancy. Diet modification is the most effective way to reduce weight gain and associated risks in pregnancy. A diet that has foods with a low glycemic index may help prevent the onset of gestational diabetes.

1.2.2.5.4 Medication:

Drugs used during pregnancy can have temporary or permanent effects on the fetus (Thangaratinam, S *et al* 2012) Anything (including drugs) that can cause permanent deformities in the fetus are labeled as teratogens (Tieu *et al* 2008) In the U.S., drugs were classified into categories A, B, C, D and X based on the Food and Drug Administration (FDA) rating system to provide therapeutic guidance based on potential benefits and fetal risks (Briggs , *et al* 2015) Drugs, including some multivitamins, that have demonstrated no fetal risks after controlled studies in humans are classified as Category A. On the other hand, drugs like thalidomide with proven fetal risks that outweigh all benefits are classified as Category X (Thangaratinam, S *et al* 2012)

1.2.2.5.5 Sexual activity:

Most women can continue to engage in sexual activity throughout pregnancy. Most research suggests that during pregnancy both sexual desire and frequency of sexual relations decrease. (Cunningham ,F *et al* 2014) In context of this overall decrease in desire, some studies indicate a second-trimester increase, preceding a decrease during the third trimester (Bermudez MP, *et al* 2001)

Sex during pregnancy is a low-risk behavior except when the healthcare provider advises that sexual intercourse be avoided for particular medical reasons. For a healthy pregnant woman, there is no *safe* or *right* way to have sex during pregnancy. Pregnancy alters the vaginal flora with a reduction in microscopic species/genus diversity (Reamy K, *et al* 1982).

1.2.2.5.6 Exercise:

Regular aerobic exercise during pregnancy appears to improve (or maintain) physical fitness.(Malarewicz A, *et al* 2006) Physical exercise during pregnancy does appear to decrease the risk of C-section (Clark , *et al* 2014) Bed rest, outside of research studies, is not recommended as there is no evidence of benefit and potential harm (Kramer MS, *et al* 2006).

The Clinical Practice Obstetrics Committee of Canada recommends that "All women without contraindications should be encouraged to participate in aerobic and strength-conditioning exercises as part of a healthy lifestyle during their pregnancy" (Domenjoz , *et al* 2014) Although an upper level of safe exercise intensity has not been established, women who were regular exercisers before pregnancy and who have uncomplicated pregnancies should be able to engage in high intensity exercise programs. In general, participation in a wide range of recreational activities appears to be safe, with the avoidance of those with a high risk of falling such as horseback riding or skiing or those that carry a risk of abdominal trauma, such as soccer or hockey (McCall , CA *et al* 2013)

The American College of Obstetricians and Gynecologists reports that in the past, the main concerns of exercise in pregnancy were focused on the fetus and any potential maternal benefit was thought to be offset by potential risks to the fetus. However, they write that more recent information suggests that in the uncomplicated pregnancy, fetal injuries are highly unlikely. They do, however, list several circumstances when a woman should contact her health care provider before continuing with an exercise program: vaginal bleeding, dyspnea before exertion, dizziness,

headache, chest pain, muscle weakness, preterm labor, decreased fetal movement, amniotic fluid leakage, and calf pain or swelling (to rule out thrombophlebitis). (McCall , CA *et al* 2013)

1.2.2.5.7 Sleep:

It has been suggested that shift work and exposure to bright light at night should be avoided at least during the last trimester of pregnancy to decrease the risk of psychological and behavioral problems in the newborn.(Davies *et al* 2003).

1.2.2.6 Complications:

Each year, ill health as a result of pregnancy is experienced (sometimes permanently) by more than 20 million women around the world (Artal R *et al* 2003) In 2013 complications of pregnancy resulted in 293,000 deaths down from 377,000 deaths in 1990. Common causes include maternal bleeding (44,000), complications of abortion (44,000), high blood pressure of pregnancy (29,000), maternal sepsis (24,000), and obstructed labor (19,000).

The following are some examples of pregnancy complications:

- Pregnancy induced hypertension
- Anemia
- Postpartum depression
- Postpartum psychosis
- Thromboembolic disorders. These are the leading cause of death in pregnant women in the US.
- PUPPP (Pruritic Urticarial Papules and Plaques of Pregnancy), a skin disease that develops around the 32nd week. Signs are red

plaques, papules, and itchiness around the belly button that then spreads all over the body except for the inside of hands and face.

- Ectopic pregnancy, implantation of the embryo outside the uterus.
- Hyperemesis gravidarum, excessive nausea and vomiting that is more severe than normal morning sickness.
- Pulmonary embolism, blood clots that form in the legs that can migrate to the lungs.(Merck , 2011)

There is also an increased susceptibility and severity of certain infections in pregnancy.

1.2.2.7 Intercurrent diseases:

A pregnant woman may have intercurrent diseases, defined as disease not directly caused by the pregnancy, but that may become worse or be a potential risk to the pregnancy.

- Diabetes mellitus and pregnancy deals with the interactions of diabetes mellitus (not restricted to gestational diabetes) and pregnancy. Risks for the child include miscarriage, growth restriction, growth acceleration, fetal obesity (macrosomia), polyhydramnios (too much amniotic fluid), and birth defects.
- Thyroid disease in pregnancy can, if uncorrected, cause adverse effects on fetal and maternal well-being. The deleterious effects of thyroid dysfunction can also extend beyond pregnancy and delivery to affect neurointellectual development in the early life of the child. Demand for thyroid hormones is increased during pregnancy which may cause a previously unnoticed thyroid disorder to worsen.

- Untreated celiac disease can cause spontaneous abortion (miscarriage), intrauterine growth restriction, small for gestational age, low birth weight and preterm birth. Often reproductive disorders are the only manifestation of undiagnosed celiac disease and most cases are not recognized. Complications or failures of pregnancy cannot be explained simply by malabsorption, but by the autoimmune response elicited by the exposure to gluten, which causes damage to the placenta. The gluten-free diet avoids or reduces the risk of developing reproductive disorders in pregnant women with celiac disease.(C. Blackwell and Seen 2008) Also, pregnancy can be a trigger for the development of celiac disease in genetically susceptible women who are consuming gluten (Tersigni *et al* 2014)
- Systemic lupus erythematosus in pregnancy confers an increased rate of fetal death *in utero*, spontaneous abortion, and of neonatal lupus.
- Hypercoagulability in pregnancy is the propensity of pregnant women to develop thrombosis (blood clots).

Pregnancy itself is a factor of hypercoagulability (pregnancy-induced hypercoagulability),as a physiologically adaptive mechanism to prevent *post partum* bleeding. However, in combination with an underlying hypercoagulable states, the risk of thrombosis or embolism may become substantial (Saccone G, *et al* 2015)

1.2.2.8 Medical imaging:

Medical imaging may be indicated in pregnancy because of pregnancy complications, intercurrent diseases or routine prenatal care. Magnetic resonance imaging (MRI) without MRI contrast agents as well as

obstetric ultrasonography are not associated with any risk for the mother or the fetus, and are the imaging techniques of choice for pregnant women. Projectional radiography, X-ray computed tomography and nuclear medicine imaging result in some degree of ionizing radiation exposure, but in most cases the absorbed doses are not associated with harm to the baby. At higher dosages, effects can include miscarriage, birth defects and intellectual disability.

1.2.2.9 Epidemiology:

About 213 million pregnancies occurred in 2012 of which 190 million were in the developing world and 23 million were in the developed world. This is about 133 pregnancies per 1,000 women between the ages of 15 and 44. About 10% to 15% of recognized pregnancies end in miscarriage. Globally 40% of pregnancies are unplanned. Half of unplanned pregnancies are aborted (Sedgh G *et al* 2014).

Of pregnancies in 2012 120 million occurred in Asia, 54 million in Africa, 19 million in Europe, 18 million in Latin America and the Caribbean, 7 million in North America, and 1 million in Oceania. Pregnancy rates are 140 per 1000 women of childbearing age in the developing world and 94 per 1000 in the developed world (Sedgh G *et al* 2014) .

The rate of pregnancy, as well as the ages at which it occurs, differ by country and region. It is influenced by a number of factors, such as cultural, social and religious norms; access to contraception; and rates of education. The total fertility rate (TFR) in 2013 was estimated to be highest in Niger (7.03 children/woman) and lowest in Singapore (0.79 children/woman) (Gresele , and Paolo 2008).

In Europe, the average childbearing age has been rising continuously for some time. In Western, Northern, and Southern Europe, first-time mothers are on average 26 to 29 years old, up from 23 to 25 years at the start of the 1970s. In a number of European countries (Spain), the mean age of women at first childbirth has crossed the 30-year threshold.

This process is not restricted to Europe. Asia, Japan and the United States are all seeing average age at first birth on the rise, and increasingly the process is spreading to countries in the developing world like China, Turkey and Iran. In the US, the average age of first childbirth was 25.4 in 2010.

In the United States and United Kingdom, 40% of pregnancies are unplanned, and between a quarter and half of those unplanned pregnancies were unwanted pregnancies.

Globally, an estimated 270,000 women die from pregnancy-related complications each year.

1.2.2.10 Society and culture:

In most cultures, pregnant women have a special status in society and receive particularly gentle care (Jayson and Sharon 2011) At the same time, they are subject to expectations that may exert great psychological pressure, such as having to produce a son and heir. In many traditional societies, pregnancy must be preceded by marriage, on pain of ostracism of mother and (illegitimate) child.

Overall, pregnancy is accompanied by numerous customs that are often subject to ethnological research, often rooted in traditional medicine or religion. The baby shower is an example of a modern custom.

Pregnancy is an important topic in sociology of the family. The prospective child may preliminarily be placed into numerous social roles. The parents' relationship and the relation between parents and their surroundings are also affected.

1.3 Previous Studies:

- (Wilkins *et al* 2004) : Gestation is a challenge to haemostasis and it is associated with significant haemostatic changes.

Several studies have evaluated von Willebrand factor in normal pregnancy.

- (Wickström K *et al* 2004): During pregnancy, significant changes occur in the hemostatic system and in the plasma levels for several plasma proteins, especially towards term. In this study changes occurring during normal pregnancy During third-trimester pregnancy, vWf was higher than in non-pregnant women, and continued to increase postpartum. The fibronectin plasma level was mostly unchanged in comparison with non-pregnant values. There is increased risk of thrombosis during pregnancy and early puerperium, with increased levels of vWf and fibronectin and decreased levels of antithrombin and free protein S.
- (Brenner B in 2004): In normal pregnancy, there is a marked increase in the procoagulant activity in maternal blood characterized by elevation of factors VII, X, VIII, fibrinogen and von Willebrand factor, which is maximal around term. Overall, there is a 4- to 10-fold increased thrombotic risk throughout gestation and the postpartum period.
- (Castaman G in 2013): VWF and FVIII increase significantly during pregnancy in normal women, already within the first trimester, reaching levels by far >100 U/dL by the time of parturition.

1 .4 Rationale:

- Thromboembolic disorders are the leading cause of death in pregnant women .
- Pregnancy itself is a factor of hypercoagulability (pregnancy – induced hypercoagulability).
- Venous thrombosis is a leading cause of morbidity and mortality during pregnancy.
- The right leg venous thrombosis have pain and swelling occur most frequently during the third trimester but left venous thrombosis is relatively equally distributed during all three trimesters.
- Increase in VWF is one of the risk factors for developing thrombosis , so it important to measure the VWF level in pregnant women's .
- To our knowledge there is no previous study addressing association between VWF and pregnancy in Sudan . so this study will try to add to the knowledge that if there is any association.

1.5 Objectives:

1.5.1 General objective:

To measure the von willebrand factor level among third trimester pregnant women

1.5.2 Specific objectives

To determine the level of vwf (Ag) in third trimester pregnant women

To correlate the level of vwf (Ag) with participant` , age.

To correlate the level of vwf (Ag) with participant weight.

To compare the vWF between case and control.

Chapter Two

Material and methods

2.1 Study design:

It is observational analytical case control study

2.2 Study area and period:

This study was carried out during the period from March to May 2017 at Military hospital.

2.3 Study population:

Sudanese pregnant Women from third trimester at different age and weight were recruited to participate in this study as well as non-pregnant women were enrolled as control group.

2.3.1 Inclusion criteria

All third trimester pregnant women.

2.3.2 Exclusion criteria

Pregnant with any disease associated with a change in vWF level

2.4 Sample size

The sample was collected from 40 third trimester pregnant women as (cases) and 40 normal individual non pregnant as (control).

2.5 Sample technique

It is convenience non probability sample.

2.6 Data collection

A questionnaire was filled for each patient by direct interviewing (see appendix 1).

2.7 Ethical consideration

All patients were informed about the aim of the study and they gave their consent.

2.8 Method of blood sample collection

2.7ml of venous blood sample were collected using vacutainer tube which contain 0.38% tri –sodium citrate and gently mixed and then separated immediately at 4500 rpm for 15 min after which platelet poor plasma (ppp) was collected in plain container and stored at -20 °C until analysis.

2.9 Estimation of Vwf: Ag

vWF level was determined for each participant by ELISA(see appendix 2) using Technozyme kit (Technoclone-Austria)

2.10 Data analysis

Data were collected manually in a master sheet and analysis was performed using computerized SPSS program.

Chapter Three

3.1 Characteristic of the studied population:

The percent study included 40 pregnant (cases) and 40 no pregnant (control) was collected.

3.1.1 The demographic data of pregnant

The studied of case characteristic are presented in table (3.1) and represent 40 pregnant of the total cases and the mean of age (years) was 27 minimum 18 and maximum 41 and the vwf mean is about .741u/ml of minimum of .5u/ml and maximum of 1u/ml. and Wight mean 78 was minimum 59 maximum 103.

Table 3.1 the demographic data of pregnant

Factor	Mean	Minimum	Maximum	STD
Age/YEAR	27.22	18	41	5.99
WEIGHT/KG	78.33	59	103	10.35
VWF U/ML	0.741	0.5	1.0	0.12

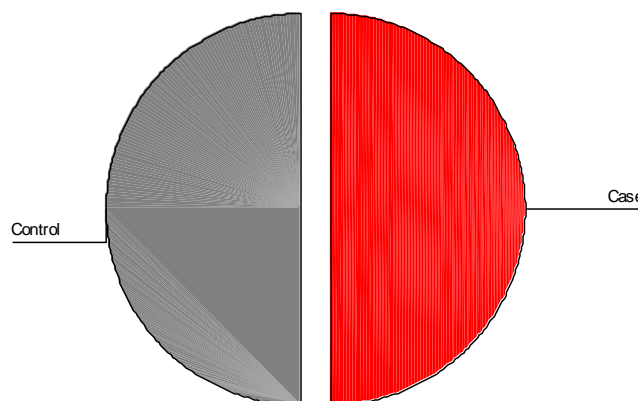


Figure (3-1): Pi chart for study group count.

3-2 vWF (Ag) among case & control groups.

The number of cases is 40 pregnant and 40 healthy individual as control of total of 80 represent in table (3.2) .The mean of vwf (Ag) concentration among pregnant was 0.770 ± 0.1203 U/ml while among control group it was 0.713 ± 0.124 U/ml ; the difference was statistically significant *P.value* : 0.039.

Table (3-2): Independent T-test for VWF means (U/mL) comparison between case & control groups.

Study group	No	Mean	S. Deviation	P. Value
Case	40	0.770	0.120	0.039
Control	40	0.713	0.124	

3.3 The Correlation betweenVwf and age and Wight among study group

The statistical analysis showed that there is positive correlation between vwf (Ag) concentration and age of the patient *p.value*=0.658 see table and Wight of patient *p.value*=0 .723 see table (3.3)

Table (3.3) The Correlation between Vwf and age and Wightamong study group

Correlations

		Old/Year	Weight/Kg	VWF U/mL
Old/Year	Pearson Correlation	1	.809**	-.072
	Sig. (2-tailed)	.	.000	.658
	N	40	40	40
Weight/Kg	Pearson Correlation	.809**	1	-.058
	Sig. (2-tailed)	.000	.	.723
	N	40	40	40
VWF U/mL	Pearson Correlation	-.072	-.058	1
	Sig. (2-tailed)	.658	.723	.
	N	40	40	80

** . Correlation is significant at the 0.01 level (2-tailed).

Chapter 4

Discussion, Conclusions and Recommendations

4.1 Discussion:

The result of our study showed significant association between pregnancy and plasma vwf level (p-value=0.039) but the result show no significant association between vwf and age (P-value=0.658). Which agrees with Brenner B in 2004 who found that in normal pregnancy, there is a marked increase in the procoagulant activity in maternal blood characterized by elevation of factors VII, X, VIII, fibrinogen and von Willebrand factor, which is maximal around term. Overall, there is a 4- to 10-fold increased thrombotic risk throughout gestation and the postpartum period (Brenner B. 2004) Also our results agrees with Castaman G in 2013 who said that the VWF and FVIII increase significantly during pregnancy in normal women, already within the third trimester, reaching levels by far >100 U/dL by the time of parturition (Castaman G.2013) . and agree with Wickström K during pregnancy, significant changes occur in the hemostatic system and in the plasma levels for several plasma proteins, especially towards term. In this study changes occurring during normal pregnancy , during third-trimester pregnancy, vWf was higher than in non-pregnant women, and continued to increase postpartum. The fibronectin plasma level was mostly unchanged in comparison with non-pregnant values. (Wickström K 2004) and agree with Lippincott Williams , Gestation is a challenge to haemostasis and it is associated with significant haemostatic changes. Several studies have evaluated von Willebrand factor in normal pregnancy,(Lippincott Williams 2003) but our results disagrees with MG Conlan who found that VWF level increases with age (Conlan MG, et al 1993).

4.2 Conclusion:

In summary we conclude that according to our result there is association between pregnancy and plasma vwf level, but the result show no significant association between vwf and age and Wight in Sudan.

4.3 Recommendations;-

- ❖ Further studies with large sample size should be carried out in vWF among pregnant Women to provide more definitive information about its effect
- ❖ Further studies must be done in the other coagulation parameter and endothelial marker to evaluate its changes in Pregnant Women.
- ❖ More intensive study must be done in all trimester.
- ❖ Study the activity of coagulation parameter in three trimester.
- ❖

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Appendix (1)

Questionnaire

Date:...../...../2017

Serial number:.....

Name:.....

Age:.....yrs

Weightkg

Type of Trimester:

First Second Third

Do you have any
disease/s?.....

Do you have a abortion?.....

Appendix (2):Equipment for performing the ELISA test

Equipment for performing the ELISA test

