VON WILLEBRAND FACTOR LEVELS AND ACTIVITY IN WOMEN WITH MENORRHAGIA IN KENYATTA NATIONAL HOSPITAL

Dr. Mutai Mercy Chepkemoi

MBChB (KU)

H58/6717/2017

A research proposal submitted in partial fulfilment for the Degree in Masters of Medicine in Human Pathology, University of Nairobi.

DECLARATION

STUDENT'S DECLARATION

This proposal is my original work and it has not been presented for a degree in any University.

Dr. Mutai Mercy Chepkemoi

MBChB (KU)

Signature…… Date…01/11/2022…

ii

SUPERVISORS' DECLARATION

Dr. FREDRICK O. OKINYI MBChB, MMed (Path)

Lecturer, Haematology and Blood Transfusion

Department of Human Pathology

School of Medicine, University of Nairobi.

Signature..... Date 1/11/2022

Dr. JAMILA RAJAB, MBChb, MMed (Path), MPH (UoN), FC Path (ECSA)

Senior lecturer, Haematology and Blood Transfusion Unit

Department of Human Pathology, School of Medicine, University of Nairobi.

Signature..... Date 1/11/2022

Dr. STEPHEN MUTISO, MBChB, MMed (Obs and Gynae)

Specialist Obstetrician/Gynaecologist, Kenyatta National Hospital,

Honorary Lecturer, Department of Obstetrics and Gynaecology

School of Medicine, University of Nairobi.

Signature..... Date 1/11/22

iii

Dedication

I would like to dedicate this dissertation to myself for the hard work and for perseverance through the study and for not giving up when work seemed impossible.

Acknowledgement

I would like to thank my parents Ernest and Lucy Mutai for their constant support during this dissertation.

I would also like to thank my supervisors Dr. Fredrick Okinyi, Dr. Jamilla Rajab and Dr. Stephen Mutiso for their valuable guidance and assistance throughout this study. I would to acknowledge my research assistants Mombo and Stacey, my statistician Ayieko and KNH and UON laboratory personnel: Anastasia, Hannington, Kaunda, Simiyu and Patrick (KAVI) for the help they gave during the study.

Finally, I would like to thank my classmates: Nyanchama, Waithera, Mwale, Wasike, Francine, Chemonges, Wang'ombe and Mbanga for working with me. You made this work bearable.

Abbreviations

APTT- Activated Partial Thromboplastin Time

BAT - Bleeding Assessment Tool

DIC –Disseminated Intravascular Coagulopathy

dL – decilitre

DNA – Deoxyribonucleic Acid

EDTA - Ethylenediamine tetraacetic acid

ELISA – Enzyme-linked Immunosorbent Assay

FFP- Fresh Frozen Plasma

FVIII: C – Factor VIII activity

GPIbα – Glycoprotein Ib Platelet Subunit Alpha

Hb- Haemoglobin

HMB – Heavy Menstrual Bleeding

INR – International Normalized Ratio

ISTH -International Society of Thrombosis and Haemostasis

IU- International Units

KNH - Kenyatta National Hospital

LOD - limit of detection

mRNA – messenger Ribonucleic Acid

MS Excel – Microsoft Excel

PFA – Platelet Function Test

PPP - platelet poor plasma

PT- Prothrombin time

RIPA – Ristocetin-induced Platelet Aggregation

SOP -Standard Operating Procedure

SPSS - Statistical Package for the Social Sciences

SSC - Scientific and Standardization Committee

TT – Thrombin Time

VWD- Von Willebrand Disease

VWF: Ag – Von Willebrand Factor Antigen

VWF: CB – Von Willebrand Factor- subendothelium collagen interaction

VWF: F VIIIB –Von Willebrand Factor- Factor VIII interaction

VWF: RCo –Von Willebrand Ristocetin Cofactor

VWF-Von Willebrand Factor

WHO- World Health Organization

Definition of Terms

Activated Partial Thromboplastin Time- A haemostasis screening test for intrinsic coagulation pathway

Prothrombin Time- A haemostasis screening test for extrinsic coagulation pathway.

Assays- Laboratory analytic procedure

Mutation- Structural change in a gene

ISTH/SSC) Bleeding Assessment Tool –A tool used to evaluate a patient for possible bleeding disorder(1)

Table of Contents

ABSTRACT

Levels and activity of Von Willebrand Factor in Women with Menorrhagia

Study Background

Von Willebrand disease (VWD) is the most prevalent inherited bleeding disorder. It is a qualitative or quantitative defect in von Willebrand factor that leads to prolonged and excessive bleeding. The global prevalence is estimated to be 1-3 %. The most common presentation of VWD in women is menorrhagia, however mild the disease is. This reduces the quality of life of these patients. The role of haemostasis is important in management of obstetrics and gynaecological conditions.

Mild forms of the disease often go undetected and eventually cause clinical complications. Women with VWD have low quality of life. Prolonged and excessive bleeding among patients with VWD is often documented after surgical procedures.

Few studies have been done on women with VWD in Africa, including Kenya. Testing for levels and activity of Von Willebrand Factor in women with menorrhagia in Kenyatta National Hospital will be a basis for specific testing for the disease. The findings of this study were meant to prompt reviews on how patients with chronic gynaecological bleeds are evaluated. Obstetrician/gynaecologists and haematologists will set up new practices when managing these patients.

Broad Objective

To determine antigen levels and activity of Von Willebrand Factor among women with menorrhagia in KNH between January 2021 and August 2021

Study design and Site

This was a descriptive cross-sectional study which took place in Kenyatta National Hospital gynaecology clinics and acute gynaecology ward (1D).

Participants and Methods

The study population was women of reproductive age (15-45 years, WHO) on management for heavy menstrual bleeding in KNH between January 2021 and August 2021. Consecutive sampling technique was applied and 50 participants were recruited for the study after signing consent and/or assent. Patients' information was collected using questionnaires. In the questionnaire, the severity of menorrhagia was assessed using the ISTH/SSC bleeding assessment tool focusing on menorrhagia. Venous blood samples were collected. Processing took place at the KNH haematology laboratory. The tests included; Haemoglobin levels,

platelet count, ABO blood group, PT, APTT, Von Willebrand antigen, activity and factor VIII levels.

Data Management

Data analysis was done using MS Excel and SPSS version 21. Frequency distribution and percentage was used in univariate analysis for categorical analysis. Chi square t-test was used to compare VWF levels antigen and activity between patients with blood group O and non-O groups. Tables and graphs were used to present data. Results were disseminated to UON Pathology department and KNH Obstetrics and Gynaecology department, where they were placed in the patients' files.

Outcomes

Out of 50 women with HMB, age ranges 17 to 49 years, 29 (58%) were over 35 years. 21 (42%) of the participants had a nonspecific clinical diagnosis of abnormal uterine bleeding (AUB) while the most common specific diagnosis was uterine fibroids that affected 19 (38%) patients. 48 had normal platelet count, one had mild thrombocytopenia and one had thrombocytosis. 4% of the patients had prolonged PT and 10% had prolonged APTT. 1 out of 50 patients had low levels and activity of VWF. The levels of VWF were significantly higher in non-O blood groups individuals than those of blood group O ($P = 0.035$) while antibody levels in O and non-O groups had no significant difference (P = 0.255). Most of the patients had menorrhagia score of 1 (32%) and 2 (32%). 10 (20%) of the patients had a score of 4 and 8 (16%) had a score of 8.

Conclusion

- In this study, 2% of patients had low levels of VWF antigen and activity
- Patients of blood group O had significantly lower VWF: Ag levels than those with non-O blood.
- Majority of the patients had low menorrhagia scores of 1 and 2 (on the ISTH tool) (32%) for each score.

Recommendations

Low VWF antigen and/or antibody levels and VWD being hereditary disorders, multicenter/ethnic studies should be done to include all age groups to determine the true prevalence of the disease in our population as this study population in KNH was mainly from Nairobi and environs.

1.0 INTRODUCTION

Inherited bleeding disorders are lifelong conditions associated with a wide range of bleeding manifestations. Von Willebrand Disease (VWD) is the most frequently occurring inherited bleeding disorder(1). It results from either quantitative or qualitative defect in von Willebrand factor (VWF). The mode of inheritance is autosomal dominance for types 1 and 2A, 2B and 2M and autosomal recessive for types 2N and 3 (2). Women with bleeding disorders experience menorrhagia as the most frequent symptom (3). The first patient to be diagnosed with VWD by Erik Von Willebrand died on her 4^{th} menstrual cycle due to heavy menstrual bleeding(HBM) (4)

Globally, the prevalence of VWD is estimated to be 1-3 %.(5). Some studies estimate the prevalence to be 1% (4). It affects male and female equally but women encounter more haemostatic challenges during childbirth and menstruation. All forms of VWD, including mild ones impair quality of life of women as they have increased absence from school and work with a resultant economic implication due to heavy menstrual bleeding (HMB). Severe cases lead to iron deficiency anaemia. (6).

A study was done in Stockholm by Govorov et al to assess the effects of HMB on women in comparison to normal controls. The quality of life between the two groups was significantly different, as those with HMB had body pain and interference of normal daily activities. Shankar et al reviewed studies on VWD among women with heavy menstrual bleeding and found prevalence ranging from 5% to 24% with a 13% overall prevalence(7). Published studies have indicated 70-90% of women with VWD have heavy menstrual bleeding, with a reported estimate of 30-100%. (2).

VWD has a prevalence of 5% to 24% among American women with chronic HMB; with Caucasians having a higher prevalence of 15.9% than African Americans at 1.3% (4).

A study on bleeding disorders in women with HMB in Kermanshah University of Medical Sciences, Japan recorded a prevalence of 55.3% (31) for VWD after excluding confounders. Of these, 45.2% had type 3, 32.3% with type 2 and the least frequent was type 1 with 22.5% (3)

A prospective cohort in 5 medical centres in Egypt detected an estimated prevalence of VWD among women presenting with menorrhagia to be 17.8% (64 out of 359). 81% of them (52/64) had VWF deficiency while 19% (12/64) had defective function (8)

A study on 94 women with heavy menstrual bleeding in KNH by Akumu in 2019 concluded that a significant number of patients had haemostatic challenges; 17% of them had thrombocytopenia, 6.4% had prolonged APTT and 16% had prolonged PT. The patients with family history of haemorrhagic disorders had a 9 fold chance of being treated for anaemia when compared to those with no family history. (9)

A case control study was done by Munoko in 2020 at Aga Khan University Hospital on the prevalence of VWD in women with HMB. 19 cases were compared to 49 controls. The cases with HMB had 0%

prevalence of VWD and 5.3% prevalence of low VWF levels, while the controls had 4.1% prevalence of VWD and 10.2% of low VWF levels. In this study the investigator noted that higher numbers of recruits would increase statistical significance of the study. (10)

In Kenyatta National Hospital, approximately 2 patients in a month are admitted due to menorrhagia. A study done on female athletes taking part in the London marathon 2015, found that 427 (54%) out of 789 women met the criteria for menorrhagia. For the participants, menstruation affected their performance with some having symptoms of anaemia. (11).

When managing patients with bleeding disorders, obstetrician/gynaecologists may fail to appreciate the role of haemostasis in obstetric events. Haematologists may not recognize gynaecological conditions that increase the possibility of VWD. (2). Prolonged, excessive bleeding is often reported after surgery. (12). Mild forms of VWD are mostly undetected and result in clinical repercussions (2). A review by Harlow and Campbell on menstrual disorders, found that there is little action taken to reduce menstrual disorders including excessive menstrual bleeding in developing countries. These disorders are excluded when dealing with disease burden globally (13)

Results

The study was done on 50 women aged between 17 and 49 years who presented to KNH with HMB. Of these patients one patient had low VWF levels and activity with VWF:Ag of 37.3%, VWF: Ab of 33.2% and FVIII activity of 57.3%.The mean and median VWF:Ag were 118.6% and 119% respectively. The mean and median VWF:Ab were 109.5% and 110% respectively while the FVIII were 210.2% and median of 198%. Patients with blood group O had significantly lower levels of VWF than none-O groups ($P = 0.035$) and lower VWF activity ($P=0.255$). Severity of menorrhagia measured using ISTH BAT (1-4) was 3 in the patient with low VWF:Ag and VWF:Ab. Out of the 50 patients 16 (32%) had scores of 1, 16 (32%) had a score of 2, 8 (16%) had a score of 3 while 10 (20%) had a score of 4.

Conclusion

There was a 2% (1 patient) prevalence of Low Von Willebrand Factor antigen and activity levels in our study. Patients with blood group O had significantly lower VWF: Ag levels than those with non-O blood. Majority of the patients had low menorrhagia scores of 1 and 2 (on the ISTH tool) (32%) for each score.

2. LITERATURE REVIEW

Haemostasis is a complex, tightly regulated mechanism that terminates vessel bleeds. It is a process that entails multiple steps. This cascade causes formation of a fibrin clot that closes the damaged part of the blood vessel, stopping the bleeding. Once the integrity of the vessel is reinstated, the clot breaks down and haemostasis is restored. Vascular endothelium disruption elicits interaction of physiological processes, which include development of a platelet plug (primary haemostasis), activation of coagulation, fibrin mesh formation (secondary haemostasis), breaking of fibrin and repair of the injured vessel.(12)

2.1.1 Von Willebrand Factor Protein

Von Willebrand factor (VWF) is a cohesive glycoprotein with multimers. It is found in megakaryocytes and Weibel Palade bodies of vascular endothelium. It was discovered in 1924 by a Finnish physician Erik von Willebrand. (14). In 1926 he described Von Willebrand Disease and differentiated it from haemophilia.(15). VWF has 2050 subunits of amino acids and functions as a factor VIII (FVIII) carrier and adhesive for platelets to sub endothelium of blood vessels. It forms VWF-FVIII complex that is not covalently bound and functions to protect FVIII from being degraded by activated protein C. It also localizes FVIII to specific sites of the platelet plug, which is followed by clot formation. It adheres platelets to damaged vascular sub endothelium. After vascular injury, VWF is immobilized by its attachment to types i and iii fibrillar collagen of sub endothelium via VWF domains A1 and A3. It in turn binds to platelets via platelets receptors the glycoprotein GPIbα in GPIb-IX-V complex and αIIbβ3 (GPIIb-IIIa complex) integrin on platelet membrane. VWF-dependent platelet adhesion occurs under fluid shear stress.(14)

Generally accepted normal levels of VWF: Ag in plasma ranges from 50-200 IU/dL. However, the reference ranges vary from one laboratory to another. It is also noted that individuals with blood group O have lower levels of VWF with ranges of 40-50 IU/dL(16)

2.1.2 Von Willebrand Disease

VWD results from quantitative or qualitative defect in Von Willebrand factor. It is classified into: VWF quantitative deficiencies which can be partial or complete deficiencies namely types 1 and 3 respectively while qualitative deficiencies are type 2. Type 1 disease is characterised by partial quantitative deficiency. Type 3 is complete deficiency of the factor. Type 2 has 4 variants (A, B, M, and N). Type 2A is characterized by decreased platelet-dependent function due to lack of VWF multimers of high and intermediate-molecular-weight. Type 2B is characterised by increase in affinity for platelet GPIb. Type 2M is identified by decreased platelet-dependent function. 2N variant has decreased affinity for factor VIII. (17). The most common these is type 1, accounting for 70-80%, type2; 20% and type 3; 5-10% (18)

Acquired Von Willebrand Syndrome presents mainly in elderly patients. It occurs as a result of cardiovascular, autoimmune, myeloproliferative and lymphoproliferative disorders. Patients with this

form of VWD have no previous history or family history of bleeding. Deficiency of VWF in these patients is thought to result from increased clearance.(19)

2.1.3 Clinical manifestation

General clinical manifestations of VWD are; easy bruising, bleeding after dental and surgical procedures and epistaxis. Other than menorrhagia and postpartum haemorrhage, women with VWD have increased incidence of developing ovarian cysts that are haemorrhagic, fibroids, endometriosis, endometrial hyperplasia and anovulation. (2)

Menorrhagia is defined as bleeding more than 80 ml in 1 menstrual cycle. (6). To fit the criteria for heavy menstrual bleeding (HMB), an individual can have 2 or more of the following; passage of large clots, use double sanitary protection, change of sanitary material every two hours or passage of the menstrual blood to clothes or bedding (11) A three-month survey by WHO in several countries identified the prevalence of menorrhagia to be 8-27% (20) The most common causes of heavy menstrual bleeding include: endometrial polyps, myometrial dysfunctions (adenomyosis & leiomyoma), endometrial malignancy & hyperplasia, coagulopathies, ovulatory dysfunction (hormonal causes), abnormalities in the endometrial stratum basalis and iatrogenic causes(21)

Diagnosis of VWD starts with acquiring a detailed clinical history and correlating it with laboratory components. The patient's history of; easy bruising, prolonged bleeding from lacerations, epistaxis, bleeding from gums, menorrhagia, excessive bleeding after child birth and dental or surgical procedure are recorded. Muscle haematomas and haemarthrosis may be present in type 3 VWD. Family history of excessive bleeding guides towards diagnosis. In some women, the only positive history is that of menorrhagia. (12) Patient examination involves assessment for the presence of haematomas, petechial haemorrhage, ecchymosis, anaemia and other signs of bleeding; splenomegaly, arthropathy and joint laxity.(2) .

2.1.3.1 Bleeding Assessment Tools

Evaluation of a patient suspected to have a bleeding disorder includes the use of bleeding assessment tools including the International Society of Thrombosis and Haemostasis (ISTH) and Scientific Standardization Committee (SSC) Bleeding Assessment Tool (BAT), The Pictorial Blood Loss Assessment Chart (PBAC) for menorrhagia and the Paediatric Bleeding Assessment tool by ISTH. Most of the BATs have evolved over time and their use has been mainly in case control studies. ISTH Bleeding assessment tools have standardized questionnaires with clinical symptoms and interventions. They function by assessing possibility of haemorrhagic disorders in both paediatric and adult groups. A scoring system is applied for 14 bleeding symptom where a patient is scored according to severity of the symptom and the level of intervention. The scores range from 1 to 4 with increasing severity of bleeding (Appendix 4). There are attempts to modify the BATs since they are long and have symptoms that are only useful in the paediatric age group and not adults. It is in this regard that a study done by Spradbrow focused on the most predictive symptoms. Epistaxis, haemarthrosis and menorrhagia have

been found to be very useful in predicting congenital bleeding disorders including VWD. Other studies therefore recommend modification of bleeding assessment tools to evaluate patients with bleeding disorders. In gynaecology, PBAC, a semi quantitative tool for menorrhagia is mostly used. The patient is scored according to the number and level of soiling of sanitary pads and/or tampons (22)(23)

2.1.4 Laboratory Tests

Abnormal haemostasis screening tests (complete blood and platelets count, PT, APTT and fibrinogen or TT) prompts the decision to perform specific tests for VWD (24). However, PT and APTT may not be reliable in screening for all types of VWD as they are mostly normal. APTT may be prolonged in types 2N and 3. Platelet Function Test (PFA 100) is sensitive for VWF but specificity for VWD is low. Diagnosis of VWD is mainly dependent on high index of suspicion (16) A group of laboratory tests are done to explore the different types of VWD. Diagnostic tests for VWD evaluate important properties of VWF: VWF antigenic level (VWF:Ag) with normal ranges being 50-150 IU/dL, interaction between platelet GPIb and VWF - (VWF:RCo), VWF and subendothelium collagen interaction (VWF: CB), VWF and FVIII interaction (VWF:FVIIIB), VWF's capacity to be form multimers and FVIII activity (FVIII:C) which reflects its protective capacity to prevent FVIII from degradation (24)

VWF ristocetin cofactor measures interaction of VWF with the GPIb receptor found on the surface of platelets. This assay utilizes ristocetin sulphate an antibiotic, to enhance in vitro interaction of VWF and GPIb. It is comparable to physiological activity and distribution of VWF multimers. This test is difficult to perform and has poor sensitivity. The tests rely on manual visualization of agglutination and platelet aggregation. The inter-laboratory coefficient of variation is about 30–40% when analysing samples with low content of VWF. The limit of detection (LOD) is high (10–20 U dL) making it difficult to identify and distinguish between types of VWD with low activities.(24)

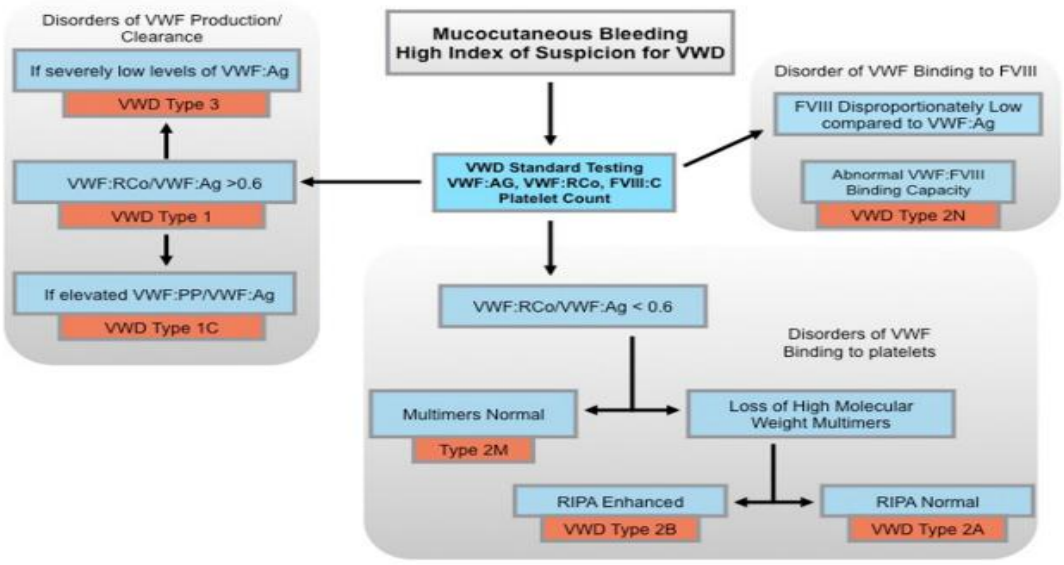
Modifications of the VWF: RCo test using micro plates/ELISA or automations for agglutination have been developed. Currently, ELISA or particle-based assays that are automated and independent of ristocetin are used. These novel assays are simpler to use. However, they have no independent assessment on all VWD types. The old method is therefore still in use.

Genetic analysis is used in patients whose specific tests fail to classify the type of VWD. In type 1, it helps in understanding pathogenicity where there are very low VWF levels. It identifies compound heterozygous individuals who have two separate mutations leading to a near total absence of VWF and those with a single dominant inherited mutation. Some individuals have rapid clearance phenotype showing elevated VWFpp/VWF:Ag ratio (mutations between exons 25 and 31) .

Type 3 diagnosis is mostly definite but may need discrimination from severe form of type 1. Mutation index is used to determine the causative mutation. Analysis of dosage and DNA sequence can single

out mutations in up to 90% of type 3 VWD alleles. Analysis of mRNA may identify absent mutations in patients whose DNA mutations are unidentified.

Genetic testing is done for patients with type 2 VWD when a disparity between VWF: RCo and VWF:Ag (≤0.6) indicate low platelet-binding activity with no clarification on typing. The starting point for analysis is exon 28. Type 2A mutation are in domains A1 and A2 of exon 28 and domain D3 of exons 22, 25–27 (>25% of 2A mutations) and rarely in cysteine knot (CK) of exon 52 and domain D2 of exons 11–17. Type 2B is a mutation that causes gain of function and is characterized by enhanced RIPA spontaneous VWF- GPIb binding. Mutations in type 2B VWD occur on domain A1 encoded by exon 28 at the 5' end. Pseudo-Von Willebrand Disease (Platelet-type) a result of GP1bA gene mutations has a similar phenotype to 2B VWD and is differentiated from it by analysing exon 2 of that gene. Mutations on exons 28-32 in type 2M affect binding to: GPIb (domain A1), collagen (domain A3) or both. Type 2N Mimics mild haemophilia A. There is reduced FVIII-VWF binding, resulting in reduced plasma half-life and levels of factor viii. It can have reduced coagulant activity of FVIII with either normal or reduced levels of VWF. This is caused by missense mutations domain D of exons 18–20, mutations close to the cleavage site of propeptide of exon 17 and in domain D3 of exons 24 and 25 (24)



Diagnostic Approach to a Patient with Von Willebrand Disease (16).

2.1.5 Treatment

Treatment regimens given to patients with HMB and VWD by obstetrician/gynaecologists and haematologists include hormonal and non-hormonal therapy. Heavy menstrual bleeding is thought to be reduced by progesterone administered orally, intrauterine or through implants or depots. Antifibrinolytics like tranexamic acid and □ aminocaproic acidmay help reduce bleeding (25).

Intranasal Desmopressin (DDAVP), a synthetic vasopressin analogue releases VWF from storage sites. Desmopressin can be given with VWF-containing concentrates (2)

2.2 Study Rationale

VWD among women presenting with menorrhagia has been associated with poor quality of life resulting in a significant economic implication. Few studies have been done on women with VWD in Africa, including Kenya. The findings of this study may form a baseline towards specific testing and define the prevalence of VWD among women with menorrhagia in this population. This may prompt obstetrician/gynaecologists and haematologists to review current practices in managing patients who present with chronic gynaecological bleeds. VWD is reported to be the most common heritable bleeding disorder in women of Caucasian race (2). The burden of this disorder is largely unknown in African populations including Kenya.

2.3 STUDY QUESTION AND OBJECTIVES

2.3.1 Study Question

What are the levels and activity of Von Willebrand Factor in women with menorrhagia in KNH between January and August 2021?

2.3.2 Broad Objective

To determine the antigen levels and activity of Von Willebrand Factor among women with menorrhagia in KNH between January and August 2021.

2.3.3 Specific Objectives

1. To document the antigen levels of Von Willebrand Factor in patients with menorrhagia in KNH
2. To determine activity of Von Willebrand factor in women with menorrhagia in KNH
3. To assess severity of menorrhagia using part of ISTH/SSC Bleeding Assessment Tool and correlate it to the levels and activity of Von Willebrand Factor

3.0 METHODOLOGY

3.1 Study design

This was a descriptive cross-sectional study

3.2 Study Site

The study was conducted in Kenyatta National Hospital which is the largest public teaching and referral hospital in Central and Sub-Sahara Africa. It is located in Upper Hill, Nairobi, about 4 kilometres from the central business district. KNH is University of Nairobi's main teaching hospital. It has 50 wards with 1800 bed capacity, 22 outpatient clinics, 24 theatres and an accidents and emergency unit. This study was conducted in gynaecology clinics and acute gynaecology ward (1D). Laboratory tests were done at the KNH haematology laboratory.

3.3 Population

The study population was women who were of reproductive age (15-49 years, WHO) on management for menorrhagia at KNH between January 2021 to August 2021.

3.3.1 Inclusion Criteria

Women who presented with heavy menstrual bleeding.

3.3.2 Exclusion Criteria

1. Patients who declined to give informed written consent to participate in the study.

2. Patients who had been transfused within 12 weeks

3. Patients who had severe thrombocytopenia (platelet levels less than 20×10^9 /L) and other known causes of bleeding disorders other than Von Willebrand disease

3.4 Sample size determination

Sample size was calculated using the Fisher's formula (Daniel WW (1999));

$$n = \frac{Z^2 x\, P(1-P)}{d^2}$$

Where,

n = Desired sample size

Z = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI)

P = expected true proportion (estimated at 17.8%, from a study conducted in Egypt by Nabil et al in 2016 of women with menorrhagia who had low levels of VWD.)

d = desired precision (0.05)

$$n_0 = \frac{1.96^2 x\ 0.178(1-0.178)}{0.05^2} = 217$$

Due to COVID 19 about 65 patients with menorrhagia were treated in KNH in the last 6 months which was a reduction from previous estimate of about 217. Adjusting the sample size for finite populations less than 10,000

$$nf = \frac{n_0}{1 + \frac{n_0 - 1}{N}} = \frac{217}{1 + \frac{217 - 1}{65}} = 50$$

The estimated sample size of 50 patients was required for the study.

3.5 Sampling Technique

Consecutive sampling method was employed

3.6 Recruitment of Participants and Consent Procedure

There was continuous sampling of patients with menorrhagia attending the gynaecology clinics and those admitted in the acute gynaecology ward. The principal investigator and/or trained research assistant processed the study subjects using a modified bleeding assessment tool as a questionnaire.

A written informed consent and /or assent (Appendix 1) was obtained from the patient after explanation of the purpose and benefits of the study procedure and specimen collection. The consent explained the study significance and the rights and responsibilities of the study participants. A copy of the consent was given to the patient.

Prior to recruitment, the principal investigator and the assistant obtained patients' information in their files before clinic to identify potential participants. All patients with heavy menstrual bleeding were recruited. Once they had been identified, they were approached at triage. Those who chose to participate in the study signed consent forms. Questionnaires were filled before consultation with the obstetrician/gynaecologists. In the questionnaire, the severity of HMB bleeding was recorded according to the ISTH/SSC bleeding assessment tool focusing on menorrhagia (Appendices 2 and 4). The presence of other bleeding symptoms was noted and recorded as present or absent for each patient. Blood samples were taken after consultation. Samples were taken only for the tests that had not already been done. Results for the tests that had been done were taken from the patients' files. These included: Hb, platelet, PT and APTT levels. Continuous recruitment of subjects was done until the sample size of 50 was attained.

3.7 Variables

Independent variables that were analysed include: Age, parity, types, sites and severity of bleeds, family history of prolonged bleeding, degree of anaemia, platelets count and ABO blood grouping.

Dependent variables were Von Willebrand factor levels, Von Willebrand Factor Activity and Factor VIII levels

3.8 Data collection

Patient social, demographic and clinical data was collected using questionnaire (Appendix2) administered by the principle investigator and research assistants. Venous blood samples were collected by phlebotomists as per phlebotomy Standard Operating Procedure (SOP). The samples were transported using a cool box to the KNH haematology laboratory within two hours of sample collection.

3.9 Laboratory Procedure

Haematological laboratory procedures were conducted as per KNH haematology lab SOPs. The tests included: Hb, platelet count, ABO blood grouping, haemostasis screening (PT and APTT) and Von Willebrand antigen, activity and factor VIII assays (Appendix 3).

3.10 Materials and Equipment

Request form, disposable Syringes(10ml) and needles(19 and 21G), gloves, elastoplasts, rack to hold specimens and sample collection bottles (EDTA (ethylenediaminetetra acetic acid) and 2.7ml of 3.2% sodium citrate.

3.10.1 ABO Blood Grouping

Tile, Antisera (anti-A, anti-B, anti-AB), droppers

3.10.2 Full Blood Count

Sysmex XN-1000 Haematology Analyzer, CD-ROM, Control blood, vials for control blood.

3.10.3 Activated Partial Thromboplastin Time (APTT)

Normal plasma 1 ml vial, distilled water, APTT-SP reagent, 1 vial 0.025M CaCl2, 2 reagent reservoirs labelled “APTT” and “calcium”, 0.5 ml sample cups, thrombolyzer XRM, wooden applicator sticks, automatic pipettes and plastic transfer pipette tips,

3.10.4 Prothrombin Time (PT)

Normal plasma, distilled water, PT-Fibrinogen HS Plus powder, 8ml PT-Fibrinogen HS plus buffer, Reagent reservoir and sample cups (0.5ml/2ml IL), automatic pipette and plastic disposable transfer pipette, thrombolyzer XRM.

3.10.5 Von Willebrand Factor levels and Activity

Latex reagent, antigen reagent, FVIII-deficient plasma, factor diluent, buffer, calibration plasma, normal control, washing solution, 0.5ml sample cups, rotors, plasma from patients with Von Willebrands Disease, ACL Elite pro coagulation analyser, HemosIL latex immunoassay. ACL Elite pro is a new fully automated assay for VWF activity from Instrumentation Lab Company. It measures

VWF:Ag, activity and FVIII Levels. A prospective study done on the assay showed 100% sensitivity and 86% specificity for VWF abnormality where the results were confirmed using a ristocetin cofactor-based assay (Salem & Van Cott, 2007)

3.10.6 Validation of HemosiL Equipment

HemosiL machine was validated by checking for accuracy and linearity to ensure its fitness for purpose. Validation was done using the standard procedures for quantitative tests. Low and normal controls of Von Willebrand Factor antigen and antibody were used. The controls were ran.

Accuracy was checked by having target values of normal and low controls. 10 points of the 2 levels were measured in duplicates. The mean of the 10 points were calculated and the findings compared to the target value. The difference between the measured value and the target value were compared to assess for acceptance. Linearity was checked to assess if the equipment could correctly measure samples with increasing concentration gradient. Different dilutions of low and normal values of both Von Willebrand Factor antigen and antibody were mixed. The manufacturer provided predetermined target values for these dilution. A graph with the target values against the measured values at different dilutions were plotted. Computing into a statistical package was done to see if reported values followed a linear gradient. The best valid linearity was a straight line on the graph.

3.11Quality Assurance Procedures

3.11.1 Pre analytical Procedures

Blood samples were taken using standard operating procedure for phlebotomy for coagulation samples (Appendix 3). The request forms and sample bottles were labelled clearly and transported to the laboratory within an hour of collection. At the laboratory the full blood count and ABO grouping was done immediately while those for PT, APTT and VWD tests were stored in a freezer at -80°c.

3.11.2 Analytical Procedure

Calibration and validation of the instrument had been done prior to our use as a similar study was on going. Quality control was done using materials from the manufacturer before running patients' samples. The QC passed.

3.11.3 Post Analytical Procedure

Data entry was done immediately the results come out to avoid transcription errors. Values were exported from the instruments to the PI's computer Ms Excel.

3.12 Ethical Consideration

The study proposal was submitted to KNH-UON Ethics and Research Committee for review and approval was given (Appendix 5). Each patient signed an informed written consent. The patient below the age of 18 signed an assent. Confidentiality was maintained by assigning a unique number. Only the PI, supervisors and statistician had access to the data. Soft copies were maintained in a personal

controlled laptop. Patients did not benefit directly. However, Full Blood Count that was done immediately may have been used in patient management. The samples for PT, APTT and Von Willebrand tests were batched and their results had no effect on the patients' management and outcome.

3.13 Data Analysis

Data collected was keyed in MS Excel and assessed for completeness and possible errors. Analysis was done using MS Excel and SPSS version 21. Frequency distribution and percentage was used in univariate analysis for categorical analysis. Continuous data was presented as means and medians. In bivariate analysis, chi square t-test was used to associate VWF levels and activity with ABO blood groups. The levels and activity of VWF were compared between patients of O and non-O blood groups and p values less than 0.05 were considered significant. Data was presented using tables, graphs and pie chart.

3.14 Dissemination of Results

Results are available in case files as part of patients' medical records. Study findings were shared with the medical fraternity of the Departments of Human Pathology and Obstetrics & Gynaecology of the UoN and KNH and professional fora. The investigator hopes to publish at least one paper in a peer reviewed journal.

3.15 Study Plan and Procedure

Prior to recruitment, the PI went through the patients' files before clinic to identify potential participants. All patient with heavy menstrual bleeding were recruited. Once they had been identified, they were approached at triage. Questionnaires were filled before consultation with the obstetrician/gynaecologists. Those who chose to participate in the study signed consents and an assent (only one patient was below 18 years of age). Blood samples were taken after consultation. Samples were taken only for the tests that had not already been done. Results for the tests that had been done were taken from the patients' files.

The principal investigator ensured SOP (Appendix 3) were followed when collecting, transporting and running of the tests.

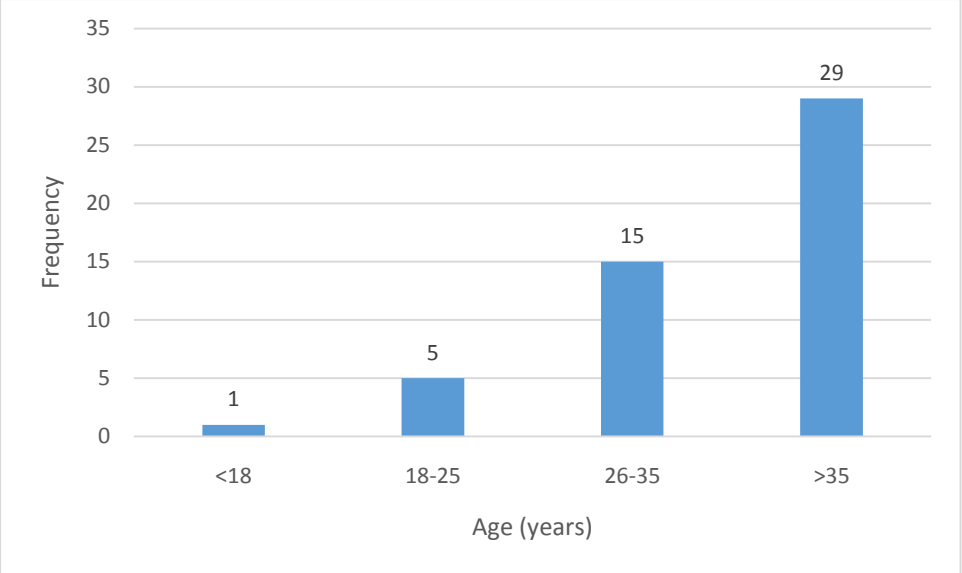
4. RESULTS

4.1 Demographic Data

The aim of this study was to determine levels and activity of von Willebrand factor in women who presented to KNH with heavy menstrual bleeding between January and August 2021 and to determine the severity of bleeding using ISTH/SSC bleeding assessment tool.

Von Willebrand factor levels and activity tests were performed on samples from 50 women. The age range was 17 to 49 years with a mean age of 36.6 and median age of 37.5. Figure 1 below shows patients' age distribution. Most of them (29) were over 35 years.

Figure 1: Age distribution



Only 5 (10%) of the patients reported family history of prolonged and/or excessive bleeding while 45 (90%) had no known family history. In regard to their parity 37 (74%) of these patients had children while 13 (26%) were nulliparous. Table 1 below shows age categories, family history of bleeding tendencies and parity.

Table 1: Age distribution, family history of bleeding tendencies and parity.

Variables	Categories	(n=50)	Percentage (%)
Age	<18	1	2.0
	18-25	5	10.0
	26-35	15	30.0
FHX	Present	5	10
	Absent	45	90.0
Parity	Nulliparous	13	26.0
	Parous	37	74.0

Table 2 below shows the number of patients presenting with other bleeding symptoms which included; epistaxis, present in 11 patients, 4 with haematuria, 4 with prolonged and /or excessive bleeding after surgery, 3 with bleeding from minor wounds, 2 with bleeding from the oral cavity, 1 patient with excessive bleeding after tooth extraction, 1 with GIT bleeding and postpartum haemorrhage affecting 1 patient.

Table 2: Other Bleeding Presentations Other than Menorrhagia (ISTH/SSC Questionnaire) N=50

Symptom	Present, *n (%)*
Epistaxis	11 (22.0)
Cutaneous	2 (4.0)
Minor wounds	3 (6.0)
Oral cavity	2 (4.0)
Gastrointestinal	1 (2.0)
Haematuria	4 (8.0)
Tooth extraction	1 (2.0)
Post-surgical Procedure	4 (8.0)
Postpartum Haemorrhage	1 (2.0)
Total	**50 (100%)**

4.2: Clinical Diagnosis of Patients with Menorrhagia

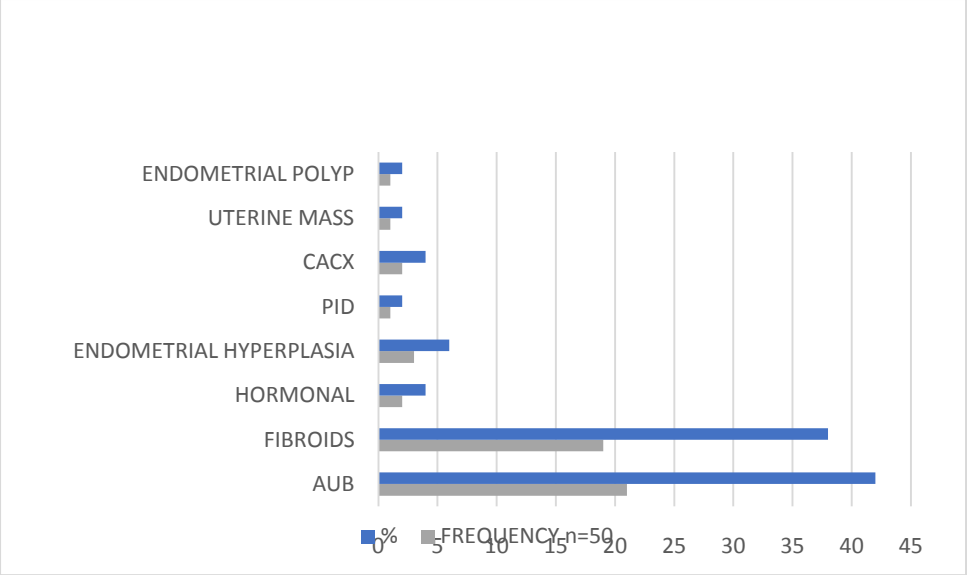
The most frequent clinical diagnosis of patients with HMB was abnormal uterine bleeding (AUB) affecting 21 (42%) patients. 29 (58%) had definitive diagnosis with 19 (38%) having fibroids, 3(6%) with endometrial hyperplasia, 2 (4%) with carcinoma of the cervix, 2 (4%) with hormonal causes, uterine mass, PID and endometrial polyp affecting one patient each.

Table 3 below show the frequency of each diagnosis and figure 2 shows frequency and percentage.

Table 3: Diagnosis n=50

DIAGNOSIS	Frequency (n)	Percent (%)
Abnormal Uterine Bleeding (AUB)	21	42
Fibroids	19	38
Endometrial hyperplasia	3	6
Carcinoma of the Cervix	2	4
Hormonal Causes	2	4
Uterine Mass	1	2
Pelvic Inflammatory Disease (PID)	1	2
Endometrial Polyp	1	2

Figure 2: Frequency Distribution for the Causes of HMB



4.3: Haematological Laboratory Findings

4.3.1 Haemoglobin Levels

Haemoglobin levels were done using Sysmex XN-1000 Haematology Analyzer, which is fully automated. The normal reference range was 12-16 g/dL. Majority of the patients (27) had normal haemoglobin levels above 12 g/dL, 15, 7 and 1 had mild, moderate and severe anaemia respectively.

Table 4: Categorization of Haemoglobin levels n=50

Levels mg/dL	Frequency (n=50)	Percent
No anaemia (>12)	27	54.0
Mild (9-12)	15	30.0
Moderate (5-8.9)	7	14.0
Severe (<5)	1	2.0

4.3.2: Haemostasis Tests

The following screening tests were done; Full blood count (platelet count), Prothrombin time and Activated partial thromboplastin.

Table 5: Haemostasis tests

Test	**Mean**	**Median**	**Min**	**Max**
PLT	300.2	288.5	94	743
PT	14.3	13.6	9.7	60.1
APTT	35.8	35.0	26.1	61.0
VWF.AG	118.6	119.5	37.3	201.0
VWF.AB	109.5	110.5	33.2	185.0
FVIII	210.2	198.0	57.3	388.0

The values of the platelet counts were derived from the full haemogram report that showed the complete blood count and other cellular haematological values and indices using sysmex XN-1000 Haematology Analyzer. The platelet levels ranged from 94-743 $\times 10^6$ /mL with mean levels of 300.2×10^6 /mL and median of 288.5×10^6 /mL. One patient had mild thrombocytopenia of 94×10^6 /mL**.** 48 (96%) of the patients had normal platelet levels and one had thrombocytosis of 743×10^6 /mL as seen on tables 5 and 6.

Table: 6: Platelet count (n=50)

Count (100-450×10⁶ /mL)	**Frequency N=50**	**Percent**
20-100	1	2.0
100-450	48	96.0
>450	1	2.0
		100.0

PT and APTT were measured using the fully automated thrombolyzer XRM which works by chromogenic and immunoturbidimetric method. PT was measured by adding thromboplastin to citrated plasma with calcium and time for fibrin clot to form measured in seconds. The control time was 14.8 seconds. The findings ranged from 9.7 to 60.1 seconds with a mean of 14.3 seconds and a median of 13.6 seconds as seen on table 5 above. Table 7 shows 2 patients with prolonged PT of more than 17.8 seconds while 48 patients had normal PT. APTT was measured by incubating plasma with phospholipid and buffer which initiated activation of the intrinsic pathway after incubation at 37°C. Calcium was added for coagulation to take place. Clotting time was measured in seconds. APTT control was of 33.2 seconds. The findings ranged from 26.1 to 61.0 seconds with a mean and median of 35.8 and 35.0 seconds respectively as seen on table 5. Table 8 below shows 5 patients with prolonged APTT of more than 43.3 seconds. Out of 50 women, one had both prolonged PT and APTT.

Table 7: Prothrombin time (n=50)

Controls 14.8 sec	**Frequency**	**Percent %**
Prolonged (>17.8)	2	4
Normal	48	96
	50	**100**

Table 8: Activated Partial Prothrombin time (n=50)

APTT (Seconds) Controls 33.2 sec	Frequency	Percent %
Prolonged (>43.2)	5	10
Normal	45	90
	50	**100**

4.3.3: ABO Blood Groups

The ABO blood group was determined in the patients that had menorrhagia. Out of 50 patients, 23 (46%) were of blood group O, 15 (30%) were of group A, 9 (18%) were of blood group B while 3 (6%) were of group AB as seen on figure 3 below.

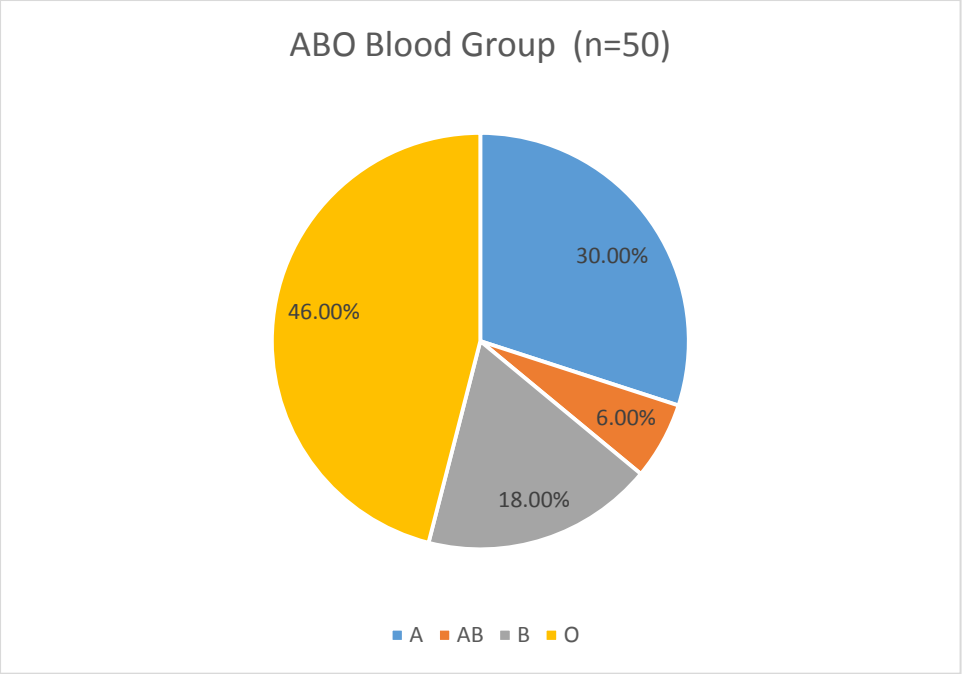
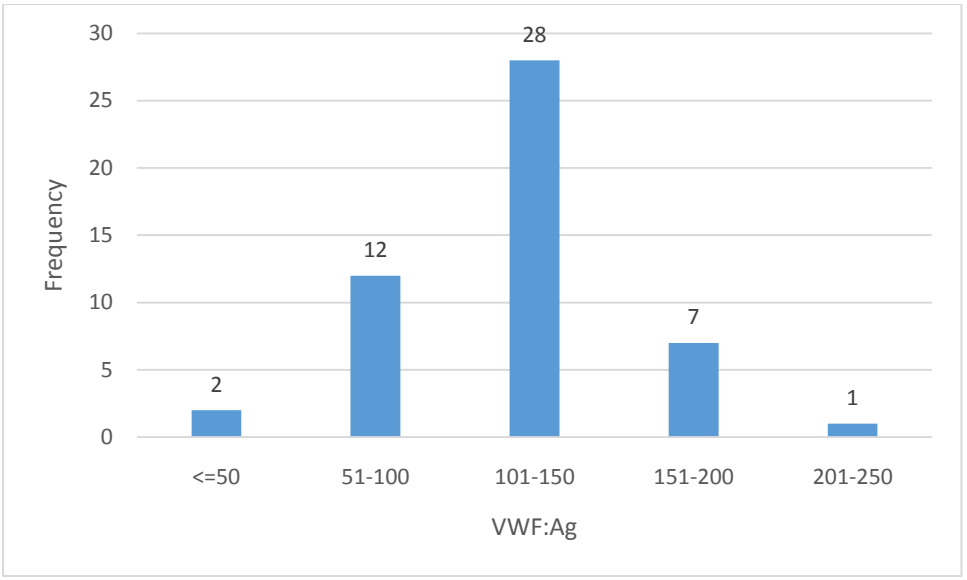


Figure 3: A, B, O Blood Group Distribution

4.3.4: Von Willebrand Factor levels (VWF: Ag)

VWF:Ag levels were determined and the normal reference range used was 50-150%.From the study, the findings ranged from 37.3 – 201% with a mean and median of 118.6% and 119% respectively. Only 2 patients had levels less than 50% as seen on table 5. Figure 4 shows distribution of VWF: Ag levels and frequency.

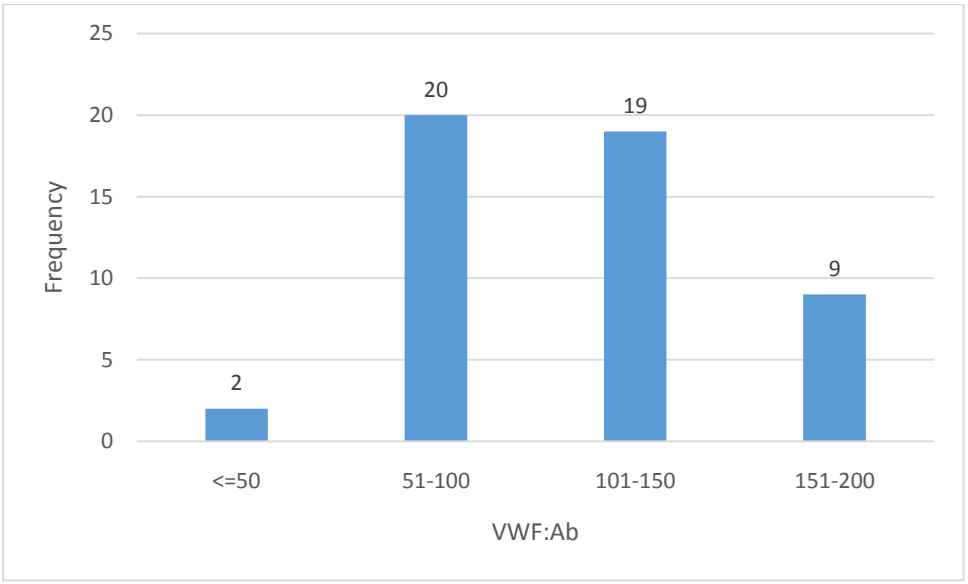
Figure 4: Von willebrand Factor Levels (VWF: Ag)
n= 50



4.3.5: Von Willebrand Factor Activity (VWF: Ab)

VWF: Ab levels with reference range used was 50-150%. The values ranged from 33.2- 185% with the mean and median of 109.5% and 110% respectively. Only 2 patients had levels less than 50% as seen on figure 5 below. The frequency distribution is seen on figure 5 below

Figure 5: Von Willebrand Factor Activity (VWF: Ab)

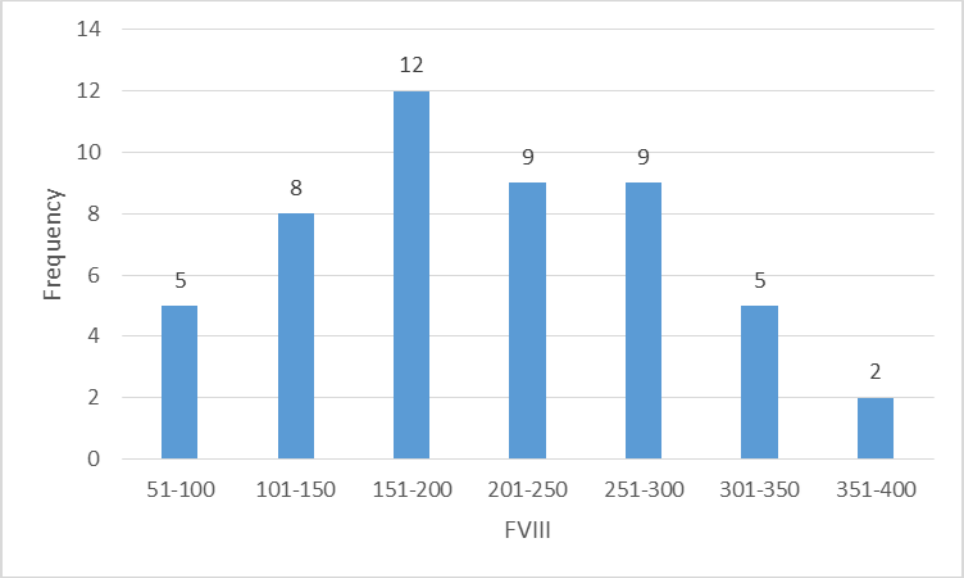


4.3.6: Factor VIII Activity

Factor VIII activity levels were determined. The normal reference range was 50-150%. In the study, the values ranged from 57.3-388% with a mean of 210.2% and median of 198%. A total of 13 (26%) patients had normal levels while 37 (74%) had high levels. None of the patients had low Factor VIII levels

Figure 6 shows frequency distribution of FVIII activity levels.

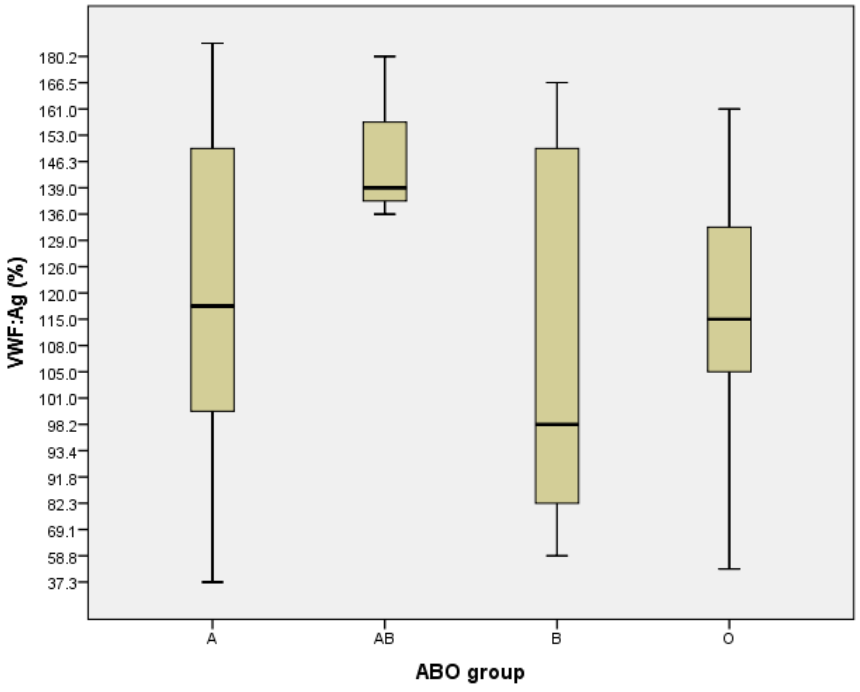
Figure 6: Factor VIII Activity



4.3.7: Relationship between Blood groups A, B, AB and O VWF: Ag (%)

Von Willebrand Factor levels among different blood groups is shown in figure 7. Out of 50 women in the study, 1 had low VWF levels of 37.3%. 41 (82%) had normal levels while 8(16%) had high levels. Among those of blood groups A, B and AB (n=27), 1 had low levels, 19 (70%) had normal levels while 7 (25.9%) high levels as seen on table 10. Those with blood group O (n=23), none had low levels while 22 (95.7%) had normal levels while 1 had high levels as seen on table 11 below. A comparison of VWF:Ag was done between O and none-O groups. Patients with blood group O had lower antigen levels than none-O groups with a (P = 0.035) as shown on table 13.

Figure 7: VWF levels Frequency Distribution among Different Blood Groups



4.3.8 Relationship between Blood groups A, B, AB and O VWF: Ab

Figure 8 below shows the distribution of VWF: Ab levels among different blood groups.

Figure 8: VWF: Ab levels among A, AB, B and O Groups

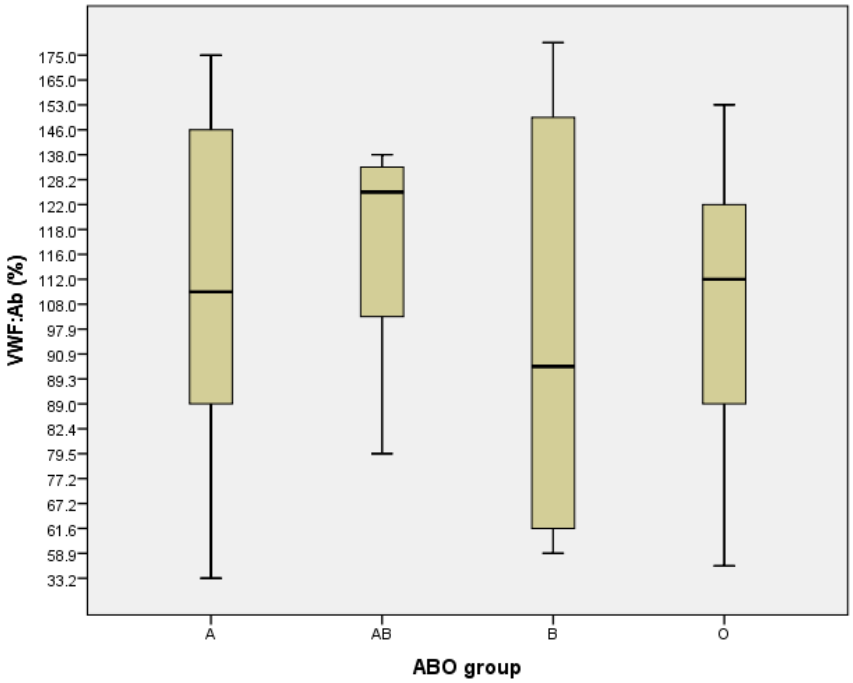


Table 10: Blood groups A, B and AB VWF: Ag (%)

Levels in %	(n=27)	Percent (%)
Low (<50)	1	3.7
Normal (50-150)	19	70.4
High (>150)	7	25.9
Total	27	100

Table 11: Blood groups O VWF: Ag (%)

	Frequency (n=23)	Percent
Normal (40-150)	22	95.7
High (>150)	1	4.3
	23	100

Table 12: Low, Normal and High levels of Von Willebrand Factor

	Frequency (n=50)	Percent
Low	1	2.0
Normal	41	82.0
High	8	16.0

Table 13: VWF: Ag levels of O and Non-O Blood group

		Low	Normal	High	p-value
Blood group	A, AB, B	1	19	7	**0.035**
	O	0	22	1	
	Total	1	41	8	

VWF: Ab levels and Blood Group Levels

VWF activity levels were lower in patients with blood group O than non-O groups. The levels were not significantly lower (p=0.255) as seen on table 14 below.

Table 14: Blood group and VWF: Ab (%)

		Low	Normal	High	p-value
Blood group	A, AB, B	1	19	7	0.255
	O	0	21	2	
	Total	1	40	9	

4.4 Menorrhagia Bleeding Assessment Score

One of the study objectives was to assess severity of menorrhagia using part of ISTH/SSC Bleeding Assessment Tool and correlate it to the antigen levels and activity of Von Willebrand Factor. The severity of bleeding among the 50 women was gauged according to the International Society of Haemostasis and thrombosis (ISTH) and Science and Standardization Committee (SSC) where a patient's bleeding symptoms are scored from 0-4 with increasing severity and magnitude of intervention. A score of 0 was given to patients with no HMB or had trivial symptoms. A score of 1 was given when patients had a consultation only, changed sanitary material more often than every 2 hours, presented with clots. A score of 2 was given to patients who had time off work or school more than twice a year or required antifibrinolytics, hormonal or iron supplements. A score of 3 was given to patients who had received treatment with antifibrinolytics combined with hormonal therapy or had the symptom since menarche and for over 12 months. Patients with history of acute menorrhagia that required admission and emergency treatment or blood transfusion or replacement, desmopressin or

endometrial ablation or dilatation and curettage or hysterectomy had a score of 4 .Out of the 50 patients with menorrhagia, 16 (32%) had scores of 1, 16 (32%) had a score of 2, 8 (16%) had a score of 3 while 10 (20%) had a score of 4. Their results are shown on the table below

Table 15: Menorrhagia Bleeding Score

SCORE (n=50)	Frequency	Percent
1	16	32
2	16	32
3	8	16
4	10	20

Menorrhagia Score and correlation with VWF Levels and Activity

One patient had low VWF:Ag of 37.3%, low VWF:Ab of 33.2% and normal FVIII activity of 57.3% with a high menorrhagia score of 3.

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study was done on 50 women who presented to Kenyatta National Hospital with menorrhagia between January and August 2021. All women presenting with menorrhagia except those with severe thrombocytopenia of less than 20×10^6 /mL were included despite some having specific clinical diagnosis as a cause of the menorrhagia. In severe thrombocytopenia of 20×10^9 /L, bleeding is attributed to the low platelet levels rather than low VWF:Ag and/or Ab levels. VWD presents with mucocutaneous bleeding including; gum bleeding, easy bruisability, menorrhagia, epistaxis, excessive and prolonged bleeding after tooth extraction and surgery(16). Some forms of VWD are so mild that they would only present if triggered by another cause. Venous blood samples were collected for haemostasis screening tests and Von Willebrand factor levels and activity tests. VW antigen levels and activity were determined using ACL ELITE Pro an automated latex enhanced immunoassay method which compares well with the traditional ristocetin-based method. The sensitivity of the immunoassay was found to be 100% and specificity was 86% according to Salem and Cott (26)

Menorrhagia is a common gynaecologic cause of ill health. WHO estimates the prevalence of menorrhagia to be 8- 27% (20) According to Shankaar et al, 12% of gynaecological referrals globally comprise women with menorrhagia (7). Heavy menstrual bleeding is a common symptom in women between the ages of 30 to 49 years according to Kushwaha et al (27) . These findings are similar to our study where 29 (58%) of the patients were older than 35 years of age. The median age was 37.5 years with a mean on 36.6 years. A case control study on the effects on menorrhagia found that an estimate of 5% of women between the ages of 30-49 years consult their general practitioners for menorrhagia more than once a year(20). In the study by Kushwaha, specific causes of menorrhagia were known in less than 50% of the patients and most patients are diagnosed with dysfunctional uterine bleeding (27) In our study, 21 (42%) of the participants had no specific cause of menorrhagia and were therefore diagnosed with abnormal uterine bleeding(AUB) while 29 (58%) had specific causes. FIGO definition of AUB is abnormal bleeding from the uterus in terms of time and/or regularity and volume (28). The most common specific diagnosis was uterine fibroids that affected 19 (38%) of the patients.

Out of the 50 patients, one (2%) had mild thrombocytopenia of 94×10^6 /mL. This differs from a study done by Akumu on women with heavy menstrual bleeding in KNH where 17% of the participants had thrombocytopenia. 4% of the patients in our study had prolonged PT and 10% had prolonged APTT which are slightly lower but comparable to Akumu's study where 6.4% had prolonged PT and 16% had prolonged APTT.

Laboratory diagnosis of VWD is difficult as VWF is an acute phase reactant that increases due to injury, stress, pregnancy, surgery and oral contraceptive use. Diagnosis however requires measurement of VWF levels, function and FVIII level (26). Traditionally, measurement of VWF levels and activity was done using Ristocetin-based assays. In this study however, immunoassay-based testing was used.

This new method measures VWF antigen levels and activity by use of VWF antibody-coated latex beads and measurement of FVIII activity. The method has 100% sensitivity and 86% specificity according to Salem and Cott (26). Our findings are therefore comparable to those of other studies that use Ristocetin.

Few studies have been done on VWF levels and activity in women with menorrhagia in Africa and Kenya in particular. In this study, 1 (2%) patient had low levels and activity of VWF. These findings are similar to a study done by American College of Obstetricians where the prevalence of VWD was higher in Caucasians compared to African Americans at 15.9% and 1.3 respectively(25). The findings are also comparable to an unpublished case control study in Aga Khan in 2020 by Munoko where none of the 19 cases of women with menorrhagia had VWD while 2 (4.1%) of 49 controls(10) had . In our study, the mean levels of VWF:Ag is 118.6 %, and high FVIII activity levels of 210 %. A study was done by Sukhu et al in Durban, South Africa on comparison of VWF and FVIII levels between Caucasians, Indians and Africans (Zulus). In the study, it was concluded that Africans had significantly higher VWF and FVIII levels than Caucasians with VWF: Ag of 118 IU/dL vs. 100 IU/dL (*P = 0.0011*), FVIII levels of 145 IU/dL vs. 126 IU/dL (P = 0.0002) and Indians with VWF: Ag mean of 118 IU/dl vs 102 IU/dL (*P= 0.0002*) and FVIII level means of 145 IU/dLvs. 129 IU/dL *(p =0.009*). There was no significant difference between the VWF antigen *(p = 0.04)* and FVIII levels *(p=0.43)* between the Caucasians and Indians and. In the two findings, it is concluded that VWF:Ag and FVIII levels are high in African race.

ABO blood group is a modifier of levels of VWF. Individuals with blood group O have approximately 30% less levels than non-O groups(29). Low VWF levels for O and non-O groups are therefore set at different reference ranges where the lower limits are at 40% and 50% respectively. The levels of VWF in our study is significantly higher in non-O blood groups individuals than those of blood group O (*P = 0.035*). These findings are similar to those of Sukhu et al study where the P value for Africans was 0.003, that of Indians was 0.02 while that of Caucasians was 0.03(30). A study by Stefan on distribution of VWF in young women had similar findings where the those with genotype O had significantly lower VWF levels compared to non-O groups (*P = 0.002))* (29). VWF: Ab levels in O and non-O groups in our study however had no significant difference (P = 0.255)

When assessing a patient with heavy menstrual bleeding, gynaecologists use the semi-quantitative Pictorial Bleeding Assessment Chart (PABC) where the patient counts the number of sanitary material (tampon or pads) and the degree of soiling(22).The ISTH/SSC bleeding assessment tool is used for assessment of patients suspected to have congenital bleeding disorders like haemophilia and VWD especially in the paediatric age group. In this study our intention was to assess the severity of HMB by using part of the tool to predict patients who were likely to have low VWF levels and activity. The scores of 3 and 4 were expected to have low levels and activity of VWF. The current view is that the BATs are long, laborious and studies have been done to modify them by picking the most predictive

symptoms. The other symptoms used in assessment of bleeding were scanty. It was therefore difficult to use the tool in predicting the occurrence of possible VWD based on the scoring. The severity of menorrhagia was recorded according to the tool in Appendix 4. Most of the patients had menorrhagia score of 1 (32%) and 2 (32%). 10 (20%) of the patients had a score of 4 and 8 (16%) had a score of 3.

In this study, one patient had low VWF levels and activity. The patient was 40 years old. She had no known family history of bleeding disorder. She was of blood group A and had history of: epistaxis, prolonged bleeding after tooth extraction and haematuria. The patient had a menorrhagia score of 3, having had menorrhagia since menarche. Laboratory tests showed low VWF:Ag of 37.3%, low VWF:Ab of 33.2% and normal FVIII activity of 57.3%. The other laboratory findings were normal with Hb of 13.6g/dL, platelet counts of 199×10^6 /mL, PT of 10.7 seconds and APTT of 39.1 seconds. It is therefore necessary that when using the BAT with focus on menorrhagia, a patient with high score should be evaluated for other causes including: Pelvic pathologies, hormonal causes, other bleeding disorders or a combination of disorders.

5.2 Conclusion

- The prevalence of Low Von Willebrand Factor levels and activity in our study is 2% (1 patient)
- Patients of blood group O had significantly lower VWF: Ag levels than those with non-O blood.
- Majority of the patients had low menorrhagia scores of 1 and 2 (on the ISTH tool) (32%) for each score.

5.3: Limitations

Due to COVID-19 pandemic, the sample size was recalculated as the number of patients attending clinic had gone down.

Combined oral contraceptives increase levels of VWF. In our study however, patients on COC were not taken into consideration while measuring levels of VWF. This may have had a contribution to patients having high levels and activity.

The study did not validate the bleeding tool within the study population.

5.6: Recommendations

Low VWF antigen and/or antibody levels and VWD being hereditary disorders, multicenter/ethnic studies should be done to include all age groups to determine the true prevalence of the disease in our population as this study population in KNH was mainly from Nairobi and its environs.

COVID-19 Prevention

The research assistant and I had face masks throughout the study. All the study participants had face masks. The questionnaires were filled by the research assistant and me. Before and after signing the consent, parental consent or assent forms, the participants were given hand sanitizers. Rubber gloves were used strictly when drawing samples with change of gloves from one patient to another. The participants, investigator and research assistant were encouraged to stand at least 1.5 meters apart except when taking blood samples.

References

1. Bucciarelli P, Siboni SM, Stufano F, Biguzzi E, Canciani MT, Baronciani L, et al. Predictors of von willebrand disease diagnosis in individuals with borderline von willebrand factor plasma levels. J Thromb Haemost. 2015;13(2):228–36.

2. James AH. Von willebrand disease in women. Thromb Res. 2009;124(1):7–10.

3. Payandeh M, Rahimi Z, Kansestani AN, Hemmati S, Aleyasin M, Zare ME, et al. Clinical features and types of Von Willebrand disease in women with menorrhagia referred to hematology clinic of Kermanshah. Int J Hematol Stem Cell Res. 2013;7(2):1–5.

4. Govorov I, National A. Karolinska Institutet , Stockholm , Sweden HEAVY MENSTRUAL BLEEDING AND OBSTETRICAL BLEEDING. 2019. 6–11 p.

5. Strong J. Bleeding disorders in pregnancy. Curr Obstet Gynaecol. 2003;13(1):1–6.

6. Govorov I, Ekelund L, Chaireti R, Elfvinge P, Holmström M, Bremme K, et al. Heavy menstrual bleeding and health-associated quality of life in women with von willebrand's disease. Exp Ther Med. 2016;11(5):1923–9.

7. James A, Matchar DB, Myers ER. Testing for von Willebrand disease in women with menorrhagia: A systematic review. Obstet Gynecol. 2004;104(2):381–8.

8. Nabil A, Torky H, Badran H, Taha T, Zein E, Bibars M, et al. Von Willebrand disease among Egyptian women suffering from menorrhagia. 2016;131–3.

9. Lidya Akumu. LydiaAkumuResultsAbstract. p. 1–2.

10. Munoko A. The prevalence of von Willebrand disease among women with menorrhagia of African descent Revised.

11. Bruinvels G, Burden R, Brown N, Richards T, Pedlar C. The prevalence and impact of heavy menstrual bleeding (Menorrhagia) in elite and non-elite athletes. PLoS One. 2016;11(2):1–8.

12. Lillicrap D, James P. Von Willebrand Disease : an Introduction for the Primary Care Physician. Treat Hemoph. 2009;(47):1–7.

13. Harlow SD, Campbell OMR. Epidemiology of menstrual disorders in developing countries: A systematic review. BJOG An Int J Obstet Gynaecol. 2004;111(1):6–16.

14. Peyvandi F, Garagiola I, Baronciani L. Role of von Willebrand factor in the haemostasis. Blood Transfus. 2011;9(SUPPL. 2):3–8.

15. Witmer CM. Von willebrand disease. 5-Minute Pediatr Consult 8th Ed. 2018;1006–7.

16. Ng C, Motto DG, Paola J Di. Diagnostic approach to von Willebrand disease. Blood. 2015;125(13):2029–37.

17. Castaman G, Goodeve A, Eikenboom J. Principles of care for the diagnosis and treatment of von Willebrand disease. Haematologica. 2013;98(5):667–74.

18. Samms V. Von Willebrand Disease and pregnancy. Pract Midwife. 2007;10(7):20–2.

19. Koike S, Gladders P, Paulus A. Diagnosis of disease. Veg Dis. 2006;(55):31–7.

20. Gokyildiz S, Aslan E, Beji NK, Mecdi M. The Effects of Menorrhagia on Women's Quality of Life: A Case-Control Study. ISRN Obstet Gynecol. 2013;2013(2010):1–7.

21. Hapangama DK, Bulmer JN. Pathophysiology of heavy menstrual bleeding. Women's Heal. 2016;12(1):3–13.

22. Herman MC, Mak N, Geomini PM, Winkens B, Mol BW, Bongers MY. Is the Pictorial Blood Loss Assessment Chart (PBAC) score associated with treatment outcome after endometrial ablation for heavy menstrual bleeding? A cohort study. BJOG An Int J Obstet Gynaecol. 2017;124(2):277–82.

23. Spradbrow J, Letourneau S, Grabell J, Liang Y, Riddel J, Hopman W, et al. Bleeding assessment tools to predict von Willebrand disease: Utility of individual bleeding symptoms. Res Pract Thromb Haemost. 2020;4(1):92–9.

24. Castaman G, Hillarp A, Goodeve A. Laboratory aspects of von Willebrand disease: Test repertoire and options for activity assays and genetic analysis. Haemophilia. 2014;20(S4):65–70.

25. Gynecologists TAC of O and. Committee Opinion. Female Pelvic Med Reconstr Surg. 2014;20(5):248–51.

26. Salem RO, Van Cott EM. A new automated screening assay for the diagnosis of von Willebrand disease. Am J Clin Pathol. 2007;127(5):730–5.

27. Kushwaha R, Kumar A, Mishra KL, Sankhwar PL, Singh R. Haemostatic disorder in women with unexplained menorrhagia: A tertiary care centre experience from north India. J Clin Diagnostic Res. 2017;11(5):EC46–9.

28. Whitaker L, Critchley HOD. Abnormal uterine bleeding. Best Pract Res Clin Obstet Gynaecol [Internet]. 2016;34:54–65. Available from: http://dx.doi.org/10.1016/j.bpobgyn.2015.11.012

29. Lethagen S, Hillarp A, Ekholm C, Mattson E, Halldén C, Friberg B. Distribution of von Willebrand factor levels in young women with and without bleeding symptoms. Influence of ABO blood group and promoter haplotypes. Thromb Haemost. 2008;99(6):1013–8.

30. Fleming AF. Ethnic variation in von Willebrand factor levels can influence the diagnosis of von

Willebrand disease. Clin Lab Haematol. 2003;25(6):413.

31. Tool BA, Questionnaire AS, Bleeding NEW, For S, Bleeding I, Of O. ISTH/SSC New Bleeding Score for Inherited Bleeding Disorders. J Thromb Haemost. 2011;8(January 2008):2063–5.

Appendix 1

1.1.1 Informed Consent

INFORMED CONSENT TO PARTICIPATE IN THE STUDY

SERIAL NUMBER …….

Background

You are being asked to participate in a research study described below. Before you decide, it is important for you to understand why the research is being done and what it will involve. Read the following information carefully and ask if there is anything that is not clear or if you would like more information. Please take time to decide whether you want to volunteer to take part in this study.

TITLE: VON WILLEBRAND FACTOR LEVELS AND ACTIVITY IN WOMEN WITH MENORRHAGIA IN KENYATTA NATIONAL HOSPITAL

PRINCIPLE INVESITIGATOR: DR MUTAI M. C.

Introduction

Von Willebrand Disease (VWD) is a disorder that causes delay in clotting of blood. It is passed down from a parent to offspring and is the most common inherited bleeding disorder. This disease is caused by deficiency or abnormal function of a component of blood called Von Willebrand Factor. It results in prolonged and excessive bleeding from menstruation, gums, skin after injury or after surgery or delivery. In women the disease presents itself during menstruation and child birth.

Purpose of the study:

Heavy menstrual bleeding in the setting of Von Willebrand Disease is associated with poor quality of life resulting in a significant social and economic implication. Few studies have been done on women with VWD in Africa, including Kenya. Information from this study may be used as a baseline towards specific testing and finding prevalence of VWD among women with menorrhagia. The study is purely for research purposes, but the findings may be used to identify gaps in the management women with heavy menstrual bleeding.

Study Procedure

The study involves filling out a questionnaire capturing your bio data and answering questions appropriately. Your responses will not be linked to you and are completely anonymous and confidential.

Blood samples will be drawn for coagulation screening (full blood count, APTT, PT) ABO blood grouping and von willebrand factor levels and activity.

Risks of Participation

There are no risks of this study except for mild pain during sample collection. The laboratory results from the tests shall be incorporated into your medical records and in case there is missing clinical history in the files, they shall be updated accordingly.

Benefits of participation

A potential benefit of the study will be improved healthcare service delivery especially in the identification of gaps which will aid in improving and creation of protocols for the better management of heavy menstrual bleeding.

Alternative Procedures

You may choose not to participate in this study and it will not affect the health care that will be provided to you.

Confidentiality

This research will be conducted in accordance with all the Kenyan laws and regulations that protect rights of human research subjects. All records and other information obtained will be kept strictly confidential and your protected health information will not be used without permission. All data collection tools will be identified by study number or otherwise coded to protect any information that could be used to identify you. Results of this study may be published, but no names or other identifying information will be released.

Voluntary Participation

It is up to you to decide whether you will take part in this study. Refusal to participate or the decision to withdraw from this research will involve no penalty or loss of benefits to which you are otherwise entitled. This will not affect your relationship with the investigators.

Right of investigator to withdraw

The investigator can withdraw you from the research without your approval.

Costs and Compensation to participants

There is no cost to you, and there is no compensation to subjects for participation in this study.

Person to Contact

If you have questions, complaints or concerns about this study, you can contact the principal investigator from University of Nairobi, School of Medicine, Department of Human Pathology, Postgraduate program: Dr. Mutai MC +254723295312 mercymutaimd@gmail.com or my Supervisor Dr Okinyi FO +254720325448 or you can contact the KNH-UoN ERC at uonknh_erc@uonbi.ac.ke.

Thank you for your participation in this research.

By signing this consent form, I confirm that I have read the information in this consent form and have had the opportunity to ask questions. I voluntarily agree to take part in this study.

Name of participant …….....

Signature/Mark……. …… Date.…….…….

Signature of investigator ……. Date…….…….

1.1.2 Fomu ya Idhini

IDHINI YA KUSHIRIKI KATIKA UCHUNGUZI

NAMBARI YA USAJILI …….

Utangulizi:
Ugonjwa wa Von Willebrand (VWD) ni moja ya magonjwa ambayo husababisha damu kutoganda. Unaweza kupitishwa kutoka kwa mzazi kwa mwanawe. Ugonjwa huu unasababishwa na upungufu wa chembechembe za damu ziitwayo "Von Willebrand Factor" ama upungufu wa kazi kwa chebechembe hizo. Hii inasababisha kuvuja kwa muda mrefu wakati wa hedhi, kuvuja kwa muda mrefu kwa fizi, ngozi baada ya kuumia ama upasuaji na kujifungua.

Unaulizwa ukubali kushiriki katika utafiti wa kiina. Kabla ya uamuzi wako, ni muhimu uelewe kiina na malengo ya uchunguzi huu na yanahusiana na utafiti husika. Soma maelezeo yafuatayo kwa makini na uliza swali ikiwa kuna jambo ambalo haliko wazi.

MADA: KIWANGO NA KAZI YA CHEMBECHEMBE ZA 'VON WILLEBRAND' KWA WANAWAKE WALIO NA HEDHI NZITO KATIKA HOSPITALI YA KITAIFA YA KENYATTA.

MDADISI MKUU: DR MUTAI M. C

Kusudi la uchunguzi huu: Hedhi nzito inayohusiana na ugonjwa wa Von Willebrand hupunguza ubora wa maisha na kupunguza matokeo ya kijamii na uchumi. Uchunguzi kidogo wa ugonjwa wa Von Willebrand umefanywa barani Afrika na nchi ya Kenya. Matokeo ya uchunguzi huu yatasaidia katika uchunguzi maalum ya ugonjwa wa Von Willebrand kwa wanawake walio na hedhi nzito. Uchunguzi huu ni wa utafiti lakini maelezo yatakayo kusanywa yataweza kutumika kutambua pengo katika huduma za wanawake.

Hatua za Zoezi

Uchunguzi utahusisha ujazaji wa hojaji itakayokuwa na maelezo ya kijumla ya afya yako kwa kujibu maswali ipasavyo. Majibu hayo hayataelekezwa kwako, na ni ya siri. Sampuli za damu zitakusanywa kwa kuchunguza ugandazaji wa damu (kiwango cha damu, 'APTT' na 'PT'), kundi la damu ya ABO na kiwango na kazi ya chembechembe ya Von Willebrand. Hii itafanywa kama sampuli hizo hazikuwa zimekusanywa hapo mbelini.

Hatari ya Uchunguzi

Hakuna hatari zitakazoshuhudiwa katika utafiti huu labda uchungu wa kiasi cha chini katika ukusanyaji wa sampuli ya viwangu vya damu. Matokeo yatahusishwa katika faili za kiafya zako na ikiwa kutakosa maelezo ya historia ya kiafya yatajazwa kwa njia inayofaa.

Manufaa ya Uchunguzi

Manufaa tarajiwa ya uchunguzi huu ni kuboreshwa kwa huduma za afya haswa katika kutambua udhaifu/pengo, ambao utasaidia katika kuimarisha matibabu ya hedhi nzito.

Uhifadhi siri ya maelezo

Utafiti huu utafanya kwa mujibu wa sheria zote za Kenya zinazolinda utafiti wa vipengele wa haki za binadamu. Rekodi na maelezo mengine yaliyokusanywa yatahifadhiwa kwa njia ya siri na maelezo ya kiafya kuhusu mwanao hayatatumika bila ruhusa rasmi. Vifaa vyote vya ukusanyaji data vitatambuliwa kwa nambari maalum ya uchunguzi huu, ili kulinda habari zozote zile zinaweza kukufichua. Matokeo ya uchunguzi huu yanaweza kuchapiswa lakini hakuna jina au maelezo yoyote ya kukutambulisha yatatumika.

Kushiriki kwa hiari:

Ni wajibu wako kuamua ikiwa utashiriki katika utafiti huu. Kukataa kushiriki au kujiondoa kutoka zoezi hii haitaleta athari mbaya kwako wala haitakuwa na hatia au adhabu yoyote ile. Pia manufaa yoyote kwako hayataathirika kwa vyovyote vile. Uhusiano wako na wadadisi utazidi kuwa mwema.

Haki ya mdadisi kuondoa

Mdadisi mkuu anaweza kukuondoa kutoka kwa zoezi hili bila ruhusa yako.

Malipo kwa washiriki

Hakuna malipo ya aina yeyote kwa wanaoshiriki katika uchunguzi huu.

Mawasiliano

Ikiwa una malalamishi au maswala kuhusu uchunguzi huu, unaweza kuwasiliana na mdadisi mkuu kutoka Chou Kikuu cha Nairobi, shule ya Udaktari idara ya patholojia. kitengo cha uzamili; Dkt. Mutai M.C +254723295312 barua pepe: mercymutaimd@gmail.com.com au msimamizi mkuu Dkt Fredrick Okinyi kupitia +254720325448 au unaweza kuwasiliana na kitengo cha KNH-UoN ERC kupitia baruapepe, anwani ambayo ni uonknh_erc@uonbi.ac.ke.

Asante sana kwa kushiriki katika utafiti huu, tunathamini sana usaidizi wako.

Idhini

Kwa kujaza fomu hii ya ruhusa, nimekubali kuwa nimesoma maelezo yote yaliyo katika fomu hii na kupata nafasi ya kuuliza maswali. Nimekubali kwa hiari yangu kushiriki katika uchunguzi huu.

Jina la mshiriki: …….

Sahihi/ Alama: …….Tarehe: …….

Sahihi ya mdadisi: ……Tarehe: ……

1.2.1 Informed Parental Consent

INFORMED PARENTAL CONSENT TO PARTICIPATE IN THE STUDY

SERIAL NUMBER …….

Background

You are being asked to let your child participate in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Read the following information carefully and ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether you want your child to volunteer to take part in this study.

TITLE: VON WILLEBRAND FACTOR LEVELS AND ACTIVITY IN WOMEN WITH MENORRHAGIA IN KENYATTA NATIONAL HOSPITAL

PRINCIPLE INVESTIGATOR: DR MUTAI M. C.

Introduction

Von Willebrand Disease (VWD) is a disorder that causes delay in clotting of blood. It is passed down from a parent to offspring and is the most common inherited bleeding disorder. This disease is caused by deficiency or abnormal function of a component of blood called Von Willebrand Factor. It results in prolonged and excessive bleeding from menstruation, gums, skin after injury or after surgery or delivery. In women the disease presents itself during menstruation and child birth.

Purpose of the study:

Heavy menstrual bleeding in the setting of Von Willebrand Disease is associated with poor quality of life resulting in a significant social and economic implication. Few studies have been done on women with VWD in Africa, including Kenya. Information from this study may be used as a baseline towards specific testing and finding prevalence of VWD among women with menorrhagia. The study is purely for research purposes, but the findings may be used to identify gaps in the management women with heavy menstrual bleeding.

Study Procedure:

The study involves filling out a questionnaire capturing your child's bio data and answering questions appropriately. Your responses will not be linked to you or the child and are completely anonymous and confidential. Blood samples will be drawn for coagulation screening (full blood count, APTT, PT), ABO blood grouping and von willebrand factor levels and activity.

Risks and benefits

There are no risks of this study except for the pain during sample collection. The laboratory results from the tests shall be in co-operated into your child's medical records and in- case there is missing clinical history in the files, they shall be updated accordingly.

A potential benefit of the study will be improved healthcare service delivery especially in the identification of gaps which will aid in improving and creation of protocols for the better management of heavy menstrual bleeding.

Alternative Procedures

You may choose for your child not to participate in this study and it will not affect the health care that will be provided to you.

Confidentiality

This research will be conducted in accordance with all the Kenyan laws and regulations that protect rights of human research subjects. All records and other information obtained will be kept strictly confidential and your child's protected health information will not be used without permission. All data collection tools will be identified by number or otherwise coded to protect any information that could be used to identify your child. Results of this study may be published, but no names or other identifying information will be released.

Voluntary Participation

It is up to you to decide whether your child takes part in this study. Refusal to participate or the decision to withdraw from this research will involve no penalty or loss of benefits to which your child is otherwise entitled. This will not affect your relationship with the investigators.

Right of investigator to withdraw

The investigator can withdraw your child from the research without your approval.

Costs and Compensation to participants

There is no cost to you, and there is no compensation to subjects for participation in this study.

Person to Contact

If you have questions, complaints or concerns about this study, you can contact the principal investigator from University of Nairobi, School of Medicine, Department of Human Pathology, Postgraduate program: Dr. Mutai MC +254723295312 mercymutaimd@gmail.com or my Supervisor Dr Okinyi F.O +254720325448 or you can contact the KNH-UoN ERC at uonknh_erc@uonbi.ac.ke.

Thank you for your child's participation in this research and we truly appreciate your help.

CONSENT

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions. I voluntarily agree to take part in this study.

Name of Child ……

Name of Caregiver / Parent…….....

Signature/Mark……. Date……

Investigator Signature……. Date…….

1.2.2 Idhini ya Mzazi

IDHINI YA MZAZI KUSHIRIKISHA MWANAO KATIKA UCHUNGUZI

NAMBARI YA USAJILI …….

Unaulizwa umkubalie mwanao ashiriki katika utafiti wa kina. Kabla ya uamuzi wako, ni muhimu uelewe kiina na malengo ya uchunguzi huu na yanahusiana na utafiti husika. Soma maelezeo yafuatayo kwa makini na uliza swali ikiwa kuna jambo ambalo haliko wazi.

MADA: KIWANGO NA KAZI YA CHEMBECHEMBE ZA 'VON WILLEBRAND' KWA WANAWAKE WALIO NA HEDHI NZITO KATIKA HOSPITALI YA KITAIFA YA KENYATTA.

Utangulizi:

Ugonjwa wa Von Willebrand (VWD) ni moja ya magonjwa ambayo husababisha damu kutoganda. Unaweza kupitishwa kutoka kwa mzazi kwa mwanawe. Ugonjwa huu unasababishwa na upungufu wa chembechembe za damu ziitwayo "Von Willebrand Factor" ama upungufu wa kazi kwa chebechembe hizo. Hii inasababisha kuvuja kwa muda mrefu wakati wa hedhi, kuvuja kwa muda mrefu kwa fizi, ngozi baada ya kuumia ama upasuaji na kujifungua.

Kusudi la uchunguzi huu:

Hedhi nzito inayohusiana na ugonjwa wa Von Willebrand hupunguza ubora wa maisha na kupunguza matokeo ya kijamii na uchumi. Uchunguzi kidogo wa ugonjwa wa Von Willebrand umefanywa barani Afrika na nchi ya Kenya. Matokeo ya uchunguzi huu yatasaidia katika uchunguzi maalum ya ugonjwa wa Von Willebrand kwa wanawake walio na hedhi nzito. Uchunguzi huu ni wa utafiti lakini maelezo yatakayo kusanywa yataweza kutumika kutambua pengo katika huduma za wanawake.

Hatua za Zoezi

Uchunguzi utahusisha ujazaji wa hojaji itakayokuwa na maelezo ya kijumla ya afya ya mwanao kwa kujibu maswali ipasavyo. Majibu hayo hayataelekezwa kwa mwanao na ni ya siri. Sampuli za damu zitakusanywa kwa kuchunguza ugandazaji wa damu (kiwango cha damu, 'APTT' na 'PT'), kundi ya damu ya ABO na kiwango na kazi ya chembechembe ya Von Willebrand. Hii itafanywa kama sampuli hizo hazikuwa zimekusanywa hapo mbelini.

Hatari na marufaa

Hakuna hatari zitakazoshuhudiwa katika utafiti huu labda uchungu wa kiasi cha chini katika ukusanyaji wa sampuli ya damu. Matokeo yatahusishwa katika faili za kiafya za mwanao na ikiwa kutakosa maelezo ya historia ya kiafya yatajazwa kwa njia inayofaa. Manufaa tarajiwa ya uchunguzi huu ni kuboreshwa kwa huduma za afya haswa katika kutambua pengo, ambao utasaidia katika kuimarisha matibabu ya hedhi nzito.

Njia Mbadala:

Unaweza kuamulia mwanao asishiriki katika uchunguzi huu ambao hautaathiri huduma ya afya atakayopewa.

Uhifadhi siri wa maelezo

Utafiti huu utafanya kwa mujibu wa sheria zote za Kenya zinazolinda utafiti wa vipengele wa haki za binadamu. Rekodi na maelezo mengine yaliyokusanywa yatahifadhiwa kwa njia ya siri na maelezo ya kiafya kuhusu mwanao hayatatumika bila ruhusa rasmi. Vifaa vyote vya ukusanyaji data vitatambuliwa

kwa nambari maalum ya uchunguzi huu, ili kulinda habari zozote zile zinaweza kumfichua mwanao. Matokeo ya uchunguzi huu yanaweza kuchapiswa lakini hakuna jina au maelezo yoyote ya kumtambulisha yatatumika.

Kushiriki kwa hiari:

Ni wajibu wako kuamua ikiwa mwanao atashiriki katika utafiti huu. Kukataa kushiriki au kumuondoa kutoka zoezi hii haitaleta athari mbaya kwa mwnao wala haitakuwa na hatia au adhabu yoyote ile. Pia manufaa yoyote kwa mwanao hayataathirika kwa vyovyote vile. Uhusiano wako na wadadisi utazidi kuwa mwema.

Haki ya mdadisi kuondoa

Mdadisi mkuu anaweza kumuondoa mwanao kutoka zoezi hili bila ruhusa yako.

Malipo kwa washiriki

Hakuna malipo ya aina yeyote ile kwa wanoshiriki katika uchunguzi huu.

Mawasiliano

Ikiwa una malalamishi au maswala kuhusu uchunguzi huu, unaweza kuwasiliana na mdadisi mkuu kutoka Chou Kikuu cha Nairobi, shule ya Udaktari idara ya patholojia.kitengo cha uzamili; Dkt. Mutai M.C +254723295312 baruapepe: mercymutaimd@gmail.com au msimamizi mkuu Dkt Fredrick Okinyi kupitia +254720325448 au unaweza kuwasiliana na kitengo cha KNH-UoN ERC kupitia baruapepe, anwani ambayo ni uonknh_erc@uonbi.ac.ke.

Asante sana kwa ushiriki wa mwanao katika utafiti huu, tunathamini sana usaidizi wako.

IDHINI

Kwa kujaza fomu hii ya ruhusa, nimekubali kuwa nimesoma maelezo yote yaliyo katika fomu hii na kupata nafasi ya kuuliza maswali. Nimekubali kwa hiari yangu kushiriki katika uchunguzi huu.

Jina la mtoto: …….

Jina la mzazi: …….....

Sahihi/ Alama: …….Tarehe: …….

Sahihi ya mdadisi: ……Tarehe: ……

Informed Assent Age 15-17 years

1.3.1 Informed Assent to Participate in the Study

SERIAL NUMBER …….

My name is DR MUTAI M.C (Principle Investigator). I would like you to participate in a research study titled VON WILLEBRAND FACTOR LEVELS AND ACTIVITY IN WOMEN WITH MENORRHAGIA IN KENYATTA NATIONAL HOSPITAL.

Introduction

What is Von Willebrand Disease (VWD)? It is a condition that causes one to bleed excessively and for a long period of time. It is passed down from a parent to a child. This condition that comes about when one has less than normal values of Von Willebrand Factor or when one has normal values but do not function properly. Von Willebrand Factor is normally found in blood. A person with VWD can have bleeding gums, bleeding excessively during menstruation, from cuts or after surgery.

Your parent(s) are aware that am talking to you about the study. I will take you through this form that will inform you about the study to help you decide whether or not you want to take part in it.

What am I being asked to do?

If you decide to be in the study, I will ask you a few questions about symptoms you get due to heavy menstrual bleeding, bleeding from other sites like the nose, while brushing teeth or prolonged bleeding after minor injuries. Your blood samples will be taken for tests that include; coagulation screening (Full blood count, APTT and PT), blood grouping and Von Willebrand factor levels and activity. The samples will only be taken for tests only for the tests that have not been done.You shall feel a little pain when the blood sample is being withdrawn, we shall ensure that bleeding has stopped before you leave.

What are the benefits to me for taking part in the study?

If you take part in this study, in case your file has some missing data I shall input the data into your file as part of the up scaling of your medical records. The laboratory results will also be added into your medical data.

Can anything bad happen if I am in this study?

I do not expect anything bad happening to you by agreeing to be part of the study. If there is a bleed during sample collection, we shall ensure we stop it promptly.

Who will know that I am in the study?

If you decide to be in the study, I will not tell anyone else how you respond or act as part of the study.

Do I have to be in the study?

No, you don’t. The choice is yours. No one will get angry or upset if you don’t want to be in the study.

What if I have questions?

If you have any questions about the study, you can ask me now or anytime during the study. You can also call me. My phone number is 0723295312 or e-mail me at mercymutaimd@gmail.com or contact my supervisor Dr Fredrick whose number is 0720325448 or you can contact the KNH-UoN ERC at uonknh_erc@uonbi.ac.ke.

Signing below means that you have understood this form and that you are willing to be in this study:

Name of the Participant:

Signature of the Participant: Date:

Investigator Signature Date

Idhini ya Mtoto miaka 15-17 years

1.3.2 Idhini ya Kushiriki Katika Uchunguzi

NAMBARI YA USAJILI ……..

Jina langu ni Dkt MUTAI M. C (Mdadisi mkuu). Ningependa ushiriki katika uchunguzi wa utafiti, mada ikiwa KIWANGO NA KAZI YA CHEMBECHEMBE ZA 'VON WILLEBRAND' KWA WANAWAKE WALIO NA HEDHI NZITO KATIKA HOSPITALI YA KITAIFA YA KENYATTA.

Utangulizi

"Von Willebrand Disease (VWD)" ni ugonjwa aina gani? VWD ni ugonjwa ambayo husababisha kutoganda kwa damu. Inaweza kupitishwa kutoka kwa mzazi kwa mwanawe. Inasababishwa na upungufu wa chembechembe amabazo hupatikana kwa damu ama chembechembe hizo zikiwa hazifanyi kazi zitakikanavyo. Ugonjwa huu husababisha kuvuja kwa fizi ama kuvuja kiasi ya juu na kwa muda mrefu wakati wa hedhi, kuumia kwa ngozi ama baada ya upasuaji.

Mzazi/wazazi wako wana ufahamu wa mazungumuzo yetu kuhusu uchunguzi huu. Nitakupa maelezo kamili kuhusi hii fomu, ambayo itakupa mwelekeo na habari Zaidi ili mwishowe ufanye uamuzi wa kushiriki au kutoshiriki katika zoezi hili.

Je ninaagiziwa nifanya nini?

Ukikubali kushiriki katika uchunguzi huu, nitakuuliza maswali kadhaa kuhusu hedhi nzito, kuvija damu kutoka sehemu za mwili kama mapua, wakati wa kupiga mswaki ama baada ya kuumia kidogo. Sampuli ya damu itachukuliwa kufanya uchunguzi wa ugandaji wa damu (kiwango cha damu , 'APTT' na 'PT'), Kundi la damu la ABO na kiwango na kazi ya chembechembe ya Von Willebrand. Sampuli

zitachukuliwa kufanya uchunguzi ambazo hazijafanywa. Kutakuwa na uchungu ya kiasi ya chini wakati wa kutoa damu. Iwapo kutakuwa na uvujaji wowote katika kukusanya sampuli, tutahakikisha kuwa tumeikomesha mara moja.

Ukishiriki katika zoezi hii na iwapo faili zako itakosa maelezo muhimu, nitayajaza maelezo hayo katika faili yako, hii ikiwa sehemu ya kuboresha rekodi zako za kiafya. Maelezo Zaidi hedhi, pia yataongezwa katika maelezo yako ya kiafya.

Kuna uwezekano wa hatari kuzuka ninaposhiriki katika zoezi hili?

Sitarajii uwezekano wa hatari au jambo mbaya kuchipuka kwa kushiriki kwako katika uchunguzi huu.

Nani atakayejuzwa kuwa ninashiriki katika zoezi hili?

Ukiamua kushiriki katika uchunguzi huu, sitamwambia yeyote kuhusu matokeo yako au sehemu yeyote ya uchunguzi huu.

Je, lazima nishiriki katika uchunguzi huu?La, sio lazima. Uamuzi ni wako. Hakuna atakayepandwa na hamaki au hasira au kuudhiwa na uamuzi wako wa kushiriki katika uchunguzi huu.

Je, ikiwa nina maswali?

Ikiwa una maswali yoyote kuhusu uchunguzi huu, unaweza kuniuliza sasa au wakati wowote ule tutakapokuwa katika zoezi hili. Pia unaweza kuwasiliana nami kupitia nambari ya simu ya rununu 0723295312 au barua pepe mercymutaimd@gmail.com au unaweza kuwasiliana na msimamizi wangu mkuu Dkt. Fredrick Okinyi kupitia nambari ya simu 0720325448 au wasiliana na kitengo cha KNH-UoN ERC kupitia anwani ya baruapepe uonknh_erc@uonbi.ac.ke.

Kwa kujaza fomu hii ina maana kuwa umeelewa maelezo yote yaliyo katika fomu hii na kuwa umekubali kwa hiari yako kushiriki katika uchunguzi huu.

Jina la mshiriki: Sahihi ya mshiriki: Tarehe:

Sahihi ya mdadisi: Tarehe:

Appendix 2 Questionnaire

Participant's code:

Socio-demographic data and Clinical characteristics

1. Age (years) ……..

2. History of transfusion ☐Yes ☐No

3. Parity: Para…..+…..

4. ☐Outpatient ☐ Inpatient

5. Family history of bleeding ☐yes ☐No

Severity Based Presentation and Intervention for Menorrhagia (31)

Menorrhagia	0	1	2	3	4
Score					

Other ISTH/SSC BAT Content

SYMPTOM	**YES**	**NO**
Epistaxis		
Cutaneous		
Bleeding from minor wounds		
Oral Cavity		
Gastrointestinal		
Haematuria		
Tooth extraction		
Surgery		
Postpartum Haemorrhage		
Muscle Haematomas		
Haemarthrosis		
CNS bleeding		
Other bleeding		

Appendix 3

Standard operating procedures for sample collection, transport, storage and test procedures were as follows;

3.1 Specimen Collection

Specimen type was whole blood.

Checking of patients' identity to ensure it corresponds to the request form details. Patient will be relaxed prior to blood collection. Cleaning of the patient skin will be done using 70% isopropyl alcohol swabs or 0.5% chlorhexidine sterile gauze swabs then allowed to dry, as a tourniquet is applied above the venipuncture site (cubital fossa). Blood will be collected in EDTA sample bottle 1.50 ± 0.25/ml of blood and mixed. Blood for routine coagulation (PT and APTT) and Von Willebrands disease will be collected in 3.2% sodium citrate bottles (9 volume of blood in 1 volume of sodium citrate). 10 ml of blood will be drawn from patients who have not had any tests done. 4ml EDTA bottles will be used for full blood count and ABO blood grouping. 2 bottles of 2.7ml sodium citrate will be taken for PT, APTT and Von Willebrand Factor testing. If all screening tests will have been done and results recorded in the file, only 2.7ml will be drawn for Von Willebrand Factor testing.

The tourniquet will be release immediately after venipuncture, a sterile swab applied on the site with pressure as the arm is elevated and an adhesive dressing applied after. The samples will be transported to the laboratory using a cool box.

3.2 Storage and Processing of Samples

Full blood count, PT and APTT will be done immediately the samples get to the laboratory will be performed immediately. Samples for von willebrand factor tests will be transferred into rotors and stored in a freezer at -80°C.

3.3 Tests

3.3.1 ABO Blood Grouping

The tile method will be used to carry out this test.1drop of anti-A, 1 drop of anti-B, 1 drops of anti-AB, are placed on 4 separate parts of a tile.1 drop of test red cell suspension is added to each drop of the typing antiserum. The cells and reagents are mixed using a clean stick. Each mixture is evenly spread on the slide over a 10-15 mm diameter area. The tile is tilted and left for 2 minutes at room temperature. The mixtures are checked for agglutination and results recorded.

The findings will be as follows where **+** represents presence of agglutination and **0** represents absence agglutination

Group	Antisera A	Antisera B	Antisera AB
A			
B			
AB			
O			

3.3.2 Full Blood Count

Specimen type: Whole blood

FBC was be done using a fully automated method. Once the QC has passed, the patients' samples will be ran. The tube holder is retracted into the analyser to enable it. When the LED is green, the rack is placed on the right sampler pool. The groove on the rack is slid into the protrusion on the right side. The sampler analyser button is clicked. The sample number is entered into the input grid, rack number, patient number and name, and the start key is pressed. Once the analysis is over, the results will be printed out automatically.

3.3.3 Activated Partial Thromboplastin Time

The test was performed using The Thrombolyzer XRM which is fully automated. This analyses the intrinsic and common pathways by evaluating factors V, VIII, XI and XII. APTT was measured by incubating plasma with phospholipid and buffer which initiated activation of the intrinsic pathway after incubation at 37°C. Calcium was added for coagulation to take place. Clotting time was measured in seconds. APTT control was of 33.2 seconds.

3.3.4 Prothrombin Time

PT was measured using the fully automated thrombolyzer XRM. The test analyses the extrinsic and common pathways of coagulation by measuring the levels of factors I, II, V, VII, X and XIII. PT was measured by adding thromboplastin to citrated plasma with calcium and time for fibrin clot to form measured in seconds. The control time was 14.8 seconds.

3.3.5 Von Willebrand Factor Level and Activity

The test for VWD was done using automated latex enhanced immunoassay (Haemosil Machine)

Principle of the Test

The measurement and comparison of von Willebrand Factor Antigen (VWF:Ag, VWF Activity and Factor VIII (FVIII) levels in plasma aid in the differentiation of quantitative(type 1 and type2) from qualitative defect(type 2). If an extremely low or undetectable level of VWF:Ag obtained, type 3 VWD is suspected.If a moderate or normal result is obtained, VWF activity and FVIII assays are performed

and compared with the VWF:Ag levels. If all three values are within the normal range, VWD and Hemophilia A are excluded.

If any of the parameters is abnormally low, the ratios VWFActivity/VWF:Ag and FVIII/VWF: Ag are calculated. If both ratios are close to 1 (some literature gives 0.7 as cut-off), a VWD type 1 may be diagnosed. When the VWF/Activity/VWF:Ag ratio is low (0.7 is also the suggested cut-off), types 2A, 2B or 2M may be diagnosed. These 3 subtypes are characterized by its abnormal multimeric pattern and/or its altered platelet affinity. Additional laboratory tests; RIPA (Ristocetin Induced Platelet Aggregation), multimeric analysis and binding assays are required to be to distinguish them. When the FVIII/VWF:Ag ratio is low (0.7 is the suggested cut-off), a type 2N or Hemophilia A may be diagnosed and a FVIII binding assay is performed to discriminate among them.

The VWF Activity kit is a latex particle enhanced immunoturbidimetric assay that quantifies VWF Activity in plasma. Activity is determined by measuring the increase of turbidity produced by the agglutination of the latex reagent. A specific anti-VWF monoclonal antibody adsorbed onto the latex reagent, directed against the platelet binding site of VWF (GP1b), reacts with the VWF of patient plasma. The degree of agglutination is directly proportional to the activity of VWF in the sample as it measures the decrease of transmitted light caused by the aggregates.

3.10.6 Validation of HemosiL Equipment

HemosiL machine was validated by checking for accuracy and linearity to ensure its fitness for purpose. Validation was done using the standard procedures for quantitative tests. Low and normal controls of Von Willebrand Factor antigen and antibody were used. Accuracy was checked by having target values of normal and low controls. 10 points of the 2 levels were measured in duplicates. The mean of the 10 points was calculated and the findings compared to the target value. The difference between the measured value and the target value were compared to assess for acceptance. Linearity was checked to assess if the equipment could correctly measure samples with increasing concentration gradient. Different dilutions of low and normal values of both Von Willebrand Factor antigen and antibody were mixed. The manufacturer provided predetermined target values for these dilution. A graph with the target values against the measured values at different dilutions was plotted. Computing into a statistical package was done and reported values followed a linear gradient.

Appendix 4

Bleeding Assessment score ((31)

SYMPTOMS (up to the time of diagnosis	**SCORE** **0**	**1**	**2**	**3**	**4**
Epistaxis	No/trivial	- > 5/year or - more than 10 minutes	Consultation only	Packing, cauterization or antifibrinolytic	Blood transfusion or replacement therapy (use of hemostatic blood components and rFVIIa) or desmopressin
Cutaneous	No/trivial	For bruises 5 or more (> 1cm) in exposed areas	Consultation only*	Extensive	Spontaneous hematoma requiring blood transfusion
Bleeding from minor wounds	No/trivial	- > 5/year or - more than 10 minutes	Consultation only*	Surgical hemostasis	Blood transfusion, replacement therapy, or desmopressin
Oral cavity	No/trivial	Present	Consultation only*	Surgical hemostasis or antifibrinolyti	Blood transfusion, replacement therapy or desmopressin

				c	
GI bleeding	No/trivial	Present (not associated with ulcer, portal hypertension, hemorrhoids, angiodysplasia)	Consultation only*	Surgical hemostasis, antifibrinolytic	Blood transfusion, replacement therapy or desmopressin
Hematuria	No/trivial	Present (macroscopic)	Consultation only*	Surgical hemostasis, iron therapy	Blood transfusion, replacement therapy or desmopressin
Tooth extraction	No/trivial or none done	Reported in ≤25% of all procedures, no intervention**	Reported in >25% of all procedures, no intervention**	Resuturing or packing	Blood transfusion, replacement therapy or desmopressin
Surgery	No/trivial or none done	Reported in ≤25% of all procedures, no intervention**	Reported in >25% of all procedures, no intervention**	Surgical hemostasis or antifibrinotic	Blood transfusion, replacement therapy or desmopressin
Menorrhagia	No/trivial	Consultation only* or - Changing pads more frequently	- Time off work/school > 2/year or - Requiring	- Requiring combined treatment with antifibrinolytics and hormonal therapy	- Acute menorrhagia requiring hospital admission and emergency treatment

		than every 2 hours or - Clot and flooding or - PBAC score>100[#]	antifibrinolytics or hormonal or iron therapy	or - Present since menarche and > 12 months	or - Requiring blood transfusion, Replacement therapy, Desmopressin, or - Requiring dilatation & curretage or endometrial ablation or hysterectomy)
Post-partum hemorrhage	No/trivial or no deliveries	Consultation only* or - Use of syntocin or - Lochia > 6 weeks	- Iron therapy or - Antifibrinolytics	- Requiring blood transfusion, replacement therapy, desmopressin or - Requiring examination under anaesthesia and/or the use of uterine balloon/package to tamponade the uterus	- Any procedure requiring critical care or surgical intervention (e.g. hysterectomy, internal iliac artery legation, uterine artery embolization, uterine brace sutures)

Muscle hematomas	Never	Post trauma, no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
Haemarthrosis	Never	Post trauma, no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
CNS bleeding	Never	-	-	Subdural, any intervention	Intracerebral, any intervention
Other bleedings^	No/trivial	Present	Consultation only*	Surgical hemostasis, antifibrinolytics	Blood transfusion or replacement therapy or desmopressin

Appendix 5

KNH-UON ERC Approval Letter

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/36

2nd February 2021

Dr. Mercy Chepkemoi Mutai
Reg. No.H58/6717/2017
Dept.of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 02 FEB 2021 KNH/UoN-ERC P. O. BOX 20723 - 00202 NAIROBI

Dear Dr. Mutai

RESEARCH PROPOSAL – VON WILLEBRAND FACTOR LEVELS AND ACTIVITY IN WOMEN WITH MENORRHAGIA IN KENYATTA NATIONAL HOSPITAL (P558/10/2020)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 2nd February 2021 – 1st February 2022.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website**http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information Dept, KNH
The Dean, School of Medicine, UoN
The Chair, Dept. of Human Pathology,, UoN
Supervisors: Dr. Fredrick O. Okinyi, Dept.of Human Pathology, UoN
Dr. Jamila Rajab, Dept. of Human Pathology, UoN
Dr. Stephen Mutiso, Dept.of Obs & Gynae, UoN