

**SJMLS - 9(2) - 009****Von Willebrand Factor Among Pregnant Women Attending Maryam Abacha Women and Children Hospital Sokoto**

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Author for Correspondence: [uchemls2013@gmail.com](mailto:uchemls2013@gmail.com)/+234-803-504-1001/.<https://dx.doi.org/10.4314/sokjmls.v9i2.9>**Abstract**

Von Willebrand factor is an important protein that plays a crucial role in haemostasis and thrombosis. Changes in von Willebrand factor levels during pregnancy may increase the risk of bleeding or thrombotic complications for both the mother and the foetus. The aim of this study was to assess von Willebrand factor (vWF) levels among pregnant women in Maryam Abacha Women and Children Hospital Sokoto, Nigeria. This was a cross-sectional study that involved 60 pregnant and 30 non-pregnant women in Sokoto. Blood samples were collected and centrifuged to get plasma and then analysed using enzyme linked immunosorbent assay (ELISA) from Nanjing PARS Biochem (China). Data were analysed using statistical package for social sciences (SPSS) version 25. Independent sample test was used to compare vWF levels in pregnant women and non-pregnant women. The results obtained showed that vWF meanSD ( $184.4 \pm 109.8$ ) of subjects were significantly higher ( $p=0.018$ ) than that of control ( $141.8 \pm 57.6$ ). The study showed no significant difference ( $p>0.05$ ) in the vWF levels of pregnant women based on trimester, gravida and parity. Also, Age and years of marriage of pregnant women have no association with vWF. Therefore, vWF levels are affected by pregnancy and pregnant women should be screened for their vWF status to prevent bleeding disorder.

**Keywords:** von Willebrand Factor, Pregnant women, Maryam Abacha Hospital Sokoto,**Introduction**

Von Willebrand factor (VWF) is a glycoprotein that plays a crucial role in haemostasis and thrombosis. It is involved in platelet adhesion to

sub-endothelium at the site of vessel injury, platelet aggregation, and the intrinsic coagulation cascade, through factor VIII stabilization (Akpan and Essien, 2016). VWF is made within endothelial cells, which line the inside surface of blood vessels, and bone marrow cells. The factor is made of several identical subunits. To facilitate binding to various cells and proteins, these subunits are cut into smaller pieces by an enzyme called ADAMTS13 (Eladly and Miesbach, 2022). VWF helps platelets stick together and adhere to the walls of blood vessels at the site of a wound. A reduction in the amount of VWF or problems with its function slows the formation of blood clots, which causes the prolonged bleeding episodes seen in von Willebrand disease (Kujovich, 2005).

von Willebrand disease (vWD) represents the most common inherited bleeding disorder. It is caused by quantitative or qualitative reductions in plasma von Willebrand factor (vWF) (Fogarty *et al.*, 2020). There are three main types of vWD that differ according to the degree of disease severity and inheritance pattern. Types 1 and 3 are characterized by quantitative defects in the vWD protein: type 1 vWD is characterized by partial quantitative vWF deficiency, resulting in a mild to moderate bleeding phenotype, whereas type 3, the most severe form of vWD, results from near complete absence of vWF. Type 2 vWD represents a group of disease phenotypes resulting from qualitative defects in vWF, affecting formation of multimers (types 2A and 2B), platelet adhesion (type 2M), or FVIII binding (type 2N) (Du *et al.*, 2023). Significant bleeding symptoms due to reduced vWF levels have been reported in approximately 1 in 1000

individuals (Fogarty *et al.*, 2020). Based on expert opinion, globally 0.6–1.3% of the general population was affected by vWD (Du *et al.*, 2023). Population-based estimates were also conducted in Nigeria, Africa where there was 2.2% prevalence of vWD (Shonde-Adebola *et al.*, 2021). Patients with vWD typically present with mucocutaneous bleeding including epistaxis, easy bruising, gum bleeding and menorrhagia, significant bleeding can also occur after trauma, delivery, or following surgical procedures, spontaneous joint bleeding is also a feature in patients with Type 3 vWD, although most types of vWD are inherited in an autosomal manner, symptomatic bleeding is more common in females, since they experience more frequent haemostatic challenges, notably menstruation and delivery (Fogarty *et al.*, 2020).

Women with vWD are at risk from a variety of bleeding complications during pregnancy, as a result of invasive prenatal diagnostic and monitoring procedures, spontaneous or elective abortions, and the haemostatic challenge of delivery. There are no prospective studies defining the risk of bleeding in these settings; estimates are based on surveys and small case series (Kujovich, 2005). Although women and men are equally likely to be affected by vWD, women continue to be disproportionately affected by the bleeding challenges. Women with vWD suffer from gender-specific symptoms, such as menorrhagia, and are at higher risk of reproductive problems and recurrent miscarriage (Luis *et al.*, 2010). Furthermore, pregnant women with vWD are more likely at higher risk of suffering from primary and secondary periportal haemorrhage and anaemia and the need for transfusions. Despite being affected by gynecologic and obstetrical bleeding, women face multiple barriers in obtaining an accurate diagnosis. This constitutes a problem that needs to be addressed, and early appropriate medical care should be ensured (Eladly and Miesbach, 2022).

Bleeding complications during pregnancy are more frequent when levels of the von Willebrand Factor antigen level is  $<50$  IU/dL (Luis *et al.*, 2010). Previous studies have identified the influence of gestational age on vWf (Castaman, 2013). There is limited information on gravida,

parity and trimester on vWF status of pregnant women. Also, previous works have not considered the effect of environmental or cultural factors on vWF but with focus on other populations or geographical locations (Castaman and James, 2019). There is limited information on von Willebrand factor levels in this specific population. Based on data available, vWF levels have never been studied specifically on pregnant women in Sokoto, in Nigeria and in Africa as whole. Therefore, the aim of this research is to determine the effect of pregnancy on von Willebrand factor of pregnant women in Maryam Abacha Women and Children Hospital Sokoto. Therefore, assessing von Willebrand factor levels in pregnant women attending Maryam Abacha Women and Children Hospital Sokoto will help to identify pregnant women at risk of bleeding complications. Also understanding how vWF levels fluctuate during pregnancy will help in prevention and management of von Willebrand Disease in pregnant women in Sokoto. In addition to the above, the research will contribute to prevention and management of pre and postpartum haemorrhage among pregnant women both in Sokoto state and beyond.

## Materials and Methods

### Study Area

The study was carried out at Maryam Abacha Children's and Women's Hospital Sokoto, Nigeria. The hospital is a 300-bed hospital that renders medical care to people in Sokoto. Sokoto is the capital city of Sokoto state, Nigeria. The state is located between longitude  $11^{\circ}30'$ ,  $13^{\circ}50'$  East and latitude  $4^{\circ}$  to  $6^{\circ}$  North. It is bordered to the North by Niger Republic, Zamfara State to the East while Kebbi State borders most of the South and Western Parts. The state falls within the Savannah vegetation zone (Mamman, 2000).

It's made up of Hausa and Fulani majority and a minority of Zabarmawa and Tuareg and other non-indigenous settlers. The two major languages in the state are Hausa and Fulfulde (spoken among the Fulani). The main occupation of the people is grain production and animal husbandry. Majority of the indigenous people practice agriculture. Crops produced include commercial crops like millet, sorghum, beans, rice and maize. Other occupations

commonly practiced are dying, blacksmithing, weaving, carving, trading, and cobbling. Sokoto ranks second in livestock production in Nigeria. Modern Sokoto city is a major commercial centre in leather crafts and agricultural products. The occupation of city inhabitants includes trading, commerce, with a reasonable proportion of the population working in private and public sectors. The vast Fadama land of the Sokoto-Rima River system dissects the plain and provides rich alluvial soil and extensive grassland fit for a variety of crop cultivation, hence farming and livestock rearing are the principal activities in the State. Other commercial activities are cement and leather production (Onuigwe *et al.*, 2017). Based on the 2006 population census, Sokoto State had a population of 3,696,999 with a projected population of 6,293,893 in 2024 based on the population annual growth rate of 3% (NPC, 2006).

### Study Population

The study population are pregnant women attending ante-natal clinic in Maryam Abacha Women and Children Hospital Sokoto, Nigeria. Apparently healthy non-pregnant women in Usmanu Danfodiyo University Sokoto age-matched served as control.

### Subjects and Selection

#### Inclusion Criteria

1. Pregnant women attending Maryam Abacha Women's and Children's hospital Sokoto who provide informed consent.

#### Exclusion Criteria

1. Pregnant women not attending Maryam Abacha Women and Children Hospital Sokoto.
2. Men and children.
3. Pregnant women attending Maryam Abacha Women and Children Hospital Sokoto who do not provide informed consent.

### Study Design

This is a comparative cross-sectional study aimed at determining the levels of von Willebrand factor in pregnant women attending Maryam Abacha Women and Children Hospital Sokoto with apparently healthy non-pregnant women of Usmanu Danfodiyo University Sokoto. Samples obtained from subjects were tested for von Willebrand factor antigen (vWF

Ag) using the Human Von Willebrand Factor ELISA Kit.

### Sample Size Determination

The sample size was determined using the formula ( $z^2pq/d^2$ ) (Fisher *et al.*, 1998)

$n$  = minimum sample size

$z$  = standard normal deviation

$p$  = prevalence or proportion of value to be estimated from previous studies

$q$  = proportion of failure ( $1-p$ )

$d$  = precision, tolerance limit, the minimum is 0.05

$n = z^2pq/d^2$

Where,  $z = 95\%$  (1.96)

$p = 4\%$  (0.04) (Sabih and Babiker, 2023).

$q = 1 - 0.04 = 0.96$

$d = 0.05$

Therefore,  $n = (1.96)^2(0.04)(0.96)/(0.05)^2$

$n = 59$

Subjects = 60

### Ethical Consideration

Ethical approval was obtained from Maryam Abacha Women and Children Hospital Sokoto.

### Informed Consent

Participants were fully informed, and their written consent was obtained before the commencement of the research.

### Questionnaire

Questionnaires were distributed to all consenting participants to collect socio-demographic and other biodata.

### Sample Collection

Four and half milliliters (4.5mls) of whole blood was collected using needle and syringe and then transferred to 3.2% sodium citrate tube. It was then mixed gently by inverting the tube 5-6 times. The sample was centrifuged to collect plasma which was then transferred to plastic vial and then frozen at -20 degrees Celsius.

### Analytical Method

**Estimation of vWF levels using enzyme linked immunosorbent assay (ELISA) (Tietz, 2012).**

Using Human vWF ELISA KIT from Nanjing Pars Biochem Co. LTD (China) with Lot no: 202310 was used for vWF testing.

**Principle**

In this assay microtitre plates are coated with a monoclonal antibody directed against part of the vWF that binds to the platelet GpIb receptor. Plasma samples [reference, tests and standards] are diluted, and aliquots added to the wells of the microtitre plate. The plates are incubated during which the immobilized antibody binds to the vWF. After incubation the bound vWF is detected with an anti-human horseradish peroxidase [HRP] labelled antibody. The bound antibody is quantified in a colorimetric reaction by adding the HRP substrate [1,2 orthophenylenediamine dihydrochloride], and measuring the absorbance at 492 nm. The absorbance is proportional to the concentration of vWF. A calibration curve is constructed by plotting on vWF: Ag against OD on linear-linear graph paper and from which the concentration of the test samples can be derived.

**Procedure**

Method: Sandwich ELISA

1. The antibody was immobilized onto a microtiter plate, that is specific for human vWF.
2. Dilution of standard was made to make concentrations of 1500pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, and 125pg/ml.
3. Ten (10) microliters of patient plasma were added to the wells and was allowed to incubate for 30mintues at 37 degrees Celsius.
4. The plate was rinsed to remove any unbound vWF.
5. Fifty (50) microliters of the second antibody conjugated to horseradish peroxidase [HRP] was added to all the wells and was allowed to incubate for 30 minutes at 37 degrees Celsius.
6. A second wash step was performed.

7. Fifty (50) microliters of the substrate for the HRP were added [Tetramethylbenzidine - TMB] was added to each well and was allowed to incubate for 15mintues at 37 degrees Celsius to develop the colour.
8. Fifty (50) microliters of the stop solution were added to all the wells.
9. The absorbance was measured at 450 nm in a microplate reader.
10. From the reference curve the concentration of vWF:Ag in the patient plasma sample was derived.

**Statistical Analysis**

Data were collected into excel spread sheet and transferred into the data editor of Statistical Package for Social Sciences (SPSS, Version 20) software. The Obtained Data of von Willebrand factor antigen levels were compared with the values of apparently healthy pregnant women using the student T-test, ANOVA was used to compare the vWF of the study subjects based on trimester, gravida, parity, age and years of marriage of pregnant women. p value 0.05 was considered significant.

**Results**

A total of 60 subjects and 30 controls were recruited and their von Willebrand factor levels were determined.

Table 1 shows Comparison of vWF levels of Subjects and Control. The vWF levels of the subjects showed a significant increase when compared to the control subjects (p=0.018) indicating a statistically significant difference. This suggests that there is a notable difference in vWF levels between the subjects and the control group.

**Table 1 : Comparison of vWF levels of Subjects and Control**

Parameter	Subjects (mean±SD) n= 60	Control (mean±SD) n= 30	T-value	P-value
vWF (pg/ml)	184.4 ± 109.8	141.8 ± 57.6	2.411	0.018*

(\*) means p-value is significant (p0.05). Key: vWF= von Willebrand Factor, pg/ml= picogram per milliliters, SD= Standard Deviation, n= number of test subjects/ number of control.

Table 2 shows Comparison of vWF of the Subjects based on Trimester, Gravida and Parity. Trimester, Gravida and Parity didn't seem to have a noticeable impact on the vWF levels of the subjects. This is determined by analyzing the data of the subjects which shows that there weren't any significant differences in the vWF levels based on these factors (p>0.05).

**Table 2: Comparison of vWF of the Subjects based on Trimester, Gravida and Parity**

Variables	Groups	vWF(pg/ml) mean±SD	F-value	P-value
Trimester	First Trimester (n=12)	154.2 ± 32.1	2.565	0.086
	Second Trimester (n=29)	216.7 ± 150.5		
	Third Trimester (n=19)	154.0 ± 19.6		
Gravida	1-3(n=9)	232.3 ± 153.6	0.796	0.516
	4-6(n=26)	174.9 ± 100.4		
	7-9(n=21)	182.3 ± 109.8		
	10-13(n=4)	148.8 ± 19.4		
Parity	1-3(n=32)	193.1 ± 121.4	0.191	0.902
	4-6(n=22)	178.6 ± 106.5		
	7-9(n=5)	158.8 ± 47.9		
	10-13(n=1)	157.4		

Key: vWF= von Willebrand Factor, pg/ml= picogram per milliliters, SD= Standard Deviation, n= number of test subjects/ number of control.

Table 3 shows Comparison of vWF of the Test Subjects based on Age and years of marriage. Age and years of marriage didn't seem to have a noticeable impact on the vWF levels of the subjects. This is determined by analyzing the data of the subjects which shows that there weren't any significant differences in the vWF levels based on these factors (p>0.05).

**Table 3: Comparison of vWF of the Subjects based on Age and Years of marriage.**

Variables	Groups	vWF(pg/ml) mean±SD	F-value	P-value
Age	15-20(n=11)	160.2 ± 27.0	1.288	0.287
	21-25(n=25)	217.1 ± 163.0		
	26-30(n=12)	160.1 ± 26.3		
	31-35(n=12)	162.6 ± 34.0		
Years of Marriage	1-5(n=23)	203.3 ± 142.1	0.415	0.743
	6-10(n=21)	176.4 ± 109.4		
	11-15(n=10)	174.4 ± 31.6		
	16-20(n=6)	155.9 ± 26.9		

Key: vWF= von Willebrand Factor, pg/ml= picogram per milliliters, SD= Standard Deviation, n= number of test subjects/ number of control

## Discussion

During pregnancy, von Willebrand factor (VWF) undergoes complex changes, impacting its levels and activity (Kadir *et al.*, 1998). Factors influencing von Willebrand factor (VWF) are diverse and include age and gender (Conlan *et al.*, 1993). Hormonal changes can also impact VWF levels, affecting the pathophysiology of von Willebrand disease (Kadir *et al.*, 1998). VWF and factor VIII levels increase two-to four-fold during pregnancy, peaking within 24 hours postpartum and returning to baseline levels by 1 month postpartum in healthy women (Sood *et al.*, 2016). These changes in VWF levels during pregnancy are reversible and are associated with advancing gestational age (Drury-Stewart *et al.*, 2014). In pregnant women with low vWF or type 1 von Willebrand disease (VWD), vWF and factor VIII (FVIII) levels increase during pregnancy, especially in the third trimester. At the time of delivery, these levels can be 2 to 3 times higher compared with baseline levels (Drury-Stewart *et al.*, 2014). Despite all these studies there is limited information on von Willebrand factor levels based on trimester, gravida and parity of pregnant women in Sokoto. In this study, it is established there's significant increase in the vWF levels of pregnant women compared to non-pregnant women when compared ( $p=0.018$ ). This is in support of a previous report (James, 2006) which reported that vWF increases during pregnancy. This could be due to the fact that high levels of estrogen (such as occur during pregnancy) are associated with an increase in von Willebrand factor (vWF) level during pregnancy and decline rapidly after delivery (James, 2006).

Also, our study showed that there is no relationship between trimester, gravida and parity with vWF levels of the pregnant women ( $p>0.05$ ). This is opposed to a study on Chinese pregnant women which found that factors such as gestational age (trimester), gravida, and parity can influence gestational coagulation parameters (Dai *et al.*, 2023). This could be because in the study only five coagulation parameters (PT, APTT, TT, Fibrinogen and D-dimer) were measured, parameters such as vWF were not measured whereas vWF levels were determined in this study.

There was also no relationship between the vWF levels of pregnant women with their age and years of marriage because there's no significant differences ( $p>0.05$ ) in vWF levels of the subjects based on age and years of marriage groups. Our finding is at variance with a previous report (Conlan *et al.*, 1993) which indicated that factors such as age can influence vWF levels. This could be as result of the fact that their study was conducted on already diagnosed von Willebrand disease patients and not apparently healthy pregnant women. So far, there is no study that associated or dissociated vWF levels with years of marriage of pregnant women.

## Conclusion

This study has shown that vWF increases in pregnancy. Age, years of marriage, trimester, gravida and parity do not affect the levels of von Willebrand Factor in pregnant women.

## Recommendations

1. There is a need to determine the prevalence of vWF disorders among pregnant women in Sokoto and their impact on maternal and foetal health.
2. There is need for large-scale epidemiological studies and clinical investigations to understand the specific challenges faced by this population.
3. There is a need to screen vWF status of pregnant women to prevent bleeding disorders.

**Conflict of Interest:** The authors have declared no conflict of interest.

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