VON WILLEBRAND FACTOR ACTIVITY AND ACTIVATED PARTIAL THROMBOPLASTIN TIME IN WOMEN WITH MENORRHAGIA AT THE GYNAECOLOGY CLINIC OF THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

By

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DECLARATION

This work/dissertation in its present form has not been submitted or accepted previously for the award of a degree or diploma at the University of Zambia or any other tertiary institution. I declare that this Dissertation contains my own work and where other authors have been cited due acknowledgement has been given. I further declare that I followed all the applicable ethical guidelines in the conduct of the research. This dissertation has been prepared in accordance with the Master of Science in Pathology (Haematology), University of Zambia guidelines.

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CERTIFICATE OF APPROVAL

The University of Zambia approved this Dissertation on "von Willebrand Factor activity and activated Partial Thromboplastin Time in women with menorrhagia at the Gynaecology clinic of the University Teaching Hospital, Lusaka, Zambia."

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ABSTRACT

von Willebrand Disease (vWD) is the most common or prevalent inherited bleeding disorder. It is caused by a deficiency or dysfunction of von Willebrand Factor, which is a glycoprotein that carries Factor VIII (Anti haemophilic factor) and an adhesive protein in platelet-vessel wall interactions. Most women with Von Willebrand disease present with menorrhagia (74 to 92%) and this can sometimes be the only symptom. Other symptoms may include easy bruising, epistaxis, prolonged bleeding on wounds, post-partum haemorrhage to mention a few.

This study aimed at determining von Willebrand Factor activity (vWF activity) and activated Partial Thromboplastin Time (aPTT) in women with menorrhagia at the University Teaching Hospital and the relationship between menorrhagia and other bleeding tendencies or the coagulation test scores (aPTT and vWF activity).

This was a case-control study undertaken at the Gynaecology clinic of the University Teaching Hospital. It included 56 cases and 112 controls of age range 18 to 45 years, (mean age of 30 +/- 7.4). The cases were women with menorrhagia and the controls women with gynaecology problems other than menorrhagia. Blood grouping, Activated Partial Thromboplastin Time and von Willebrand Factor Activity test were performed on both study groups. A questionnaire was also used to obtain demographic information from both groups.

The study findings were the following, von Willebrand Factor Activity levels were higher in individuals without menorrhagia (97.8 ± 53.1) than women with menorrhagia (66.6 ± 31.5) $p < 0.001$. Further, aPPT levels in women without menorrhagia were 30.395 ± 7.733) and 31.092 ± 8.259 in women with menorrhagia, with a non-statistically significant difference of $p = 0.5924$. Based on pathological scores in both VWF activity and a PTT, vWD was diagnosed in 1.8 percent of controls and 10.7 percent of cases. Other significant findings were in Epistaxis and menorrhagia association ($p <0.001$), Menorrhagia and family history of menorrhagia ($p=0.003$). There were no significant findings between Blood groups and VWF activity.

Our study showed that vWF activity levels were associated with menorrhagia while aPTT was not associated with menorrhagia. Further, vWF activity levels did not depend on the presence of a specific Blood Group. Our study also showed that the prevalence of vWD was significantly higher in participants with menorrhagia. Further that repeated epistaxis and a positive family history of menorrhagia pointed to a higher risk of menorrhagia.

These results highlight the need for haemostatic evaluation in women with excessive menstrual bleeding. This could help in better management of these patients and avoid complications during and after child-birth and surgery.

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ABBREVIATIONS

aPTT	activated Partial Thromboplastin time
BT	Bleeding Time
ECM	Extra Cellular Matrix
GP1b	Glycoprotein 1b
HB	Haemoglobin
HMB	Heavy Menstrual Bleeding
HTC	Haemophilia Treatment Centre
INR	International Normalized Ratio
ISTH	International Society on Thrombosis & Haemostasis
ITP	Immune Thrombocytopaenic Purpura
IUD	Intra Uterine Device
OB/GYN	Obstetrics and Gynaecology
OR	Odds Ratio
OVB	Owren's Veronal Buffer
PBAC	Pictorial Blood Assessment Chart
PPH	Post-partum Haemorrhage
PTT	Partial Thromboplastin Time
RiCoF	Ristocetin Cofactor (Platelet)
SHP	Standard Human Plasma
UNZABREC	University of Zambia Biomedical Research Ethics Committee
USA	United States of America
UTH	University Teaching Hospital
vWD	von Willebrand Disease
vWF Ac	von Willebrand Factor Activity
VWF Ag	von Willebrand Factor Antigen
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Background

von Willebrand Disease (vWD) is usually autosomal dominant and results from mutations in the von Willebrand Factor gene. This results in a deficiency or functional abnormality of von Willebrand Factor (vWF). VWF is a glycoprotein that acts as a carrier protein for Factor VIII (anti Haemophilic Factor) and as an adhesive protein in platelet - vessel wall interactions (Tossetto et al., 2006). vWF has two main haemostatic functions. In primary haemostasis at the site of injured vessel walls, it facilitates platelet adhesion to sub-endothelial structures such as exposed collagen fibres and supports platelet aggregation and thrombus formation. As part of secondary haemostasis, vWF acts as a carrier protein for coagulation Factor VIII (FVIII), stabilizing and protecting FVIII pro-coagulant activity. vWD is the most important disease of platelet adhesion discovered in 1926 by Dr Eric von Willebrand. It is the most prevalent inherited bleeding disorder worldwide, affecting 1 to 3 percent of the world population (Ciesla., 2007).

Individuals with vWD are at an increased risk for muco-cutaneous bleeding that includes epistaxis, easy bruising, prolonged bleeding after trivial cuts, excessive bleeding with dental procedures, excessive bleeding from the oral cavity, gastrointestinal bleeding, excessive post-operative bleeding, and reproductive tract bleeding (Tosseto et al. 2006).

von Willebrand Factor activity (vWF ac) measures the activity of vWF. It is expressed as percentage of the normal. activated Partial Thromboplastin Time (aPTT) is a laboratory based test for haemostasis. It measures the intrinsic and common pathway of coagulation. It is prolonged in vWD and is used as one of a panel of tests for diagnosis of vWD. Normal ranges are 30 to 40 seconds (Hoffbrand et al., 2005).

Normal coagulation processes are needed to stop blood loss due to the shedding of the endometrial lining and blood vessels. In some cases, bleeding can be worsened by blood disorders involving the platelets (as in immune thrombocytopenic purpura formerly idiopathic thrombocytopenic purpura), coagulation mechanisms (as in vWD), or anticoagulant medication such as warfarin. Normal Prothrombin Time (PT) and aPTT

do not rule out an underlying bleeding disorder, but they are inadequate for screening for severe rare clotting factor deficiencies. These tests have poor sensitivity, specificity, and positive and negative predictive values for detecting an underlying bleeding disorder (Fricke et al., 2004).

Clinical features of vWD include bleeding typically from membranes, (mouth, epistaxis, menorrhagia, excess blood loss during trauma or surgery. Diagnosis includes prolonged aPTT, Factor VIII and VWF levels are low with a prolonged bleeding time (Mehta., 2014). Despite being the most prevalent bleeding disorder, only a fraction of people are diagnosed with vWD with more than 75 percent of those diagnosed being women. Women encounter more episodes at which they are likely to bleed than men and therefore have more opportunities to face bleeding challenges. Women bleed during menstruation, during childbirth, and after childbirth and are therefore more likely to manifest with a bleeding disorder as they have more opportunities to experience bleeding challenges in their lifetime than men do (Women's Health, 2012). Most studies have used menorrhagia as an indicator for bleeding disorders because it is the commonest symptom in women with a disorder and could even be the only presenting symptom. In the United States, 1.5 to 4 million women have a bleeding disorder, and up to 2 million do not know it (Ragni et al., 1999).

There are three main types of vWD and diagnosis is based on both qualitative and quantitative testing. The signs and symptoms depend on the type and severity of the disease. Most people with Type 1 have bleeding that is mild to moderate in severity, does not require routine transfusions or other treatments and is not life-threatening. Life-threatening bleeding that involves the brain or gastrointestinal tract can occur in individuals with type 3 disease, in some individuals with type 2 disease and rarely in individuals with type 1 disease. Bleeding within deep tissues, such as muscles and joints may occur in individuals with type 3 disease (Nicholas et al. 2008).

Table 1: Types of von Willebrand Disease

TYPE	MECHANISM
Type 1	Quantitative deficiency of vWF
Type 2A	Abnormal Platelet dependent vWF function
Type 2B	Increased Platelet dependent vWF function
Type 2M	Abnormal Platelet dependent vWF function
Type 2N	Decreased affinity of vWF for Factor VIII
Type 3	Near complete deficiency of vWF

The commonest symptom women with vWD experience is menorrhagia. Menorrhagia is defined as greater than 80 cubic centimeters (cc) of blood loss per cycle which is the amount of blood loss that would result in iron deficiency anaemia. The following findings are associated with menorrhagia and include: soaking through a pad or tampon within 1 hour, soaking through bed clothes, below normal ferritin, anaemia, and a Pictorial Blood Assessment Chart (PBAC) score of greater than 100. The PBAC was developed as a way to assess menstrual blood loss without collecting menstrual pads and tampons (James et al., 2009).

In a review of studies in the United States of America (USA), there was a prevalence of menorrhagia in 32 – 100% of women with vWD (Nicholas et al., 2008). Additional bleeding symptoms include epistaxis, bleeding after dental extraction, ecchymosis, bleeding from minor cuts or abrasions, gingival bleeding, haemarthrosis and gastrointestinal bleeding. A number of studies have evaluated the presence of bleeding disorders in women with menorrhagia. Findings vary among studies and ranges for vWD in women with menorrhagia are 5 – 20 % (James 2010).

Studies done in South Africa by Sukhu et al and in Sudan by Omer et al found that different racial groups have varying amounts and functions of von Willebrand Factor. Both studies found that people of African descent to have more vWF antigen and activity

(Sukhu et al., 2003; Omer et al., 1973). Studies have also found that various blood groups have differing amounts and function of vWF with blood group O having the lowest (Gill et al., 1987).

There are no recorded studies of vWD in Zambia. vWD, despite being the most common inherited bleeding disorder in the world is not a routine test for patients who visit the hospital with bleeding abnormalities. This study aims to determine whether women experiencing bleeding problems have underlying vWD and also to determine and compare the levels of vWF activity and aPTT in women with menorrhagia and those without.

1.2 Statement of the Problem

von Willebrand Disease is the most common bleeding disorder and menorrhagia is a presentation in over 75 percent of women with vWD (Goodman-Gruen et al., 2001). Despite being a common gynaecologic problem, more than 50 percent of women are not diagnosed with a specific cause for their menorrhagia (Kadir et al., 1998). Excessive bleeding in menorrhagia may affect the quality of life of affected women and may also result in anaemia, with complications such as fatigue, dizziness, shortness of breath and may cause problems in future pregnancies for women. Patients need to be made aware of any bleeding disorders they may have especially Haemophilia and vWD which run in families (Gupta., 2007).

Africa has the highest prevalence of anaemia at 67.6 percent according to the World Health Organization in 2005. It is also estimated that 14 million women suffer severe blood loss during child birth. Around 140 000 die as a result, about 12 percent develop severe anaemia. This means each year 1.6 million women suffer from long lasting debilitating consequences of anaemia. Undoubtedly, some of these haemorrhages are due to bleeding disorders (Abouzhar., 2003).

Though not properly documented, Zambia records a number of deaths from uncontrolled bleeding after child delivery in women; this is according to records from registers in referral hospitals such as The University Teaching Hospital, Kitwe Central Hospital and Ndola Central Hospital. A number of women also visit Gynaecology clinics due to menorrhagia with or without other forms of excessive or abnormal bleeding. Some of these cases may be due to bleeding disorders such as vWD. It is, therefore, imperative to have a known prevalence of bleeding disorders among women in Zambia which is currently unknown.

1.3 Research Question

What are the levels of von Willebrand Factor Activity and Activated Partial Thromboplastin Time in women with menorrhagia at the Gynaecology clinic of the University Teaching Hospital?

1.4 Objectives

1.4.1 General

The study aims to determine the levels of von Willebrand Factor activity and activated Partial Thromboplastin Time in women with menorrhagia and estimate the prevalence of von Willebrand Disease in these women.

1.4.2 Specific

1.5.2.1 To determine and compare vWF activity and aPTT in women with menorrhagia, the “cases” and women without menorrhagia, the “controls”.

1.5.2.2 To evaluate the association between vWF activity and Blood groups in cases and controls.

1.5.2.3 To estimate the prevalence of von Willebrand Disease based on vWF activity and aPTT in Cases and Controls.

CHAPTER 2: LITERATURE REVIEW

vWD is a coagulation disorder caused by a deficiency in, or a dysfunction of von Willebrand Factor. It is the most important disease of platelet adhesion discovered in 1926 by Dr. Eric von Willebrand. It is the most prevalent inherited bleeding disorder worldwide, affecting 1 to 3 percent of the world population by conservative estimates (Ciesla., 2007).

Individuals with vWD are at an increased risk for muco-cutaneous bleeding that includes epistaxis, easy bruising, prolonged bleeding after trivial cuts, excessive bleeding with dental procedures, excessive bleeding from oral cavity, gastrointestinal bleeding, excessive postoperative bleeding, and reproductive tract bleeding (Tosseto et al., 2006). The most common symptom women with vWD experience is menorrhagia (Ragni et al., 1999).

vWF activity and aPTT are among a number of tests used to diagnose vWD. Activated Partial Thromboplastin Time is a laboratory based test for haemostasis. It measures the intrinsic and common pathway of coagulation (Mannucci et al., 2004).

There is no literature recorded on the prevalence of bleeding disorders in women or men in Zambia; however, a number of studies in some parts of Africa, Europe, Asia and America have been done on vWD and other bleeding disorders in women.

Most studies have used menorrhagia as an indicator for bleeding disorders particularly vWD, in women because it is the commonest bleeding symptom in women with bleeding disorders and could be the first and only presenting symptom. A tool was designed in form of a questionnaire to make easier and uniform the evaluation of women with menorrhagia (Phillips et al., 2011).

In 2004, a systematic review of eleven studies was done by Shankar et al. to determine the prevalence of vWD in women with menorrhagia. Eleven studies were included from different parts of the world and they included 988 women with menorrhagia. The study concluded that the prevalence of vWD was increased in women with menorrhagia and was an underlying cause in a significant group of women with menorrhagia across the world. 131 women were diagnosed as having vWD and individual study prevalence

ranged from 5 – 24% (Shankar et al., 2004). An evaluation of other bleeding disorders was also done in the same study.

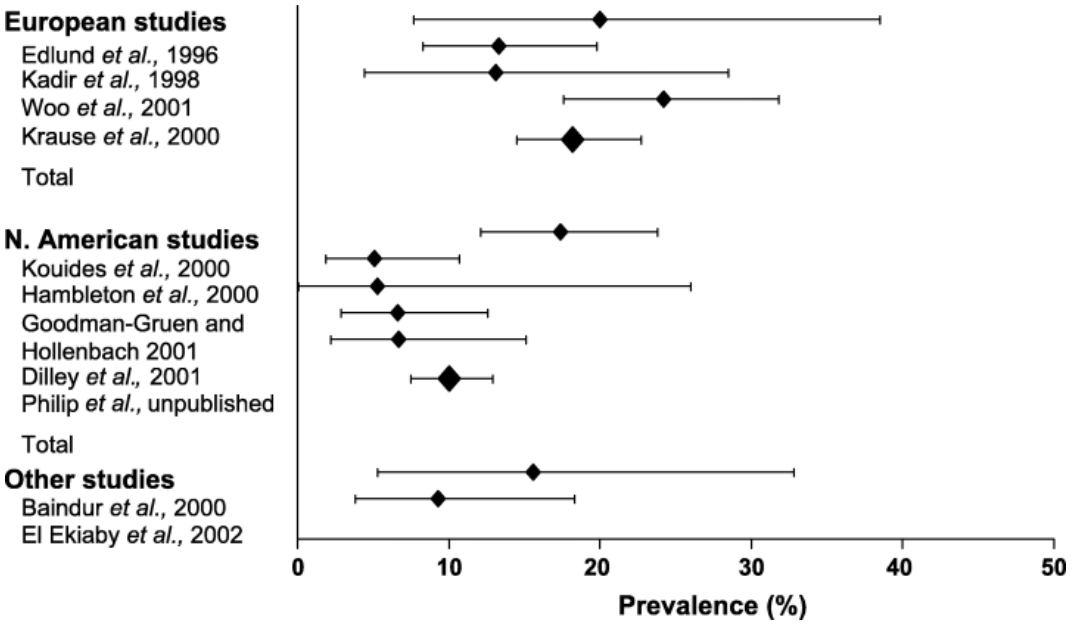

Figure 1 A review of studies, Prevalence of vWD with respective Confidence Intervals

In the United States of America Dilley et al carried out a study to determine the prevalence of vWD and other bleeding disorders in women with menorrhagia (Dilley et al., 2001). The study population was of reproductive women aged 18 to 45 years. Blood types and race specific range were determined. 121 cases and 123 controls were recruited for the study and 6.6 percent of cases and 0.8 controls were diagnosed with vWD. A higher prevalence was found in Caucasian women compared to African American women. A study was carried out in the south-east of the United States by Dilley et al which included one hundred and twenty-one women (121) with menorrhagia and one hundred and twenty-three (123) controls. The women were tested primarily for vWD but also for other coagulation disorders. The test group was compared by race and blood types against controls. vWD, a factor deficiency or a platelet abnormality were found in 10.7 percent of the menorrhagia patients and in 3.2 percent of the controls. vWD was found in eight of the patients and in one of the controls that is 6.6 percent and 0.8 percent respectively. In the same study separate analyses of race revealed a prevalence of 15.9 percent among white and 1.4 percent among black menorrhagia

patients. The study concluded that vWD was lower in black women and that it was important to consider bleeding disorders as a cause of menorrhagia. (Dilley et al., 2001). A study conducted in the USA to estimate the incidence of bleeding events and other complications during pregnancy and childbirth in women with vWD found that women with vWD are at an increased risk of bleeding events and possibly death during pregnancy and child delivery. This study was conducted from 2000 to 2003 on 4067 women with vWD as well as a control group by James AH and Jameson AG. The maternal mortality rate was ten times higher than that of other women (James AH and Jameson AG, 2007). The women in this study also had a fivefold risk of being transfused. It is well known that transfusions come with complications and risks on their own. Greer et al also reported that women with vWD were more likely to experience a rare vaginal delivery complication known as perineal haematoma (Greer et al., 1991). According to a 2000 study in New York, United States of America published in Haemophilia, 7 percent of women diagnosed with vWD underwent a hysterectomy prior to their diagnosis that did not treat the underlying bleeding disorder. Nearly three times more women with vWD had undergone a hysterectomy than similar women without a bleeding disorder (26 percent vs. 9 percent), according to a study published in Haemophilia in 2003, hysterectomies do not help with either diagnosis or treatment of vWD. In a study published in September 2007 in Haemophilia, researchers from the Rochester Haemophilia Treatment Centre (HTC) reviewed the records of 61 adolescents between 11 and 19 years old with a history of menorrhagia that were referred to the center. They found that 36% had a deficiency in von Willebrand Factor. Another 7 percent had defects in their platelets, leading to other clotting problems. These results highlight the importance of closely evaluating adolescents with heavy periods for bleeding disorders.

A study to determine population differences in vWF in Atlanta Georgia by Miller et al.in 2001 determined that vWF was higher in African-American women than in Caucasians. Study participants were 123 women enrolled in a health maintenance organisation who had menorrhagia and 123 randomly enrolled controls that had no menorrhagia diagnosis. A total of 70 (56 percent) cases and 76 (62 percent) controls were African-American.

The finding of higher vWF and FVIII in African-American women than in Caucasian women, confirmed findings from previous American studies (Colan et al. 1993) that black women were less affected by vWD and similar observations were made in women of African descent in studies done in Britain (Kadir et al., 1999), South Africa (Gomperts et al., 1976) as well as Brazil (Colonia et al., 1979). A study on adolescents with menorrhagia in New York found that 36 percent of these girls had a deficiency in vWF and 7 percent had platelet aggregation abnormalities (Mikhail et al., 2007).

In Europe studies done in Stockholm Sweden by Edlund et al used menorrhagia as a predictor for coagulation disorders found an increased prevalence of vWD, 20 percent in women with menorrhagia. The study concluded that menorrhagia is an excellent predictor for vWD and platelet disorders (Edlund et al., 1996) and in Clamart, France a similar study by Veyradier et al which included 50 cases and 50 controls, found no statistical difference between the two groups, but 8.6% of the cases had mild VWD. A study in Maramo Sweden aimed to describe the prevalence of perceived bleeding symptoms, including menorrhagia, in young healthy females was done. One thousand and nineteen girls consented to answer the questionnaires distributed. The mean age of the girls was 16.7 years. Eight girls had a previously diagnosed bleeding disorder and had a higher frequency of most bleeding symptoms. Seventy-three percent of girls experienced at least one bleeding symptom, 43 percent had more than one symptom, 23% were troubled by more than two symptoms, and 10 percent more than three symptoms. Thirty-seven percent experienced heavy menstruation. Thirty-eight percent had a family history of heavy menstruation and half of them suffered from heavy menstruation themselves. The study concluded that bleeding symptoms were relatively prevalent in this population and similar to other population-based studies (James et al., 2009).

In Asia a study from Kemanshah in Iran in 2013 by Merdhad et al, was done on clinical features and types of vWD in women with menorrhagia. The study comprised 482 women with menorrhagia, 56 of these were evaluated for inherited bleeding disorders and 31 of these (55.3 percent) of these were diagnosed with vWD. Type 3 being the

most frequent subtype (Merdhad et al., 2013). In Pakistan, a study on vWD in women with heavy menstrual bleeding found that 14.5 percent of the 200 enrolled women had low levels of vWF which is an indicator for vWD (Anjum et al., 2016).

Most studies done based on ethnic variations have found that women of African descent, have higher levels of von Willebrand Factor than Caucasians. A study by Miller et al in 2006 found that levels of vWF antigen (vWF Ag) were significantly higher in African Americans than in their Caucasian counterparts. A study done in South Africa by Gomperts et al in 1976 aimed at studying the levels of vWF in two ethnic groups Blacks and Whites found that people of Black descent had higher levels than Caucasians. In Sudan, a study to evaluate the effects of menorrhagia in the coagulation profile by Mohamedahmed et al in 2016 concluded that the coagulation profile can be affected by menorrhagia. In Egypt a study was done to find a mode to reach out to women with von Willebrand Disease by targeting women with menorrhagia. The study included 76 women with menorrhagia against 38 age-matched otherwise healthy women. The study indicated a high prevalence of vWD in women with menorrhagia at 6.5 percent (Sherif et al., 2014).

Studies have revealed that vWD diagnosis is also dependent on Blood groups. Studies have found that Blood Group O individuals generally have lower levels of vWF therefore vWD diagnosis in Blood Group O individuals is based on lower levels of VWF activity and vWF Ag than in non-blood Group O (Blood Groups A, B, AB) individuals (Gill et al., 2016).

The studies above highlight the need for a known prevalence of vWD in a population as well as a thorough assessment of women reporting menorrhagia at Health Facilities in order to ensure that proper management and therapy are provided to patients.

CHAPTER 3: METHODOLOGY

3.1 Study design and Study site

This was an unmatched Case-Control study. It included women who visited the Gynaecology and Obstetrics' (Ob/Gyn) clinic of the UTH with menorrhagia as cases and women who visited the Ob/Gyn clinic without menorrhagia or other bleeding problems as the controls. The study site was the University Teaching Hospital.

3.1.1 Target Population

Women at the Gynaecology clinic of the UTH with menorrhagia.

3.1.2 Study Population

Women at the Gynaecology clinic of the UTH. Approximately, 35 women visit the Gynaecology clinic on a daily basis. Therefore, an estimated study population is: **35 women x 5 days per week x 3 months of sample collection = 525.**

3.2 Sample Size

A total sample size of 168 women with 56 cases and 112 controls. This was calculated using sample size for unmatched case control study using Open epi statistical software. The computation was as below.

Sample Size for Unmatched Case-Control Study

For:

Two-sided confidence level(1-alpha)	95
Power(% chance of detecting)	80
Ratio of Controls to Cases	2
Hypothetical proportion of controls with exposure	1
Hypothetical proportion of cases with exposure:	10
Least extreme Odds Ratio to be detected:	11.00

	Kelsey	**Fleiss**	**Fleiss with CC**
Sample Size – Cases	56	66	82
Sample Size – Controls	112	132	164
Total sample size:	168	198	246

References Kelsey et al.

CC = continuity correction

Results are rounded up to the nearest integer.

Results from OpenEpi, Version 3, open source calculator--SSCC

Therefore, using Kelsey, 56 cases and 112 controls, 168 participants were included in the study.

3.2.1 Sampling Method

Convenient sampling was used. Patients visiting the Gynaecology clinic with menorrhagia were selected as they came. These patients were either referred from clinics

or self-referrals. The patients were told about the study and presented with an Information sheet. If patients were willing to take part in the study, they were given a Consent form to sign and were also presented with a Questionnaire. The participants either filled in the Questionnaire themselves or were interviewed and the interviewer filled in the Questionnaire depending on what they felt comfortable with.

The Controls were also patients at the Gynaecology clinic but without menorrhagia. Willing patients were also presented with an Information sheet, a Consent form and a Questionnaire.

3.2.1.1 Inclusion criteria

Inclusion criteria for the study were:

Cases:

i) Women aged between 18 and 45 years inclusive with menorrhagia for three or more months.

ii) Women with gynaecological problems, menorrhagia for cases and non-specified but not bleeding related for controls.

Controls:

i) Women aged between 18 and 45 years inclusive with gynaecological problems other than menorrhagia.

3.2.1.2 Exclusion criteria

Exclusion criteria for the study were:

Cases

i) Women were excluded if they had known conditions which also cause menorrhagia such as uterine fibroids or were using Intra Uterine Devices (IUDs) for birth control. Women were only included in the study after ruling out Fibroids by the attending Physician.

ii) Women below 18 years and above 45 years were also excluded from the study.

Controls

i) Women were excluded if they had experienced any prolonged bleeding during their menstrual cycle or from a wound during their lifetime.

3.3 Materials and Methods

3.3.1 Screening

A screening tool was constructed as in previous studies done in the United States of America, Spain and others (James et al., 2009). The tool contained questions in the following, regulatory occurrence of menstruation, (regular or irregular) duration and severity of menstrual bleeding, previous patient's history of abnormal bleeding, treatment history and family history of abnormal bleeding. The Questionnaire was then either given to participants to fill in themselves or for those unable to read or write, the questions were read to them in the language they preferred and their responses were entered on the Questionnaire.

3.3.2 Sample Collection and Storage

An Information sheet was given to selected patients and those willing to take part in this study were also presented with a Consent form to sign in the presence of a witness (any relative to the participant or medical personnel that was around). After the consent form was signed, the participants were given a Questionnaire to fill in.

After the Questionnaire was filled in, the participant was made comfortable and approximately 4mls of blood was collected from the forearm vein into a sodium citrate (blue top) container labelled with an identification number. The container was inverted five times to mix the blood and transported to the Haematology Laboratory.

The samples were centrifuged for 15 minutes at 1500 rotations per minute (rpm) in a centrifuge and the plasma was separated from the blood cellular components and stored in serum vials at a temperature of -20^0C for analysis on a later date. The separated Red blood cells were used for blood grouping.

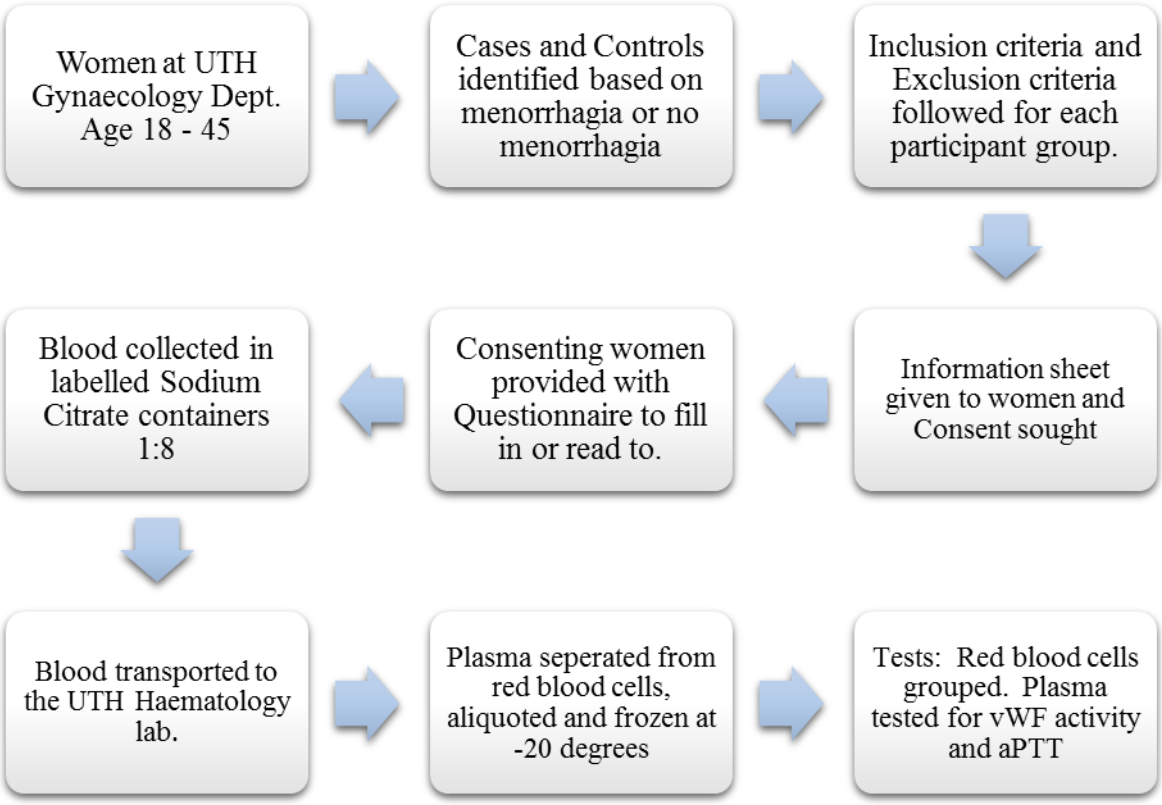

Figure 2 Flow Chart showing the process from participant recruitment to sample testing.

3.3.3 Quality Control

To ensure accurate and reliable results, quality control was performed on all the analytical instruments and analysers used for any purpose during specimen analysis according to the UTH quality control guidelines. Quality control included equipment calibrations and analytical control runs on every analyser before each test analysis.

3.3.4 Specimen Analysis

3.5.1 Blood Grouping

3.5.1.1 Sample Collection

Samples were spun in a centrifuge at 1500 rotations per minute (rpm) for 15 minutes. The serum was separated from the red blood cells which had settled at the bottom of the

vacutainers. The red blood cells that were separated from serum after spinning and separating serum were used for blood grouping.

3.5.1.2 Test procedure

The glass slide method was used where blood was grouped on a glass slide. Three drops of blood from each sample were placed on glass slides. To each drop Anti-A, Anti-B and Anti D (Rhesus Factor) were placed on the respective drops. A wooded applicator stick was used to mix the blood and the grouping sera. The glass slide was gently shaken for about one minute and agglutination was noted to determine blood groups.

Table 2: Blood Group determination.

Blood group	Antigen(s) present on Red Cell surface	Antibodies present in serum	Genotype(s)	Rhesus Factor
A	A	Anti B	AA or AO	+/-
B	B	Anti A	BB or BO	+/-
AB	A and B	None	AB	+/-
O	None	Anti A and Anti B	OO	+/-

3.5.2 Activated Partial Thromboplastin Time Test protocol

3.5.2.1 Summary

Dade Actin FS Activated PTT Reagent: This test was used primarily to evaluate coagulation abnormalities in the intrinsic pathway, it also detected abnormalities in Factors II, V, X or fibrinogen.

3.5.2.2 Principle of the method

Factors of the intrinsic pathway were activated by incubating the plasma with optimal amount of phospholipids and a surface activator. The addition of calcium ions triggered the coagulation process, and the clotting time was then measured.

3.5.2.3 Specimen Collection and Preparation

Nine parts of freshly collected blood was mixed with one part of 0.11 or 0.13mol/L of Sodium Citrate. The blood was centrifuged at 1500 rpm for 15 minutes. Plasma was separated from the cells and stored in plastic cryo-vials at -40 degrees Celsius.

3.5.2.4 Sample Running

Samples were removed from the freezer and thawed at room temperature. They were then run on the C1500 coagulation machine.

3.5.3 von Willebrand Factor Activity Test

3.5.3.1 Summary

Innovance vWF AC is a particle enhanced assay for the automated determination of von Willebrand Factor activity in human citrated plasma.

3.5.3.2 Principle of the Procedure

The assay principle makes use of the binding of vWF to receptor Glycoprotein 1b (GP1b) which is the main VWF receptor on platelets. Polystyrene particles are coated with antibody against GP1b. Recombinant GP1b (two gain of function mutations included) is added and binds to the antibody as well as to the vWF of the sample. This vWF binding induces a particle agglutination which can be measured as an increase in extinction by turbidimetric measurements.

3.5.3.3 Quality Control

Normal range: Control Plasma N

Pathological range: Control plasma P

Two levels of quality control material were measured at the start of the test run, with each calibration, upon reagent vial changes and at least every eight hours on each day of testing. Calibration was done using Standard Human Plasma (SHP) and a standard curve was generated by automatic determination of different dilutions of Standard Human Plasma and Owren's Veronal Buffer (OVB).

3.5.3.4 Results

The Innovance vWF ac assay was automatically carried out by the coagulation machine, C1500. Assay calibration was performed with Standard Human Plasma which was calibrated against vWF:RCo value of the International Standard for blood coagulation Factor VIII and von Willebrand Factor in Plasma. The results were reported as a percentage of the normal.

Table 3: Normal expected range of values of vWF activity based on blood group.

Blood Group O (% of normal)	Blood Group Non-O (% of normal)	Blood Group independent (% of normal)
46.3 to 145.6 %	61.4 to 179.1 %	47.8 to 173.2%

3.4 Ethical Consideration

Ethical clearance was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) before the commencement of the study. Patient information and results were confidential and access to this information was restricted to the Researcher and Supervisors.

3.4.1 Ethical permission

The research proposal was submitted to the University of Zambia Biomedical Research Ethics Committee (UNZABREC) for approval and was approved with **Ref. No. 004-01-16**. obtained on the 8th April, 2016.

3.4.2 Facility Permission

Permission to conduct the study in the Haematology laboratory was sought from the Medical Superintendent at the University Teaching Hospital. Permission to use equipment and facilities in the Department of Pathology and Microbiology in the UTH was obtained from the Head of the Department of Pathology and Microbiology at the University Teaching Hospital.

3.5 Data Analysis

All data processing was performed using a commercial software package MATLAB version R2016b (MathWorks Inc., Natick, MA, USA).

The Shapiro-Wilk test was used to check for normality, and histograms and box plots were examined to verify the normality of distribution of measurement data, with non-normally distribution data, was transformed using the Box-Cox transformation.

The independent sample t-test was used to evaluate the mean difference of vWF activity and aPTT between women with and without menorrhagia, and of vWF activity between blood group O and non-blood group O women.

A one-way ANOVA was used to evaluate mean differences of vWF activity and aPTT in participants with varying menses flow rate. Furthermore, the chi-square test or Fisher's exact test where appropriate were used to assess associations between women

with a family history of menorrhagia either in menstruation flow rate or VWF activity, and menorrhagia with epistaxis.

All statistical tests were performed at the 5% significance level, and differences were considered significant if 2-tailed $p < 0.05$ for all test applied.

Data are mean ± standard deviation, unless otherwise stated. vWF activity and aPTT levels for each level of groups were non-normally distributed, as assessed by Kolmogorov-Smirnov test ($p > 0.05$) for VWF activity and aPTT levels, respectively, in both comparison groups. Thus, the data were transformed using the Box-Cox transformation to achieve normality. vWF activity and aPTT levels showed no outliers in the transformed data as assessed by inspection of a boxplot (Figure 12, Appendix 7).

CHAPTER 4: RESULTS

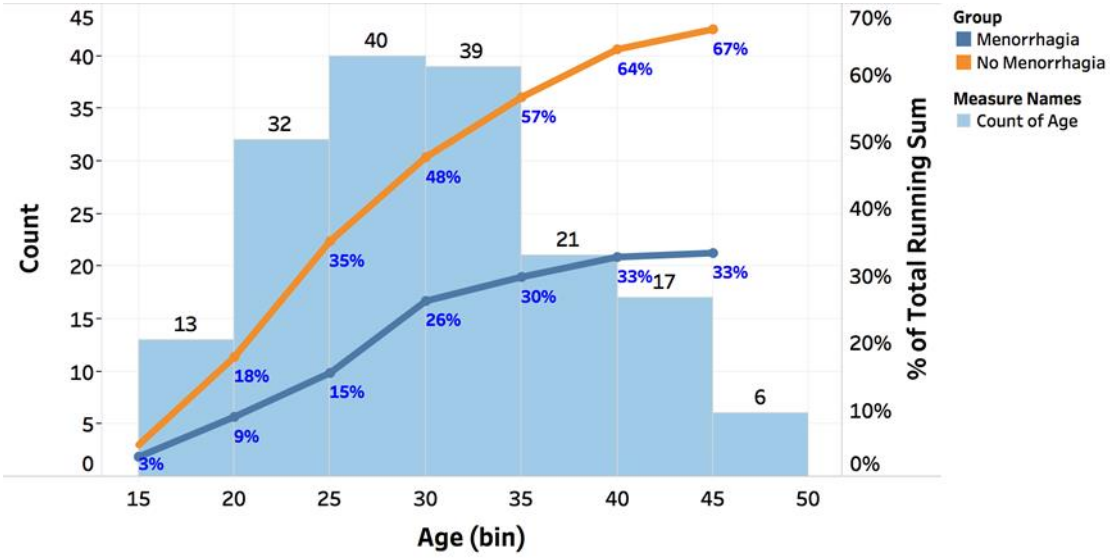

Figure 3 The trends of the age of participants (first y-axis) and percentage of the total running sum of age (second y-axis) for Age (bin) on the x-axis. The histogram shows counts for each bin of age. For pane percentage of the total running sum of age: colour shows details about the group.

Table 4. Clinical and Demographic details of participants' n = 168.

CHARACTERISTIC	**Menorrhagia participants (N=56)**	**Non menorrhagia participants (N=112)**
Ethnicity	African (100%)	African (100%)
Age	29.82 ± 0.91	30.03 ± 0.72
Nosebleeds	(13) 23%	(5) 0.04%
Other Bleeding problems	(10) 18%	0
Medical attention	(4) 0.07%	0
Family history of menorrhagia	(10) 18%	(5) 0.04%
Family history of abnormal bleeding	(3) 0.05%	(4) 0.04%

4.1 von Willebrand Factor activity and activated Partial Thromboplastin Time

Our study found that women with menorrhagia had a statistically lower mean VWF activity (66.6 ± 31.5) percent, than women without menorrhagia (97.8 ± 53.1) percent. A statistically significant difference of 0.272 (95% CI, 0.148 to 0.396), *t* (4.346) = 123.3, p < 0.001 (Figure 4a). Furthermore, our study found that mean aPTT levels in women with menorrhagia was (31.092 ± 8.259) seconds and (30.395 ± 7.733) seconds in women without menorrhagia, A non-statistically significant difference of -0.001 (95% CI, -0.003 to 0.002), *t* (-0.537) = 95.392, p = 0.593 (Figure 4b). Group descriptive statistics for these comparisons are as summaries in Table 5.

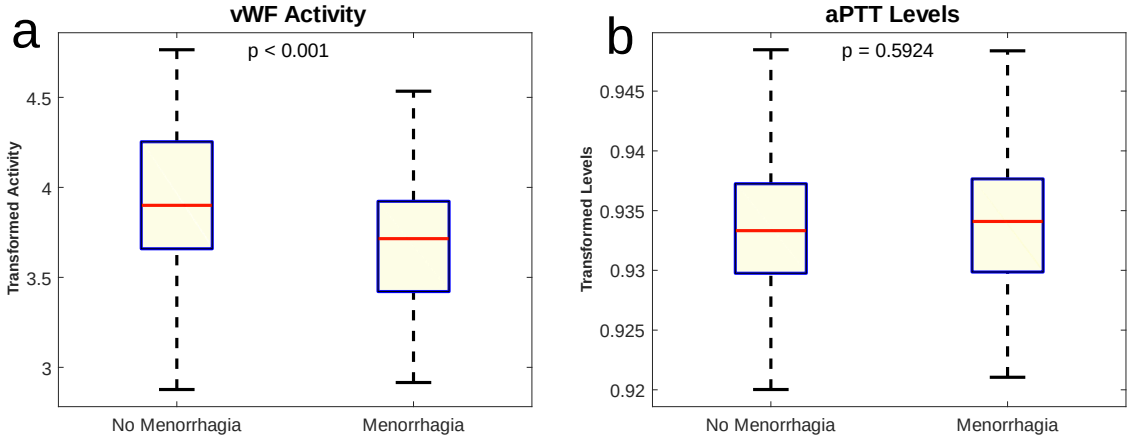

Figure 4 The mean vWF activity in women with menorrhagia was lower (66.6%) than that of women without menorrhagia (97.8%) p <0.001. This may explain why these women had menorrhagia. The mean aPTT of the women with menorrhagia was only slightly but not significantly higher (31.09s) than that of women without menorrhagia (30.40s) p = 0.593.

Table 5: Descriptive Statistics of vWF Activity and aPTT Levels. Mean vWF activity was higher in cases than controls. Mean aPTT was higher in cases than controls.

Measurements	Condition	N	Mean	Median	Std. Dev.	Range	Min	Max
vWF Activity	No Menorrhagia	112	97.8	80.4	231.7	53.1	22	253.7
	Menorrhagia	56	66.7	63.3	162.5	31.5	23.1	185.6
aPTT Levels	No Menorrhagia	112	30.4	28.7	42.8	7.7	18.3	61.1
	Menorrhagia	56	31.1	29.5	40.7	8.3	20	60.7

4.2 Variations in vWF activity across Blood Groups

Our study found that that vWF activity was not statistically different in Blood Group O women and Non Blood Group O women (Blood groups A, B and AB). Blood group O women had a lower mean vWF activity of (86.3 ± 51.3) percent and Non blood group O women had a higher mean vWF activity of (88.0 ± 47.5) percent. A non-statistically significant difference of -0.048 (95% CI, -0.178 to 0.082), t (-0.729) = 147.146, p = 0.467.

We also found no association between the two blood groups (O and non-O) and laboratory levels of vWF activity (low, normal and high) using the chi-square test of independence $\chi 2(2) = 3.0951$, p = 0.213 (Figure 5).

Corresponding descriptive statistics of Blood Group O and non-blood group O vWF activity presented in Table 6.

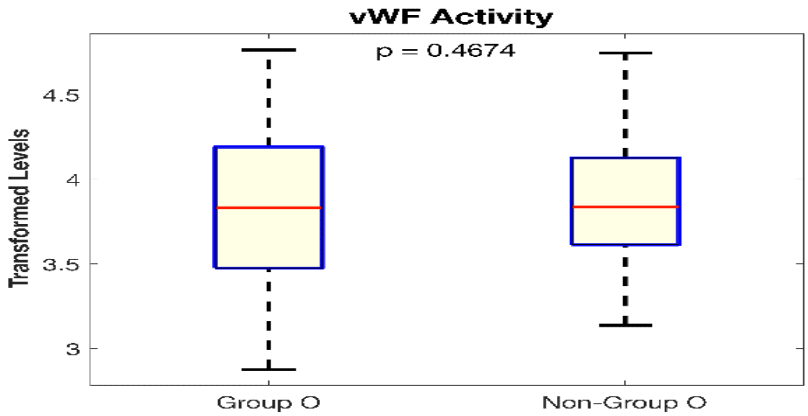

Figure 5 The mean vWF activity levels in Blood group O women was slightly but not significantly lower (86.3%) than in the other women with Blood groups A, B and AB (88.0%) p = 0.467.

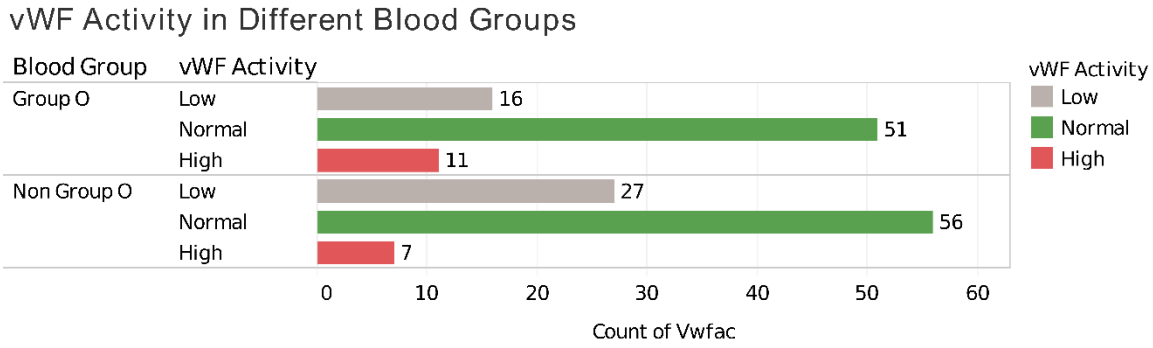

Figure 6 Blood group type did not influence the levels of vWF activity p = 0.213.

Table 6: Descriptive Statistics of vWF activity Distribution across Blood Group O and non-blood group O.

Measurement	Blood Group	N	Mean	Median	Std. Dev.	Range	Min	Max
vWF Activity	Group O	76	86.3	73.7	51.3	231.7	22	253.7
	Non-Group O	89	88	74.1	47.5	216.9	30.5	247.4

4.3 Prevalence of von Willebrand Disease in study participants

Our findings based on pathologic values of vWF activity and aPTT indicated that 2 controls out 112 (1.78%) had VWD and 6 out of 56 cases (10.71%) had vWD. (Figure 7). We found that women with menorrhagia had 6.6 odds of having vWD than women without menorrhagia. Odds ratio 6.6, 95% Confidence Interval (1.2868 – 33.850) p=0.024. A Fishers exact test revealed that the number of women with vWD in the menorrhagia group was statistically higher than women with vWD in the no menorrhagia group p=0.017.

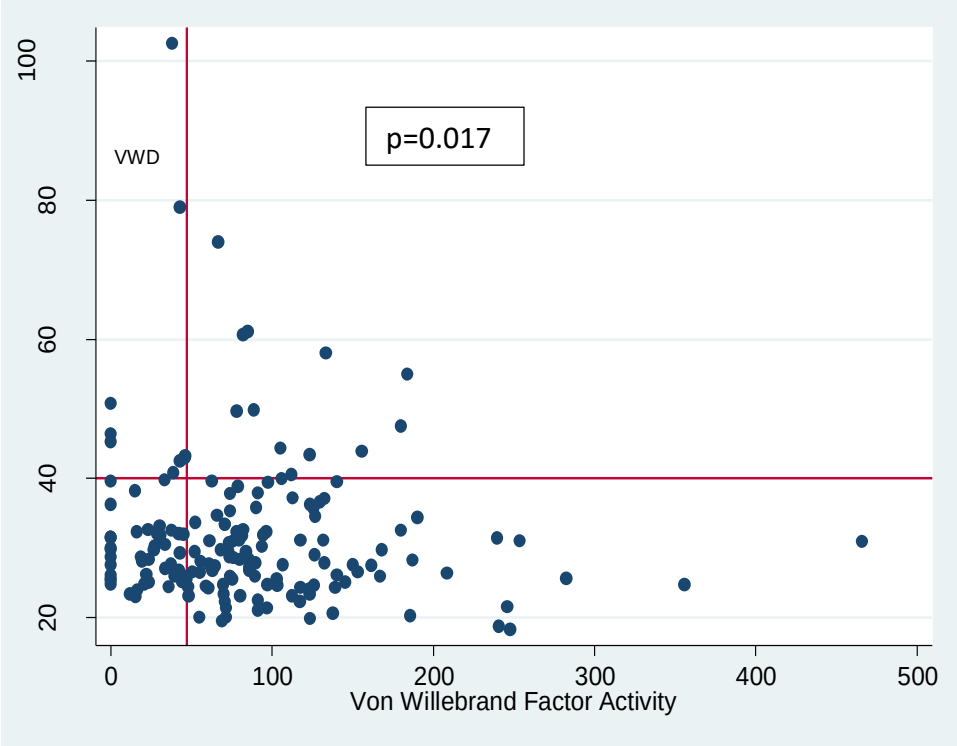

Figure 7 A total of eight women were diagnosed with vWD. A higher prevalence of vWD was seen in women with menorrhagia compared to women without menorrhagia. This means that menorrhagia could be used to predict vWD.

Table 7: Descriptive statistics for Odds Ratio of vWD in Women with menorrhagia and women without menorrhagia.

Odds ratio	6.6000
95 % CI:	1.2868 to 33.8503
z statistic	2.262
Significance level	P = 0.024

Table 8: Characteristics of women who were diagnosed with vWD

	Ethnicity	**Age (years)**	**Blood group**	**aPTT (s)**	**VWF activity (%)**	**History of bleeding**	**Family history bleeding**
Control	African	30	A^+	43.2	46.0	No	No
Control	African	18	O^+	42.5	42.8	No	No
Case	African	41	A^+	40	33.5	No	Yes
Case	African	28	O^+	45.3	<15	No	No
Case	African	24	O^+	46.4	<15	No	No
Case	African	27	A^+	102.5	38.2	No	No
Case	African	29	A^+	50.8	<15	Yes	Yes
Case	African	27	O^+	43.0	45.5	No	No

4.4 Differences in clinical features between Cases and Controls.

a) Difference of vWF activity and aPTT in Different Menses Flow Rate:

As we had four different classes of menstrual flow rate categories low, medium (no menorrhagia) and heavy, very heavy (menorrhagia group) refer to Questionnaire, Appendix 5, we conducted a one-way ANOVA test and found that vWF activity increased from heavy (62.29 ± 33.49), very heavy (75.62 ± 34.76), to medium (107.82 ± 73.32) to light (107.82 ± 43.93) flow rate of menses, in that order, but the differences between these menstrual flow rate groups was not statistically significant, $F(3,161) = 2.259$, $p = 0.08$ (Figure 8a).

Additionally, aPTT decreased from the heavy (34.22 ± 15.38), very heavy (32.47 ± 13.08), medium (30.78 ± 8.01), to light (28.44 ± 4.72), flow rate of menses, in that order, but the differences between these menstrual flow rate groups was not statistically significant, F (3,159) = 1.33, p = 0.267 (Figure 8b).

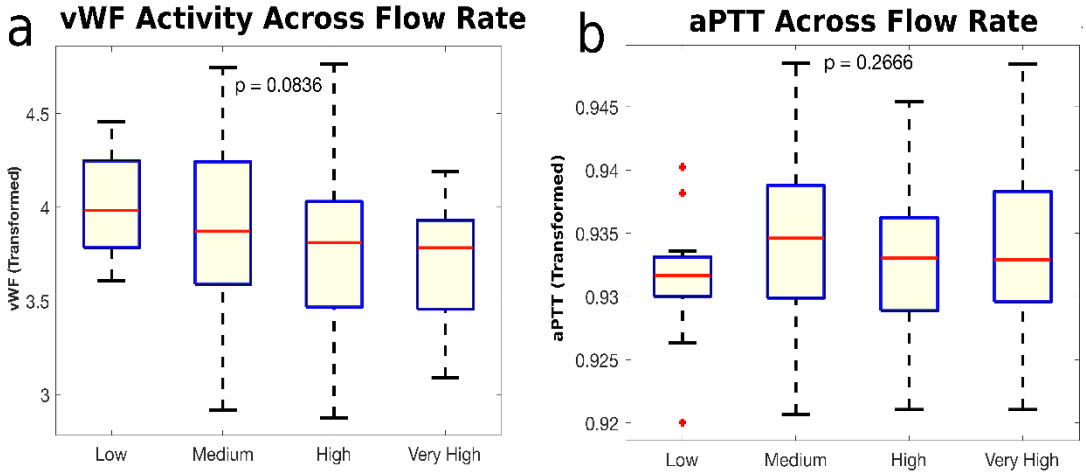

Figure 8: Comparisons of vWF activity and aPTT across menstrual flow rates. A decrease in vWF activity could be seen with a reduction in menstrual flow. This difference was however not significant. The differences in aPTT were also not significant among the different flow rates p = 0.267.

b) Association between Menorrhagia and Epistaxis.

The chi-square test of independence was conducted between a participant history of epistaxis and the presence of menorrhagia. We found a statistically significant association between Epistaxis and menorrhagia $\chi 2(1) = 13.72$, $p < 0.001$ (Figure below).

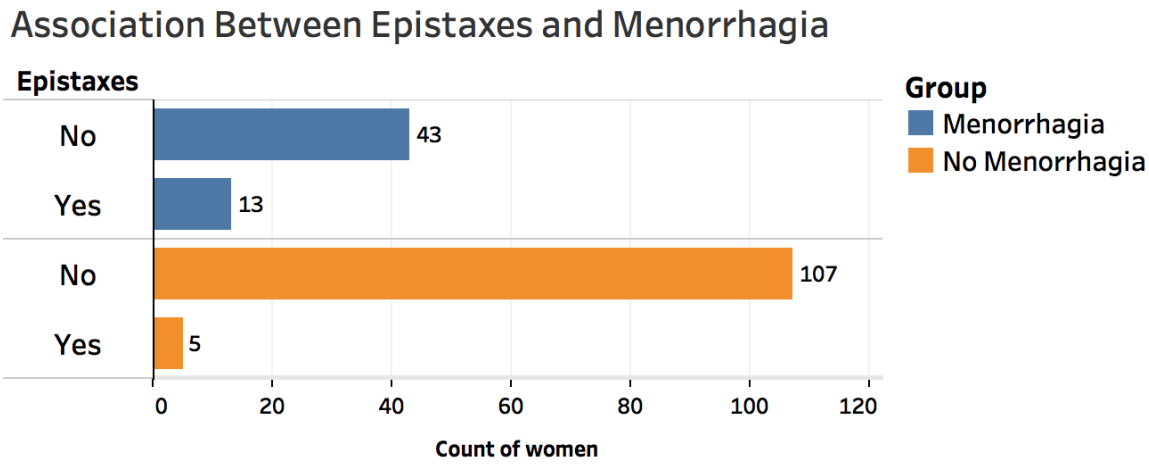

Figure 9 **:** A higher number of women with menorrhagia (13) reported to have suffered epistaxis compared to women without menorrhagia (5) There was a positive association between the menorrhagia and epistaxis p <0.001.

c) Association between a family history of menorrhagia and participant menorrhagia or vWF activity

Of the 168 women randomly recruited to the study, there were 154 women with no family history of menorrhagia and 14 with a family history of menorrhagia. For women with a family history of menorrhagia, 10(6.0%) experienced menorrhagia, and 4(2.4%) did not experience menorrhagia. Whereas for the women without a family history of menorrhagia, 46(27.4%) experienced menorrhagia, and 108(64.3%) did not experience menorrhagia. There was statistically significant association between the presence of menorrhagia and the family history of menorrhagia as assessed by Fisher's exact test, p = 0.003 (Figure 10)

Furthermore, of women with no family history of menorrhagia, 18(11.7%) had high vWF activity, 99(64.3%) had normal vWF activity, and 37(24.0%) had low vWF activity. For women with a family history of menorrhagia, 8(57.1%) had normal vWF activity, and 6(42.9%) had low vWF activity. There was no association between von Willebrand Factor activity and a family history of menorrhagia as assessed by Fisher's exact test, p = 0.196 (Figure 11).

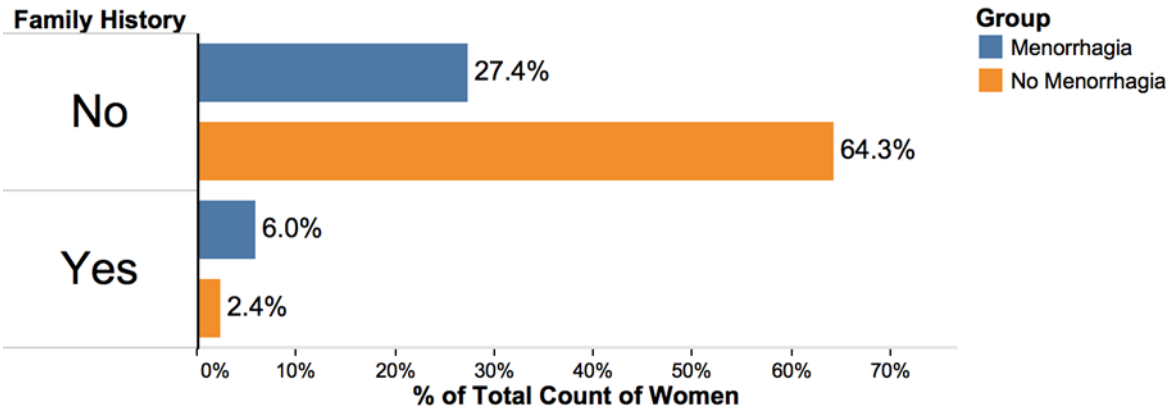

Figure 10: There was a significantly higher percentage of women with menorrhagia (6%) that reported a family history of menorrhagia compared to women without menorrhagia (2.4%) p = 0.003 meaning a positive Family history of menorrhagia increased the chances of having menorrhagia.

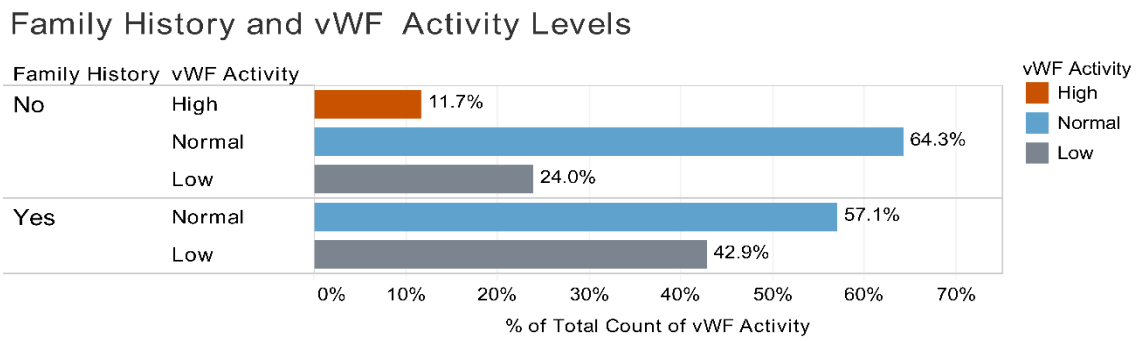

Figure 11: There was no association between levels of vWF activity and a Family history of menorrhagia as assessed by Fisher's exact test p = 0.196.

CHAPTER 5: DISCUSSION

Menorrhagia is a common presenting symptom in women of reproductive age with bleeding disorders (Payandeh et al., 2013). Other common causes include fibroids, intrauterine devices (IUD) and some forms of contraceptive (Phillip et al., 2005). In cases where the cause is unknown vWD should be suspected. The frequency of vWD is equal in both males and females but it is more apparent in women due to inevitable occurrences of bleeding during menstruation, child birth, caesarean sections to mention a few (Ragni et al., 1999).

The mean age of the cases was 29.82 years and that of controls, 30.03 years. Menorrhagia is often reported at menarche which is normally between ages 11 and 16 (Perry et al., 2009). Our study however, only included women between reproductive ages 18 and 45. The total participants mean age was 29.96 +/-7.37 as shown in (Figure 3). As menstruation is thought to be a topic of taboo in the African set-up, a lot of women affected by it would rather not seek medical help early or resort to traditional medicines before finally visiting a Health Centre at a later stage. Nearly 100 percent of the participants were of African ethnicity with only one control being mixed race. vWF levels have been found to be lower in African women than in women of Caucasian and Asian race. We did not do a racial comparative study. However, of the 168 women we included we found an overall mean vWF activity of (84.65±68.51) percent, which is within normal expected values of vWF activity. A study by Miller *et al* found a mean vWF activity of 99 percent in African women, their finding was not statistically different between African and Caucasian women in their study. The source of the race difference is not known but studies in South Africa, Britain, America and others have found similar results.

Von Willebrand Factor Activity

The diagnosis of VWD is based on abnormalities in specific tests of von Willebrand factor (vWF), including vWF antigen (vWFAg) and vWF cofactor activity (Flood et al., 2010). vWF activity is reported as a percentage of normal (Gill et al., 1987).

Our study found an association between vWF activity and menorrhagia. There was a significant difference between the mean vWF activity of the cases and controls (p=0.001). (Figure 4). vWF activity is expected to be less in women with VWD and consequently menorrhagia as it is the most common presentation. Therefore, our findings of less vWF activity in women with menorrhagia than those without was consistent with current literature and other studies. In a systematic review, Shankar *et al* reviewed studies of vWD in women with menorrhagia across the world. Nine out of these eleven studies had used vWF activity as one of the tests for diagnosis of vWD and found that 131 (13%) women out the 988 women included in the study were diagnosed as having vWD (Shankar et al., 2004). Our study found that 6 (10.71%) women out the 56 women with menorrhagia had vWD indicating significantly higher odds, OR=6.6 (Table 7) of vWD in the menorrhagia group compared to the no-menorrhagia group.

We also tested for an association between menstrual flow rates and vWF activity but the association was not significant (p = 0.196). (Figure 8). However, vWF activity levels reduced from light menstrual flow to very heavy menstrual flow. This observation though not significant explained why women with menorrhagia had more pathologic values of vWF activity just as other studies have suggested low vWF activity in menorrhagia patients and disorders of prolonged bleeding.

Activated Partial Thromboplastin Time

aPTT is a laboratory based test for haemostasis. It is prolonged in vWD and is used as one of a panel of tests for diagnosis of vWD. Normal ranges are 30 to 40 seconds (Hoffbrand et al., 2005).

We did not find a significant difference between the mean value of aPTT in women with menorrhagia and in those without, (p = 0.592) Figure 4. Our findings were in contrast to a study by Mohamedahmed *et al* who reported a significant difference in aPTT between cases and controls (Mohamedahmed et al., 2016). However, our study showed a decrease in aPTT across self-reported menstrual flows by our study participants from heavy, very heavy, medium and finally light flow had the lowest levels of aPTT which fall in the acceptable range (Figure 8).

The insignificant findings in differences between cases and controls aPTT in this study could have been due to our limited sample size. However, as some previous studies have reported a significant difference, it would suggest that a prolonged aPTT could be used as a marker for a coagulopathy.

Blood Groups and Von Willebrand Factor Activity

A number of studies have confirmed that plasma levels of vWF Antigen and activity are significantly affected by ABO blood group. Blood group O candidates have lower levels of vWF antigen (vWF ag) and activity therefore, pathological values for this group are lower than non-blood group O candidates (O'Donnell et al., 1987). Galliriono *et al* explained that this difference across blood groups could be due to the shorter vWF survival or elimination half-life in plasma compared to non-blood group O candidates vWF (Gallinaro et al., 2008). It is suggested that this difference across the ABO blood group is due to the presence of blood group antigens on vWF itself, which may affect its interaction with platelet receptors or its clearance from the circulation (Matsui et al., 1993). Our study however, was in variance to this hypothesis as we did not find a significant difference between blood group O women and non-blood group O women in terms of vWF activity. ($p = 0.467$). (Figure 5). A much larger sample size may have been required as observed in other studies like Gill et al who had 1,117 participants in their study of the effect of ABO blood groups on the diagnosis of vWD which showed that Blood Group O individuals had the lowest amounts of vWF ag at 74.8 U/dL (Gill et al., 1987). A hypothesis could be tested whether higher amounts of VWF activity was common in Africans.

von Willebrand Disease

The mean age of women affected with vWD in our study was 28 and 4(50%) of them were blood group O Rhesus positive while the other four (50%) were Blood Group A rhesus positive. Interestingly our findings were very similar to Dilley *et al* who also found that the women with vWD were of Blood Group O and A in almost equal proportions (Dilley et al., 2001). Out of the six cases, two had a family history of

abnormal bleeding while one had suffered previous episodes of bleeding through the nose, easy bruising and prolonged bleeding.

We diagnosed vWD based on abnormal vWF activity and an abnormal aPTT. This principle was also used by Dilley *et al* in their study whose study subjects were classified as having von Willebrand disease if two or more tests of von Willebrand Factor tests were abnormal based upon the control range. Our study found that two controls out of one hundred and twelve (1.78%) had vWD and six out of fifty-six cases (10.71%) had vWD (Figure 7). We found an Odds ratio of 6.6, women with menorrhagia had higher odds of having vWD than women without menorrhagia. Our findings tended to support the observation that menorrhagia was an important indicator for bleeding disorders in women. Our findings are similar to a number of studies that had found a higher prevalence and odds ratio of vWD in women with menorrhagia compared to women without. Dilley *et al* found a prevalence of 6.6 percent in women with menorrhagia and 0.8 percent in women without menorrhagia and an Odds Ratio of 8.6 (Dilley at al., 2001). In addition, they reported that 10.7% women with menorrhagia had inherited bleeding disorders with 61.5% of these patients having vWD. A study by Halimeh *et al* found that the number of women with a coagulation disorder was noticeably higher in the Heavy Menstrual Bleeding (HMB) group (151/199, 76%) than the control group (4/106, 4%). Among the 151 HMB women with bleeding disorders, the most common diagnosis was vWD in 118 (78%) (Halimeh et al., 2016). Payandeh *et al* found a prevalence of 55 percent of vWD in women with menorrhagia. Their study did not include a control group for comparison but we can still see the strong association between vWD and menorrhagia (Payandeh et al., 2013). Studies in Egypt and Sudan also indicated that women with HMB had higher prevalences of vWD at 6.5 percent and 32 percent respectively (Mohamed., 2015; Sherif et al., 2014). Finally, a systematic study by Shankar et al concluded that VWD in women with menorrhagia is increased and VWD is a significant cause of menorrhagia in women (Shankar et al., 2004).

Clinical comparisons between Menorrhagia and no Menorrhagia group

We found a statistically significant difference between the cases and controls history of epistaxis ($p<0.001$). Figure 9. Our findings suggest that women with menorrhagia were more likely to experience epistaxis compared to women who did not suffer from menorrhagia. Similar to our study, Payandeh *et al* found a significant association between a history of muco-cutaneous bleeding and menorrhagia, nose bleeding was also found to be statistically higher in women who were further diagnosed with vWD (Payandeh et al., 2013). These findings highlighted the need to assess patients with bleeding abnormalities such as menorrhagia for coagulopathies. Our results further suggested that for a Physician, taking a good patient history could assist in the detecting the presence of bleeding disorders in patients. This would also prove cost effective for a low income country like Zambia as only selected patients would be considered for coagulopathy testing. vWD is associated with muco-cutaneous bleeding and low levels of vWF activity which could be an indicator for the vWD could therefore also result in muco-cutaneous bleeding (Mehta et al., 2005). Therefore, recurrent Epistaxis could be used as a predictor for coagulation disorders in patients with menorrhagia.

As vWD is genetically inherited, it is expected that people with vWD should have a family history of abnormal bleeding (Mehta et al., 2005). We found a significant association between participant's family history of menorrhagia and participant menorrhagia, (Figure 10) but no significant association between family history of menorrhagia and an abnormal vWF activity, (Figure 11). Of a total 168 recruited women 154 (92 percent) had no family history of menorrhagia while 14 (8 percent) did. Similar results were found by Dilley et al who found no significant association between a family history of abnormal bleeding or a disorder and vWD. They also found no significant difference between cases and controls family history of bleeding. (Dilley et al., 2001). Payandeh, *et al* found an association as all 3 of the women with menorrhagia that had family history of abnormal bleeding also had a haemostatic disorder (Payandeh et al., 2013). Payandeh *et al* however, did not include a control group so their findings were on women with menorrhagia only. Mohamed *et al* in Sudan also found that a family

history of bleeding tendencies was not a predictive finding for abnormal coagulation tests and menorrhagia (Mohamed., 2015).

5.1 Conclusion

Our study showed that vWF activity levels were associated with menorrhagia while aPTT was not associated with menorrhagia. Further, vWF activity levels did not depend on the presence of a specific Blood Group. Our study also showed that the prevalence of vWD was significantly higher in participants with menorrhagia. Further that repeated epistaxis and a positive family history of menorrhagia pointed to a higher risk of menorrhagia.

5.2 Implications and Recommendations

The results of this study highlight the need for haemostatic evaluation in women with excessive menstrual bleeding without obvious pelvic abnormalities like fibroids or contraceptive methods that cause excessive menstrual bleeding. Further studies on bleeding abnormalities and their prevalence in Zambia could prompt funding in such areas as well in drug research such as Transenamic acid which has been recently found to be useful in Post-partum Haemorrhage.

It is recommended that future studies are undertaken that have a larger sample size and include multiple health centers in various provinces of the country and evaluate more variables (as there is inadequate data in the literature on this topic in Zambia).

5.3 Limitations/Weaknesses and Assumptions

- A lot of women were apprehensive when approached with the topic of menstruation due to taboos on such topics in Zambia and Africa as a whole. This resulted in a much longer time taken recruiting participants.
- Despite having collected phone numbers of participants for communication, it was again a challenge to have a discussion due to reason listed above, hence it was impossible to collect full information on morbidity and mortality data of participants.
- A full panel of tests for von Willebrand Disease would have enhanced our study had funding been sufficient. These tests include Bleeding Time, Platelet count, vWF ag, Factor VIII as well Genotyping and would have enabled us to diagnose vWD types based qualitative and quantitative abnormalities (Table 1).

5.4 Future Direction of similar studies

Follow up studies would be important to establish a full picture of bleeding disorders in both men and women in Zambia as a whole.

REFERENCES

Abouzhar, C. (2003). Global burden of maternal mortality. British Medical Bulletin: 67:1-11.

Anjum, N., Shaheen, Z., Altaf, C., Shaheen, K., & Bashir, K. (2016). Frequency of von Willebrand disease in patients of heavy menstrual bleeding. *Age (In Years), 32*, 8-505.

Anurag Gupta, Joel B. Epstein, Robert J. Cabay. Bleeding Disorders of importance in Dental Care and Related patient management. www.cda-adc.ca/jcda/vol-73/issue-1/77.html. (30 Jan, 2015).

Bleeding disorders in women: consensus on diagnosis and management from an international expert panel. Am J Obstet Gynecol (2009); 201:12. e1- 8.

Charan, Jaykaran, and Tamoghna Biswas. "How to Calculate Sample Size for Different Study Designs in Medical Research?" Indian Journal of Psychological Medicine 35.2 (2013): 121–126. PMC. Web. (22 Feb, 2016).

Ciesla B, (2007). Haematology in Practice, F.A Davis and Company, Maryland, USA, 16; 249 – 250.

Colonia VJ, Roisenberg I. (1979). Investigation of associations between ABO blood groups and coagulation, fibrinolysis, total lipids, cholesterol, and triglycerides. Hum Genet, 48: 221 - 230.

Conlan MG, Folsom AR, Finch A, Davis CE, Sorlie P, Marcucci G, Wu KK. (1993) Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study.Thromb Haemostasis, 70: 380 - 385.

Connie HM, Dilley A, Richardson L, Hooper WC, and Evatt BL. (2001). Population Differences in von Willebrand Factor Levels Affect the Diagnosis of von Willebrand Disease in African-American Women American Journal of Hematology, 67:125 – 129.

Dell RB, Holleran S and Ramakrishrank. (2002) Sample size determination, the institute for Laboratory Animal Research Journal 43: 207 - 213.

Dilley A, Drews C, Miller C, Lally C, Austin H, Ramaswamy D, et al. (2001) The von Willebrand disease and other inherited bleeding disorders in women with diagnosed menorrhagia. Obstet Gynaecol, 97:630-6.

Edlund, M., Blombäck, M., Von Schoultz, B. O., & Andersson, O. (1996). On the value of menorrhagia as a predictor for coagulation disorders. American journal of hematology, 53: 234-238.

Federici, A. B., Bucciarelli, P., Castaman, G., Baronciani, L., Canciani, M. T., Mazzucconi, M. G. & Iorio, A. (2011, July). Management of inherited von Willebrand disease in Italy: results from the retrospective study on 1234 patients. In Seminars in thrombosis and hemostasis; 37: 511-521.

Frederici AB, Manucci PM. (2002). Advances in the genetics and treatment of von Willebrand disease. Curr Opin Pediatr; 14:23 – 33.

Flood, V. H., Gill, J. C., Morateck, P. A., Christopherson, P. A., Friedman, K. D., Haberichter, S. L., ... & Hoots, W. K. (2010). Common VWF exon 28 polymorphisms in African Americans affecting the VWF activity assay by ristocetin cofactor. Blood, 116(2), 280-286.

Fricke, W., Kouides, P., Kessler, C., Schmaier, A. H., Krijanovski, Y., Jagadeesan, K., & Joist, J. (2004). A multicenter clinical evaluation of the Clot Signature Analyzer. Journal of Thrombosis and Haemostasis, 2(5), 763-768.

Gallinaro, L., Cattini, M. G., Sztukowska, M., Padrini, R., Sartorello, F., Pontara, E., ... & Casonato, A. (2008). A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. Blood, 111(7), 3540-3545.

Gill, J. Cox, Janet Endres-Brooks, Patricia J. Bauer, WJ Jr Marks, & Montgomery, R. R. (1987). The effect of ABO Blood group on the diagnosis of Von Willebrand Disease. Blood, 69(6), 1691-1695.

Gomperts ED, Fatti LP, van der Walt JD, Feesey M, Hartman E, Snell RJ. (1976). Factor VIII and factor VIII related antigen in normal South African blacks and a black carrier group. Thrombosis Research 9(3), 293 - 299.

Greer IA, Lowe GD, Walker JJ, Forbes CD. (1991). Haemorrhagic problems in obstetrics and gynaecology in patients with congenital coagulopathies. Br J ObstsetGynaecol, 98: 909 -918.

Gupta M, Bhattacharyya M, Choudhry VP, Saxena R. (2005). Spectrum of inherited bleeding disorders in Indians. Clin Appl Thromb Hemost. 11(3):325-330.

Halimeh, S., Rott, H. and Kappert, G. (2016), PBAC score: an easy-to-use tool to predict coagulation disorders in women with idiopathic heavy menstrual bleeding. Haemophilia, 22: e217–e220. doi:10.1111/hae.12886

Hoffbrand A. V, Moss P. A. H, Petit J.E, (2006) Essential Haematology, Blackwell Publishing Ltd, Massachusettes, USA: 278.

Hoffbrand V A, Pettit J E, (2000) Colour Atlas of Clinical Hematology, third edition, Mosby, Spain: 272.

James, A. H., & Jamison, M. G. (2007). Bleeding events and other complications during pregnancy and childbirth in women with von Willebrand disease. Journal of Thrombosis and Haemostasis, 5(6), 1165-1169.

James, A. H., Kouides, P. A., Abdul-Kadir, R., Edlund, M., Federici, A. B., Halimeh, S., ... & Peyvandi, F. (2009). Von Willebrand disease and other bleeding disorders in women: consensus on diagnosis and management from an international expert panel. American journal of obstetrics and gynecology, 201(1), 12-e1.

James, A. H. (2010). Women and bleeding disorders. Haemophilia, 16(s5), 160-167.

Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. (1999). Variations in coagulation factors in women: effects of age, ethnicity, menstrual cycle and combined oral contraceptives. ThrombHaemostasis 82:1456 - 1461.

Kadir RA, Economides DL, Sabin CA, Pollard D, Lee CA (1999). Assessment of menstrual blood loss and gynecological problems in patients with inherited disorders. Hemophilia; 40-48.

Kadir RA, Kingman CE, Chi C, Lee CA, Economides DL. (2007) Is primary postpartum hemorrhage a good predictor of inherited bleeding disorders? Haemophilia; 13:178-181.

Kalikiki Abel, (2013). Maternal and Child Health Outcomes, Ministry of Health.

Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-15 Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19

Kouides, P. A., Phatak, P. D., Burkart, P., Braggins, C., Cox, C., Bernstein, Z., ... & Howard, F. (2000). Gynaecological and obstetrical morbidity in women with type I von Willebrand disease: results of a patient survey. Haemophilia, 6(6), 643-648.

Kumar V, Cotran R S, Robbins S L, (2007) Basic Pathology, eighth edition, W.B. Saunders Company, Philadelphia, USA 65 - 66.

Mackie I, Bull H, Kennedy J, Brozovic M. (1982) Ristocetin-induced platelet agglutination in Afro-Caribbean and Caucasian people. Br J Haematol 50:171-173.

Mannucci PM, Duga S, Peyvandi F. (2004). Recessively inherited coagulation disorders. Blood. 104:1243–1252.

Matsui T, Fujimura Y, Nishida S, Titani K. (1993) Human plasma a2-macroglobulin and von Willebrand factor possess covalently linked ABO (H) blood group antigens in subjects with corresponding ABO phenotype. Blood; 82:663–668.

Mikhail, S., Varadarajan, R., & Kouides, P. (2007). The prevalence of disorders of haemostasis in adolescents with menorrhagia referred to a haemophilia treatment centre. *Haemophilia*, *13*(5), 627-632.

Miller CH, Haff E, Platt SJ, Rawlins P, Drews CD, Dilley AB, Evatt B. (2003). Measurement of von Willebrands Factor activity: relative effects of ABO blood type and race. J TThrombHaemost. 1: 2191 - 2197.

Mohamedahmed, K. A., Sayedahmed, S. A., Abdualbagy, I. I., & Alsaeed, A. Y. Evaluation effects of menorrhagia in coagulation profile (PT, INR, PTT, and platelets count) in Sudanese females (2015).

Mohamed, L. (2015). Prevalence Bleeding Disorders among Women Presenting with Menorrhagia in Khartoum Teaching Hospital (Doctoral dissertation, UOFK).

Mosby's Medical Dictionary, 8th edition. © 2009, Elsevier.

Nichols, W. L., Hultin, M. B., James, A. H., MANCO-JOHNSON, M. J., Montgomery, R. R., Ortel, T. L., ... & Yawn, B. P. (2008). von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA) 1. Haemophilia, 14(2), 171-232.

O'donnell, J., & Laffan, M. A. (2001). The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. Transfusion Medicine, 11(4), 343-351.

Omer, A., & Ziada, M. (1973). Haemophilia and allied disorders in the Sudan. British journal of haematology, 25(1), 69-75.

Payandeh, M., Rahimi, Z., Kansestani, A. N., Hemmati, S., Aleyasin, M., Zare, M. E., ... & Hashemian, A. H. (2013). Clinical features and types of von Willebrand disease in women with menorrhagia referred to hematology clinic of kermanshah. *IJHOSCR*, *7*, 1-5.

Perry, J. R., Stolk, L., Franceschini, N., Lunetta, K. L., Zhai, G., McArdle, P. F., ... & Cherkas, L. (2009). Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. Nature genetics, 41(6), 648-650.

Philipp, C. S., Faiz, A., Dowling, N. F., Beckman, M., Owens, S., Ayers, C., & Bachmann, G. (2008). Development of a screening tool for identifying women with menorrhagia for hemostatic evaluation. American journal of obstetrics and gynecology, 198(2), 163-e1.

Ragni MV, Bontempo FA, Hassett AC. (1999). Von Willebrand disease and bleeding in women. Haemophilia. 5; 313 – 317.

Sadler JE, Budde U, Eikenboom JC, et al. (2006) Update on the patho-physiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. J Thromb Haemost. 4(10):2103-2114.

Sadler, J.E., Mannucci, P.M., Berntorp, E., Bochkov, N., Boulyjenkov, V., Ginsburg, D., Meyer, D., Peake, I., Rodeghiero, F. and Srivastava, A., (2000). Impact, Diagnosis and Treatment of von Willebrand Disease*. ThrombHaemost, 84(2), pp.160-174.

Shankar, M., Lee, C. A., Sabin, C. A., Economides, D. L., & Kadir, R. A. (2004). von Willebrand disease in women with menorrhagia: a systematic review. *BJOG: An International Journal of Obstetrics & Gynaecology*, *111*(7), 734-740.

Sherif, N., Goubran, H., Hassan, A., Burnouf, T., & El-Ekiaby, M. (2014). An approach to outreach patients with von Willebrand disease in Egypt by targeting women with heavy menstrual bleeding and/or bleeding symptoms. *Haemophilia*, *20*(2), 238-243.

Srivastava, A., & Rodeghiero, F. (2005, December). Epidemiology of von Willebrand disease in developing countries. In *Seminars in thrombosis and hemostasis* (Vol. 31, No. 05, pp. 569-576). Copyright© 2005 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.

Sukhu, K., Poovalingam, V., Mahomed, R. and Giangrande, P.L., (2003). Ethnic variation in von Willebrand factor levels can influence the diagnosis of von Willebrand disease. Clinical & Laboratory Haematology, 25(4), pp.247-249.

Tosetto, A., Rodeghiero, F., Castaman, G., Goodeve, A., Federici, A. B., Batlle, J., ... & Eikenboom, J. (2006). A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD). Journal of Thrombosis and Haemostasis, 4(4), 766-773.

Toxqui L, Granados AMP, Rojo RB, Wright I, Vaquero MP. (2014) A simple and feasible questionnaire to estimate menstrual blood loss: relationship with hematological and gynecological parameters in young women. BMC Women's Health, 14(71)

Vincentelli A, Susen S. N Engl J Med 2003; 349: 343 – 349.

Vo KT, Grooms L, Klima J, Holland-Hall C, O'Brien SH. (2012). Menstrual bleeding patterns and prevalence of bleeding disorders in a multidisciplinary adolescent hematology clinic. Haemophilia. doi: 10.1111/hae. 12012.c

WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy (2010). 15 Aug, 2015.

Women Bleed TOO: http://www.womenbleedtoo.org.uk/index.php?pub_content_id=4 21 Feb, 2015.

APPENDICES

Appendix 1 Information Sheet

Introduction

My name is Miyoba Munsanje. I am studying for a Master of Science degree in Pathology - Haematology at the University of Zambia, School of Medicine in my final year.

I am carrying out a research as a requirement for fulfillment of the Master's degree.

About the study

This study will examine two factors of the blood clotting system, namely von Willebrand Factor and factor VIII (activated Partial Prothrombin Time). Abnormalities with these factors are associated with bleeding disorders. The ability of blood to form a clot and stop bleeding is reduced. This study is concerned with women experiencing bleeding abnormalities in the form of menorrhagia to check if these women may in fact be affected by von Willebrand Disease. Women are more likely to experience problems with bleeding due to aspects of their lives which involve bleeding like menstruation and child birth.

Participating in the study

You have been invited to participate in this study because you are a woman who has come to the University Teaching Hospital Obstertrics and gynaecology clinic with mennorhagia (or for any other reason for control group).

You can decide whether you want to join this study or not. You are free to say yes or no. If you decide to take part in the study, you will be asked a few questions and also requested to give 4 mls of blood. This blood is required because it will be used to measure the substances that have been mentioned above and will only be done once and you will not need to come back for any more collections for this study. The collection

of blood will be done by a qualified person in blood collection. You are free to ask any questions you may deem personal or otherwise you are free to withdraw from the study at any time without repercusions. The study requires 56 cases(women with menorrhagia) and 112 controls(women without mennorhaggia). A total of 168 participants.

Confidentiality

Any information obtained from you will be strictly confidential and your identity will remain anonymous. Records will also be kept safe and used only for the purpose of this research.

Risks/benefits/discomforts

The problem that participants may experience in this study are mostly those related to venipuncture. The following are some of the risks of venipuncture:

- Occasionally individuals may bleed longer than normal after a venipuncture.
- Some patients may develop a haematoma (an accumulation of blood) under the skin.
- More often a bruise will be noted
- A risk of infection is present whenever the skin is broken. It is a rare complication of venipuncture and more likely when fluids are given than during a blood draw.
- A common complication of venipuncture is pain. This is usually mild but occasionally the needle will strike a nerve causing severe pain.

All study related injuries will be treated and every effort will be made to reduce the pain that you feel as the blood is collected. In addition all your personal medical records will be kept confidential.

You have the right at any point during this process to withdraw if you feel uncomfortable or seek clarification should you be unsure of anything.

<u>FOR FURTHER INFORMATION, CONTACT:</u>

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Appendix 2 Information Sheet (Vernacular)

PEPALA LA NKANI

Chiyambi

Dzina langa ndine Miyoba Munsanje. Ndi chita ma phunziro akuya a Master of Science degree mu Pathology-Haematology pa sukululalikulu la University of Zambia. Ndilimu chaka cho siliza maphunziro.

Phunziro yomwe ndiku chitandi yo funnikira kuti ndisiridze maphunziro yanga ya Master's degree.

ZamphunziroIye

Mphunzilori idza yanganapo adzinthu dziwirizo funkira pa kuumamaga zi mthupi). Madzinaochewa von Willebrandndi Factor VIII Prothrombin Time). Zo nyantsa za mabvuto yomwe yalikupedzeka pa kulimba pa gazi kumachoka. Luntha lamagazi khumandi kuleka li khudza azimai amene amakale ndi bvuto sa kuchoka magazi mwina angakhale ndi nthenda la von Willebrand. Azimai ambiri amapezeka ndi vuto ili chifukwa cha mo mwealila pa umoyo wao pape dzake ama pita kumwezi ndi kubweka ana.

OtengapoMbaliMuphunzilo

Inu ndi nuo yitanidwa kutengapo mbali mu mphunziro ili chifukwa cha kuti ndi nuo dwala ndi punso mwabwela kuno ku chipatala cha chikula cha University Teaching Hospital. Mwa bwela kuno ku chipatala chifukwa cha bvuto yaku chota magazindi zina zake.

Ndinu ololedwa kutengapo mbali ngati mu funamwinaso ngati simufuna ndinuoma sukaku sa tengapo mbali. Ngatimudza fune kutenga po mbali mudza fundiswa ma funso ndipo nso muza yenekela ku patsa magasi ag'onochabe(4mls). Magaziyo mwemu dzapatsa ndi yufunika kuti awone zomwe zili kulankulidwapo pa phunziroili. Mudziwe kuti mu katuptsa kamodzi magazi ndipo si mu dzafunika kupatsa ngogazi pa phunziloili. Katengedwe ka gazi kwainu kadza citikandi aka swili kuno ku chipatala. Mufunika kudzi waku tindi nuo masuka ku funsa mafunso aku mtimandiposo ndi nuo loledwaku leka kutenga kombali nthawi iliyonse kopanda bvuto lililonse. Phunziloli yifunika anthu ali makhumi as anu ndilimodzi (60) omweanga khale ndimutenga awandi poene makhumi asanu ndi limodzi alibe mabvuto awa. Bantu onsepa modzi adzakhala dzana ndi mukumi awili.

Zachisinsi

Nkani iliyonse yomwe mu dza kambapo idza sungidwa muchisinsi ndiponso dzinalanuli dzasungi dwanso muchisinsi. Zonse idzizi dza sungidwa bwino kute lo kuti nkani idzi ndizo funikila pa phunziroili chabe.

Chiopsedza/ChopindulaZosowetasMtendere

Mabvuto omwe anthu anaga pedzeke nalyo ndi banthu alindivuto la venipunture zomwe zili pansi pa ndi ziopsezo.

- Odwala kumatenda awa angapedzeke ndi magazi ochuli ka a mbili pansi pa nkanda.
- Pa nthawi za mbili kumulidwa kuma oneka.
- Ngati nkanda ya n'gambi ka chiopse dzochi matendaena yakhodza kulowa mthupi iyi bvuto ndiyo sowa mabvuto la venipuncture ndiponso ngatimaga zimwine zochoka mthupi.
- Zofunika kudziwa ndikuti matenda ya venipuncture ndikuwawa osati kupitilila kama nsingano italasa mthupi ikodza kuwawitsa zambili.
- Ngati kudza pezeka ngozi ili yonse kuli aliyense omwe atengapo mbali muphunzilo ili adza patsidwa mankwala ndiponso mapepala onse po lembedwa za omuyo wa nuadza sungidwapa bwino kwambili kotelo kuti kulibe adzaya gwilitsa nchito lili lonse ndinu omasuka kuleka kutengapo mbali panthawi ili yonse.

<u>NGATHI MULI NDIFUNSO ILIYONSE PEDZANI NTHWI KUONA MWINE KUTUMWA LAMYA KU ANTHU AWA.</u>

The Chairperson

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Appendix 3 Informed Consent Form

TITLE OF PROJECT**: von WILLEBRAND DISEASE IN WOMEN WITH MENORRHAGIA AT THE GYNAECOLOGY CLINICS OF UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.**

NAME OF RESEARCHER: Miyoba Melinda Munsanje

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1. I confirm that I understand the Plain Language Statement for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.

3. I agree / do not agree (delete as applicable) to take part in the above study and give an amount of blood (4 mls).

Statement by Researcher of person taking consent:

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the research procedure. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

NOTE: The participant will be provided with a signed and dated copy of this consent form. It will help him/her remember what we discussed today.

Participant Signature	*Date*	*Participant Thumb Print*
Witness	*Date*	*Signature*
Researcher	*Date*	*Signature*

Appendix 4 (Consent Form Vernacular)

MUTHU WANKHANI: KUFUNA KUDZIWA NTENDA LA von WILLEBRAND MU ADZIMAI ALI NDIVUTO LO CHOKA MAGAZI OBWELA KUCHIPATALA CHA UTH

NDIZA LA OCHITA PHUNZILO ILI: Miyoba Melinda Munsanje

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1. Nditsimiidza ndi kumvetesa zo funikela pa phunziro nili kuyanganapo ndipo ndinali ndimapata ofunsa mafunso.

2. Ndivettsetsa kuti kutengapo mbalipaphunziro ili ndi zofuna zanga ndipo ndine omasukakuleka pa nthawi lili yonse.

3. Ndizomeredza po tengapo mbali pa phunziro ili ndiponso ndizapatsa magazia n'gonochabe.

KULONGOSULA KWA NKHANI IYI KU ANTHU OTENGAPO MBALI NDIPU LOLA

Ndawerenga bwino zonse zankhani ili iye kuonseo otenga po mbali ndiponso ndiindikiza kutianthu a kudziwa zofunika zomeredza kuti anthu anthuwa adzapatsidwa pa nkaniyi. Atafunsaaz ayankedwa mukwana ndizomeredzanso kuti kulibe omwe adza patikizidwa kutengapo mbali.

CHIZIWITSO: Aliyense otengapo mbali adzapatsidwa pepala lo lembedwa.

Otengapombali	*Tsiku*	*Kufwatika*
Odzindikira	*Tsiku*	*Signacha*
Mwinewaphunziro	*Tsiku*	*Signacha*

Appendix 5 Questionnaire

THE UNIVERSITY OF ZAMBIA

PATHOLOGY AND MICROBIOLOGY DEPARTMENT

PROJECT TITLE: von WILLEBRAND DISEASE IN WOMEN WITH MENORRHAGIA AT THE GYNAECOLOGY CLINICS OF UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

NOTE: You are free not to answer questions you may deem personal or otherwise and you are also free to withdraw from study at any time without any repercussions

AGE: …….RESIDENTIAL AREA……

ETHNICITY: African: Caucasian: Asian: Mixed Race:

MEDICAL HISTORY AND GENERAL HEALTH

1.1 Have you ever had spontaneous nose bleeding?

0. No
1. Yes

1.2 If yes, did you require Medical attention?

0. No
1. Yes
2. Not Applicable

1.3 Please specify:

0. Consultation only
1. Treatment with medicine e.g. Iron Therapy
2. Blood Transfusion
3. Not Applicable

1.4 Approximately how many episodes have you experienced

0. One in two years
1. One per year
2. One to five every six month
3. One to three every month
4. One every week
5. Not Applicable

2.0 Do you experience prolonged bleeding on wounds or easy bruising?

0. No
1. Yes

2.1 Have you required medical attention for this?

0. No
1. Yes
2. Not applicable

3.0 How many days does your menstruation/ period last?

0. One to three days or less
1. Three to seven days
2. Eight to ten days
3. Ten days or more

3.1 How would you describe your flow of period?

0. Light
1. Medium
2. Heavy
3. Very heavy

3.2 Are you on any form of medical or traditional contraceptive medicine?

0. No
1. Yes

3.3 Do you know of any woman in your immediate family that experience very heavy, prolonged and painful periods?

0. No
1. Yes

3.4 Do you know of any close family members (men and women) that experience problems with any other kind of bleeding?

0. No
1. Yes

Appendix 6 Cases and Controls Raw data

id	age	b_group	aptt	vwfac	nose_bleed	medatte	attention	noepisodes	otherbleed	medatte2	mensdays	flow	contra	fam_hist	fam_hist2
controls	26	O Rhesus positive	29.3	42.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	38	O Rhesus positive	20	54.9	Yes	Not Applicable	Not applicable	1-5/six months	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	45	B Rhesus positive	21.3	71.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	30	B Rhesus positive	34.7	65.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	27	A Rhesus positive	39.6	62.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	42	AB Rhesus positive	31.5	40	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	30	A Rhesus positive	30	40	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	36	B Rhesus positive	29.5	51.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	31	O Rhesus positive	26.2	22	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	20	AB Rhesus positive	26.7	62.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	No	No	No
controls	23	O Rhesus positive	24.7	69.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	Yes	No
controls	27	O Rhesus positive	25.9	89.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	Yes	No	No
controls	21	A Rhesus positive	39.6	40	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	25	B Rhesus positive	24.7	96.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	28	A Rhesus positive	32.3	77.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Light	No	No	No
controls	26	O Rhesus positive	24.5	59	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	23	A Rhesus positive	49.7	78	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	No	No	No
controls	19	A Rhesus positive	32	44.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	37	O Rhesus positive	35.8	90.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	No	No	No
controls	39	B Rhesus positive	27.4	64.3	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	41	B Rhesus positive	28.7	40	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	Yes	No
controls	23	AB Rhesus positive	27.7	37.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	41	O Rhesus positive	24.4	35.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	23	O Rhesus positive	24.6	103	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Light	No	No	No
controls	34	O Rhesus positive	25.1	23.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Light	Yes	No	No
controls	26	A Rhesus positive	26.5	50.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	Yes	No	No
controls	45	A Rhesus positive	27.6	106.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	No	No	Yes
controls	42	B Rhesus positive	29	126.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	19	O Rhesus positive	32.5	180	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	22	O Rhesus positive	28.4	85	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	23	AB Rhesus positive	30.9	75.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	39	B Rhesus positive	23	55.3	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Light	Yes	No	No
controls	26	O Rhesus positive	31.9	80.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	35	A Rhesus positive	29.7	76.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	Yes	No	Yes
controls	28	A Rhesus positive	23.4	71.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	34	O Rhesus positive	20.6	137.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	35	A Rhesus positive	31.1	79.3	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	36	O Rhesus positive	24.1	123.3	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Light	Yes	No	No
controls	45	B Rhesus positive	19.5	68.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	42	A Rhesus positive	36.3	123.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	34	O Rhesus positive	27.5	161.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	No	No	No
controls	36	O Rhesus positive	31	253.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No

controls	54	B Rhesus positive	54.6	126.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Light	Yes	No	No
controls	27	B Rhesus positive	24.5	139	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Light	Yes	No	No
controls	26	A Rhesus positive	26.8	42	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	20	O Rhesus positive	26.4	55.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	28	B Rhesus positive	29.8	65	Yes	Not Applicable	Not applicable	one per year	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	31	O Rhesus positive	25.9	69.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	30	O Rhesus positive	27.9	69.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	58	B Rhesus positive	24.8	70.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	21	B Rhesus positive	37.8	74.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	21	O Rhesus positive	27.9	152.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-5days/less	Medium	No	No	No
controls	30	O Rhesus positive	25.5	75.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	42	O Rhesus positive	28	69.3	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Light	No	No	No
controls	56	O Rhesus positive	59.4	97.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	39	A Rhesus positive	59.5	140	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-5days/less	Medium	Yes	No	No
controls	37	O Rhesus positive	61.1	84.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	29	B Rhesus positive	37.2	112.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	40	A Rhesus positive	37.9	91.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	32	A Rhesus positive	25.9	167.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Light	Yes	No	Yes
controls	29	A Rhesus positive	19.8	125.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	33	O Rhesus positive	31.5	50	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-5days/less	Medium	Yes	No	No
controls	36	O Rhesus positive	24.7	355.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	31	A Rhesus positive	26.7	39.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	22	O Rhesus positive	26.5	206.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	25	O Rhesus positive	55	165.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	45	B Rhesus positive	45.9	155.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	Yes	No
controls	18	A Rhesus positive	47.5	160	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-5days/less	Medium	Yes	No	No
controls	27	A Rhesus positive	25.5	102.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-5days/less	Medium	Yes	No	No
controls	29	A Rhesus positive	27.6	150.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	24	O Rhesus positive	33.7	52.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	41	O Rhesus positive	34.4	169.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Heavy	Yes	No	No
controls	41	A Rhesus positive	26.5	155.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	50	A Rhesus positive	45.2	46	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-5days/less	Medium	Yes	No	No
controls	25	O Rhesus positive	55	155.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	26	O Rhesus positive	26.6	129.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	18	O Rhesus positive	42.5	42.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	Yes
controls	59	A Rhesus positive	50.9	50.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	33	O Rhesus positive	25.1	145.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	32	O Rhesus positive	29.7	66.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-5days/less	Medium	Yes	No	No
controls	45	O Rhesus positive	21.5	245.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	25	B Rhesus positive	56.9	76.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	Yes	No	No
controls	26	A Rhesus positive	35.7	125.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	21	A Rhesus positive	28.6	76.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	5-7days	Medium	No	No	No
controls	30	O Rhesus positive	23.4	70.2	Yes	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-5days/less	Medium	Yes	No	No

controls	24	A Rhesus positive	31.4	239.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	18	B Rhesus positive	37.1	132.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Light	No	No	No
controls	22	O Rhesus positive	31.1	131.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	18	O Rhesus positive	32.5	57.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	24	O Rhesus positive	25.4	45.6	Yes	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	No	No	No
controls	33	A Rhesus positive	18.3	247.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	44	O Rhesus positive	23.4	123.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	43	O Rhesus positive	40.8	58.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	22	A Rhesus positive	23.1	80.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	27	A Rhesus positive	22.5	91.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	29	O Rhesus positive	25.6	282.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	Yes	No	No
controls	31	B Rhesus positive	28.2	187.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	23	A Rhesus positive	18.7	240.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	27	O Rhesus positive	23.1	112.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	No	Yes	No
controls	24	O Rhesus positive	29.7	168.3	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Light	No	No	No
controls	29	B Rhesus positive	31.9	94.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	32	O Rhesus positive	32.6	81.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Light	Yes	No	No
controls	43	O Rhesus positive	38.2	55.1	Yes	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	26	AB Rhesus positive	26.1	140.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	25	O Rhesus positive	27.2	86	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	27	O Rhesus positive	24.6	126	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	1-3days/ less	Medium	Yes	No	No
controls	29	O Rhesus positive	32.1	42	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
controls	21	A Rhesus positive	30.9	466	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	19	B Rhesus positive	28.7	73.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	19	A Rhesus positive	21.1	91.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	20	B Rhesus positive	31	60.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	No	No	No
controls	30	O Rhesus positive	33.3	70.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Medium	Yes	No	No
cases	30	A Rhesus positive	35.3	73.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Very heavy	Yes	Yes	Yes
cases	23	AB Rhesus positive	24.8	45.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	Yes	No	No
cases	30	A Rhesus positive	23.9	36.5	Yes	No	Not applicable	1-5/six months	No	Not Applicable	8-10days	Heavy	No	No	No
cases	45	O Rhesus positive	26.1	47.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	Yes	No
cases	20	A Rhesus positive	25.6	62.8	No	Not Applicable	Not applicable	Not Applicable	Yes	Yes	8-10days	Very heavy	Yes	No	No
cases	28	AB Rhesus positive	28.7	68.8	Yes	No	Not applicable	one per year	No	Not Applicable	10days/ more	Heavy	No	No	No
cases	38	O Rhesus positive	27.6	45.9	Yes	No	Not applicable	Not Applicable	Yes	Yes	8-10days	Very heavy	Yes	No	No
cases	30	A Rhesus positive	28	55.7	No	Not Applicable	Not applicable	Not Applicable	No	Yes	8-10days	Heavy	Yes	Yes	No
cases	20	O Rhesus positive	28.3	79.9	No	Not Applicable	Not applicable	Not Applicable	No	No	8-10days	Very heavy	Yes	No	No
cases	34	B Rhesus positive	49.9	88.9	No	Not Applicable	Not applicable	Not Applicable	No	No	8-10days	Very heavy	No	No	No
cases	18	B Rhesus positive	20.3	185.6	No	Not Applicable	Not applicable	Not Applicable	Yes	No	10days/ more	Very heavy	No	No	No
cases	34	O Rhesus positive	43.4	123.1	No	Not Applicable	Not applicable	Not Applicable	Yes	Yes	10days/ more	Very heavy	No	No	No
cases	39	A Rhesus positive	21.3	96.5	Yes	No	Not applicable	one per year	No	Not Applicable	8-10days	Very heavy	Yes	No	No
cases	34	B Rhesus positive	32.3	96.3	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Very heavy	Yes	No	No
cases	40	B Rhesus positive	79	42.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Very heavy	No	No	Yes
cases	42	O Rhesus positive	33.1	30.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Heavy	No	No	No

cases	41	B Rhesus positive	39.8	33.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	Yes	No
cases	36	A Rhesus positive	40.6	112.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Heavy	No	No	No
cases	18	O Rhesus positive	30.7	73.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Very heavy	No	No	No
cases	29	O Rhesus negative	31.8	81.2	Yes	No	Not applicable	one per year	No	Not Applicable	8-10days	Very heavy	No	No	No
cases	24	O Rhesus positive	32.6	23.1	No	Not Applicable	Not applicable	Not Applicable	Yes	Not Applicable	10days/ more	Heavy	No	No	No
cases	39	A Rhesus positive	24.2	60.2	Yes	No	Not applicable	one in twoyears	No	Not Applicable	10days/ more	Very heavy	Yes	No	No
cases	24	A Rhesus positive	24.4	47.9	No	Not Applicable	Not applicable	Not Applicable	Yes	Yes	8-10days	Heavy	No	No	No
cases	28	O Rhesus positive	45.3	39.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	No	No
cases	24	A Rhesus positive	27.7	60.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Heavy	No	No	No
cases	40	B Rhesus positive	27.2	36.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	No	No
cases	24	A Rhesus positive	29.6	73	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Very heavy	No	No	No
cases	20	B Rhesus positive	30.5	33.8	No	Not Applicable	Not applicable	Not Applicable	Yes	No	8-10days	Heavy	No	No	No
cases	33	O Rhesus positive	28.3	23.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Very heavy	No	Yes	No
cases	21	B Rhesus positive	30.2	93.9	Yes	No	Not applicable	one per year	Yes	No	8-10days	Very heavy	No	No	No
cases	18	B Rhesus negative	36.3	63.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Very heavy	No	No	No
cases	29	O Rhesus negative	25.5	46.8	Yes	No	Consultation only	1-5/six months	No	Not Applicable	8-10days	Very heavy	No	Yes	Yes
cases	24	O Rhesus positive	46.4	39.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	No	No
cases	29	O Rhesus positive	30.3	27.3	Yes	No	Not applicable	1-3/ month	Yes	No	8-10days	Heavy	Yes	Yes	No
cases	30	O Rhesus positive	32.3	56	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	Yes	No	No
cases	19	O Rhesus positive	31.1	117.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Very heavy	No	No	No
cases	37	O Rhesus positive	25.9	46.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Very heavy	Yes	No	No
cases	32	O Rhesus positive	24.3	117.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Very heavy	Yes	No	No
cases	33	A Rhesus positive	44.3	105.4	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Heavy	Yes	No	No
cases	30	A Rhesus positive	22.3	117	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	Yes	No	No
cases	19	O Rhesus positive	39.9	105.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	Yes	No
cases	26	B Rhesus positive	20	71.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	No	No
cases	40	O Rhesus positive	27.1	33.9	Yes	No	Treatment with medici	one in twoyears	No	Not Applicable	8-10days	Heavy	Yes	No	No
cases	32	O Rhesus positive	32.1	28.7	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Very heavy	No	Yes	No
cases	32	O Rhesus positive	29.5	83.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	3-7days	Heavy	No	No	No
cases	29	O Rhesus positive	74	66.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	No	No
cases	33	A Rhesus positive	26.7	85.8	Yes	No	Treatment with medici	1-5/six months	No	Not Applicable	8-10days	Heavy	Yes	No	No
cases	31	O Rhesus positive	22.2	70.6	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Very heavy	Yes	No	No
cases	32	O Rhesus positive	25.2	44.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Very heavy	No	No	No
cases	36	A Rhesus positive	23.1	48	Yes	No	Consultation only	one per year	Yes	No	8-10days	Very heavy	Yes	No	No
cases	29	B Rhesus positive	33.4	70.8	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	Yes	No	No
cases	27	A Rhesus positive	102.5	38.2	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	No	No
cases	29	A Rhesus positive	50.8	67.6	Yes	No	Treatment with medici	one per year	No	Not Applicable	10days/ more	Heavy	No	Yes	No
cases	31	O Rhesus positive	25.9	74.1	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	No	No
cases	30	AB Rhesus positive	60.7	81.9	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	10days/ more	Very heavy	No	No	No
cases	27	O Rhesus positive	43	45.5	No	Not Applicable	Not applicable	Not Applicable	No	Not Applicable	8-10days	Heavy	No	No	No

Appendix 7 Transformation of Raw data to achieve normality.

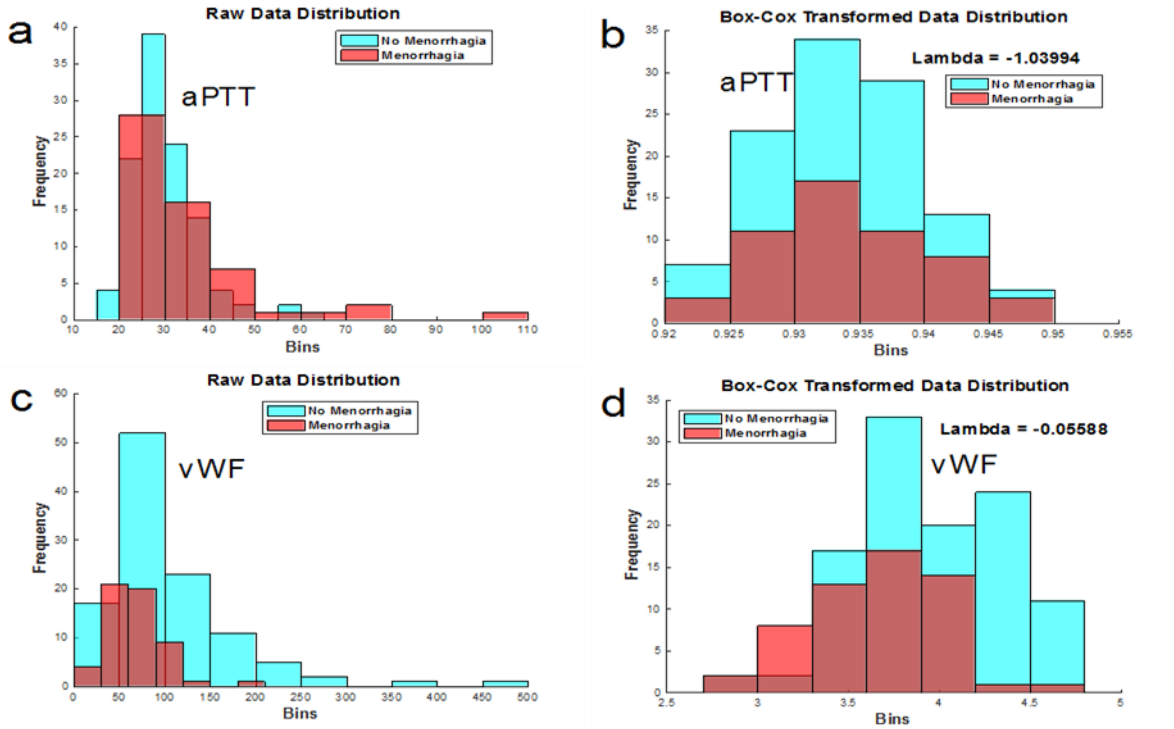

Figure 12: Raw data distribution of vWF activity and aPTT and the distribution of Box-Cox transformed data and Turkey's outlier removal method to achieve normality of distribution of vWF activity and aPTT.