EVALUATION OF GAMMA-GLUTAMYL CARBOXYLASE LEVEL AND BLEEDING TENDENCY AMONG PREGNANT WOMEN ATTENDING FEDERAL MEDICAL CENTRE KATSINA

[1]Mba, C., [2]Prof. A. Kulia-Gwarzo., [3]Garba, N., Yusuf, A., Yandutse, M. I., Jarmai, M. M., Hamza K. H., Lawal, U., and Ibrahim, A.O.

[1]Department of Medical Laboratory Science, College of Health Science, Faculty of Allied Health Science, Bayero University, P.M.B 3011, Kano State, Nigeria.

[2]Department of Haematology, Faculty of Medicine, Bayero University Kano, Aminu Kano Teaching Hospital P.M.B 3452, Kano State, Nigeria

3Department of Medical Laboratory Science, College of Health Science, Faculty of Allied Health Science, Bayero University, P.M.B 3011, Kano State, Nigeria.

Corresponding Author: Mba Chinedu

e-mail:Cmentor348@gmail.com; +2348038847686

Received: 31st October, 2022 **Accepted:** 4th December, 2022 **Published:** 8th December, 2022

ABSTRACT

Background: Postpartum haemorrhage contributes significantly to maternal mortality and morbidity in Nigeria and globally with retained placenta being identified as the leading cause in Nigeria.

Aim: This study was designed to establish the relationship between gamma-glutamyl carboxylase (GGCX) levels and bleeding tendencies among pregnant women.

Methodology: The study was conducted at Federal Medical Centre (FMC) in Katsina, Nigeria. A total number of 180 subjects were sampled after obtaining their informed consent. The ELIZA method was used to analyze the gamma-glutamyl carboxylase enzyme while a semi-automated coagulometer was used to analyze the prothrombin time (PT) and activated partial thromboplastin time (APTT).

Results: The results obtained were analyzed using SPSS version 20. The mean ages of the participants were 27±5.13years and about nineteen (10.6%) were in the first trimester of their pregnancy, ninety-one (50.6%) in the second trimester, and seventy (38.9%) were in the third trimester. At the end of the study, it was established that, at $P< 0.05$ level of significance, there is a weak positive correlation and no linear relationship ($r = 0.06$, $n = 180$, $P = 0.935$) between gamma-glutamyl carboxylase (GGCX) and prothrombin time (PT) and a negative correlation and no linear relationship ($r = -0.093$, $n = 180$, $P = 0.212$) between gamma-glutamyl carboxylase (GGCX) and activated partial thromboplastin time (APTT). About eighty-three (46.1%) of the subjects sampled showed a high risk of bleeding with a prothrombin time >15 seconds and about twenty-three (12.8%) showed a high risk of bleeding with an APTT >37 seconds and none of the subjects showed an absolute deficiency of gamma-glutamyl carboxylase enzyme.

Conclusion: The assessment of gamma-glutamyl carboxylase and prothrombin time might be very useful in identifying patients at risk of postpartum bleeding and thereby reducing the incidents of maternal mortality and morbidity due to postpartum haemorrhage.

Keywords: Postpartum haemorrhage, gamma-glutamyl carboxylase, Vitamin-K, prothrombin time, activated partial thromboplastin time, bleeding

INTRODUCTION

The enzyme gamma-glutamyl carboxylase (GGCX) is produced by the gamma-glutamyl carboxylase gene, and is found on the reverse strand of chromosome 2 at 2p11.2.

The gene carries an integral membrane protein of the rough endoplasmic reticulum that carboxylates glutamate residues of Vitamin-K-dependent proteins to gamma carboxyl glutamate, a modification that is essential for their activity (Derek and Anthony, 2018).

Gamma-glutamyl carboxylase enzyme (GGCX) catalyzes the conversion of specific glutamate (Glu) residues to gamma-carboxyglutamate (Gla) residues, a process called gamma-carboxylation (Tie and Stafford, 2016).

In humans, the gamma-glutamyl carboxylase enzyme is most highly expressed in the liver. Hepatic synthesis of clotting factors II, VII, I, X and requires gamma-carboxylation of the glutamic residues within the clotting factors by gamma-glutamyl carboxylase (Derek *et al*., 2018). During this process, Vitamin K is an essential co-factor for the post-translational modification of these gamma carboxyglutamic acid clotting factors (GLA) which are important in binding calcium to a phospholipid membrane and in forming the coagulation complex necessary for the formation of thrombin (Thomas and Charles, 2013). Gamma carboxylation is very crucial in the activation and proper functioning of vitamin-k-dependent proteins involved in blood clotting and coagulation (FII, VII, IX, X) including natural anti-clotting agents like protein C, protein S, and protein Z (Suleiman *et al.*, 2013). In 1998, gamma-glutamyl carboxylase was first linked to human disease by Brenner *et al*., (1998), in four patients with a combined deficiency of all vitamin-k dependent blood coagulation factors (FII, VII, IX, X, and protein S and protein C) due to a homozygous missense mutation in gamma-glutamyl carboxylase gene. The disease was coined vitamin-k dependent clotting factor deficiency-1 (VKCFD1), an autosomal recessive disorder characterized by a mild to severe bleeding tendency and a moderate predisposition to a thrombotic event (Brenner *et al*., 1998).

Research has also shown that gamma-glutamyl carboxylase homozygous null mutants manifested dramatic effects on development with partial midembroyonic loss and postnatal hemorrhage. More so, functionally critical substrates for gamma-carboxylation are primarily restricted to components of the blood coagulation cascade (Randal *et al*., 2018).

During pregnancy, the physiological changes that occur make the entire process of haemostasis more intricate with fluctuating coagulation factor concentrations and physiological anaemia which alter the balance between bleeding and clot formation in preparation for par partum blood loss (Collis and Collins, 2015). The understanding of these changes in coagulation is crucial not just for labor and cesarean section but also for the management of hemorrhage which is common in the parturient (Dutton *et al*., 2014).

Postpartum hemorrhage is a major cause of maternal morbidity and mortality worldwide with the highest incidence in developing countries. According to World Health Organization (WHO), obstetrics hemorrhage causes 127,000 deaths annually and is the leading cause of maternal mortality (WHO, 2008). In Africa, obstetric hemorrhage is responsible for 30% of total maternal deaths. Postpartum hemorrhage is the excessive loss of blood per vaginam after the delivery of the baby and up to 42 days postpartum. It can either be primary or secondary (Khan *et al.,* 2006). Primary postpartum hemorrhage is the loss of more than 500ml of blood within the first 24hrs of delivery or the loss of any amount that is enough to cause haemodynamic instability. Secondary postpartum hemorrhage is any bleeding in excess of lochia after 24hrs and up to 6 weeks postpartum. Since deaths from postpartum hemorrhage are preventable it is, therefore, important to identify risk factors to eliminate or reduce maternal mortality (Elbourn *et al.,* 2001).

MATERIALS AND METHODS

Study Area

The study was conducted at the antenatal clinic of Federal Medical Centre (FMC), Katsina, which is a tertiary health care center located in Katsina Local Government Area of Katsina State. It was established in 1996 with a current bed capacity of five hundred (500). It is the tertiary referral center in Katsina state. The analytical procedure was carried out in the haematology department of the Federal Medical Centre (FMC), Katsina.

Katsina State is a state in the North-West geopolitical zone of Nigeria and was created in 1987. It shares borders in the East with Kano and Jigawa States, in the West with Zamfara State, and in the South with Kaduna State. It also shares an international boundary in the north to the Niger Republic. It is located at coordinates $12^0$15N and $7^0$30E with an Elevation of 519 above sea level. It is known as the home of Hospitality.

Study Population

The target population for this study was pregnant women who were attending the antenatal clinic of Federal Medical Centre, Katsina.

Ethical Consideration

Ethical clearance was sought and obtained from the research and ethical committee of Federal Medical Center, Katsina with the number FMCNHREC.REG.N003/08/2012. More so, written informed consent was obtained from the research subjects and a questionnaire was administered appropriately.

Sample Collection

All pregnant women attending the antenatal clinic of Federal Medical Centre, Katsina, who met the inclusion criteria, were sampled.

The venipuncture technique was employed. A blood sample was collected from the ante-cubital fossae using a syringe and needle. The blood was collected into a 3.2% tri-sodium citrate container and a plain container (Lavery and Ingram, 2005). Samples collected were transported to the laboratory for processing and then storage.

Techniques

Gamma-glutamyl carboxylase (GGCX) Estimation (Using ELISA method)

Principle: This is based on GGCX antibody-antigen interactions (immunosorbency) and an HRP (horseradish peroxidase) colorimetric detection system to detect GGCX antigen targets in samples. (mybiosource.com)

Prothrombin Time (PT)

Principle: Tissue thromboplastin in the presence of calcium and factor VII activates the extrinsic pathway of coagulation when a mixture of tissue thromboplastin and calcium is added to the anticoagulated plasma (Monica 2006).

Activated Partial Thromboplastin Time (APTT)

Principle: When citrated plasma is mixed with PTT reagent; coagulation is triggered by contact activation or the intrinsic pathway. The time required for a clot to form is the activated partial thromboplastin time (Monica 2006).

RESULTS

In this study, all participants (180) resided in an urban area. The mean age of the participants was 27.18±5.13 years. Most of the women (96.1%) were literate with 87(48.3%) at the tertiary level of education, 73(40.6%) at the secondary level of education, and 13(7.2%) at the primary level of education while only 7(3.9%) never had formal education (Table 2).

A total of 70% of the participants were housewives while 21.7% were civil servants (Table 2.0). Some of the participants (33.3%) were primipara and 66.7% were multiparous. In terms of stage of pregnancy, 19(10.0%) of the women were in their first trimester, 91(50.0%) in their second trimester, and 70(40.0%) in the third trimester (Table 3).

All (100%) participants showed no absolute deficiency of the gamma-glutamyl carboxylase enzyme. About 92.8% of the subjects produced ≥10 ng of gamma-glutamyl carboxylase enzyme and this provided an efficient haemostatic function of the clotting factors.

About 26.1% of the subjects produced ≤ 2 ng of GGCX and this produced the same effect on the clotting factor mechanism as those who produced 20-70 ng of the enzyme (3.0% of patients), hence, the effect of the GGCX enzyme is based on all or non-principle.

One of the subjects who had 18 children did not attain formal education and the PT (13seconds) and APTT (35 seconds) were within the normal range with a GGCX level of 12ng.

Table 1 shows the age distribution and number of Children among subjects. The majority of the subjects were between the ages of 27-29years followed by those between the ages of 24-26years. Most of the subjects were pregnant for the first time and contributed about 60(33.3%) of the subjects.

Table 2 shows the Socio-demographic Characteristics among participants. The majority of the subjects were educated up to the tertiary level of education. Only about 7(4%) of the subjects did not attain formal education. More so, the majority of the subjects were housewives, about 39(22%) were civil servants and 15(%) were self-employed.

Table 3 Shows Trimester and Parity distribution among Subjects. The majority of the subjects were in their second trimester only about 10% were in their first trimester. More so, about 121 of the subjects were multipara and only about 33% were just pregnant for the first time.

Table 4 Shows Prothrombin time and APTT Category among Subjects. The prothrombin time of about 47(26.1%) of the subjects showed hypercoagulability with PT ≤13seconds while about 83(46.1%) showed a high risk of bleeding with a prothrombin time of 15 seconds. The APTT of about 27(15%) of the subjects showed hypercoagulability with APTT ≤30 seconds while about 23(12.8%) of the subjects showed a high risk of bleeding with APTT ≥ 37seconds.

Table 5 shows Pearson Correlation between Gamma-glutamyl carboxylase, APTT, and PT. At P< 0.05 level of significance, the table shows a weak positive correlation and no linear relationship between GGCX and PT, and a negative correlation and no linear relationship between GGCX and APTT. The results, therefore, indicate that gamma-glutamyl carboxylase is a better predictor of prothrombin time (PT) than activated partial thromboplastin time (APTT).

Table 6 Shows One way Anova of GGCX, Prothrombin Time (PT), and APTT in Relation to Trimester. Prothrombin time (PT) shows a high F value of 1.347 with a P value of 0.263 which is higher than the critical value of 0.05; this indicates that there is no statistically significant difference in the means between the groups of the factor. In the first trimester, the patients had a mean prothrombin time of 14.68±1.49 and the least prothrombin time in the third trimester with a mean value of 14.16±1.28.

APPT shows a high F value of 1.515 with a P value of 0.223 which is higher than the critical value of 0.05; this indicates that there is no statistically significant difference in the means between the groups of the factor. In the first trimester, the patients had a mean APTT of 34.42±2.65 and the least mean APTT in the second trimester with a value of 33.19±2.65

GGCX shows a high F value of 1.228 with a P value of 0.295 which is higher than the critical value of 0.05; this indicates that there is no statistically significant difference in the means between the groups of the factor. In the second trimester, the subjects had a mean GGCX of 6.15±8.69 and the least GGCX in the first trimester with a mean value of 4.21±2.84.

Table 1: Age distribution and Number of Children among subjects.

AGE	Frequency	Percent	Cumulative%
18-20	22	12.2	12.3
21-23	24	13.4	25.6
24-26	39	21.7	47.3
27-29	41	22.8	70.1
30-32	26	14.5	84.6
33-35	18	10.0	94.6
36-38	7	3.9	98.5
39-41	1	0.6	99.1
42-44	2	1.2	100
Total	**180**	**100**	
Number of children			
1	60	33.3	33.3
2	31	17.2	50.5
3	34	18.9	69.4
4	22	12.2	81.6
5	21	11.7	93.3
6	5	2.8	96.1
7	2	1.1	97.2
8	3	1.7	98.9
10	1	0.6	99.5
18	1	0.6	100
Total	**180**	**100**	

Table 2: Socio-demographic Characteristics among Participants

	Frequency	Percent	Cumulative %
Level of Education			
Informal	7	4	4
Primary	13	7	11
Secondary	73	41	52
Tertiary	87	48	100
Total	**180**	**100**	
Occupation			
Civil Servant	39	22	22
House Wife	126	70	92
Self Employed	15	8	100
Total	**180**	**100**	

Table 3: Trimester and Parity Distribution among Subjects

	Frequency	Percent	Cumulative %
Trimester (TRM)			
1ST TRM	19	10	10
2nd TRM	91	50	60
3rd TRM	70	40	100
Total	**180**	**100**	
Parity			
Primipara	59	33	33
Multipara	121	67	100
Total	**180**	**100**	

Legend: TRM- Trimester

Table 4: Prothrombin time and APTT Category among Subjects.

	Hypercoagulability	Low Risk	High Risk	Total
PT	13.0sec	≤14.0sec	>15.0sec	
	(26.1%)	(27.1%)	(46.1)	100%
APTT	30.0sec	≤36.0sec	>37.0sec	
	(15.0%)	(72.2%)	(12.8%)	100%

Legend: PT-Prothrombin time, APTT- Activated partial thromboplastin time

Table 5: Pearson Correlation between Gamma-glutamyl carboxylase, APTT, and PT

	n	P-value	r-value
APTT	180	0.212	-0.935
PT	180	0.935	0.06

Legend: * Correlation is significant at 0.05 levels (2-tailed). PT=Prothrombin, APTT=Activated partial thromboplastin time, GGCX=Gamma-glutamyl carboxylase enzyme.

Table 6: One way ANOVA of GGCX, Prothrombin time, and APTT in Relation to Trimester

PARAMETER	1st TRM	2nd TRM	3rd TRM	P-VALUE
PT (secs)	14.68 ± 1.49	14.37 ± 1.29	14.16 ± 1.28	.263
APTT (secs)	34.42 ± 2.65	33.19 ± 2.65	33.37 ± 2.95	.223
GGCX (ng)	4.21 ± 2.84	6.15 ± 8.69	4.63 ±4.95	.295

Legend: *P<0.05 indicates a statistically significant difference; all values are expressed as mean ± standard deviation. TRM= Trimester, PT=Prothrombin, APTT=Activated partial thromboplastin time, GGCX=Gamma-glutamyl carboxylase enzyme.

DISCUSSION

Obstetric haemorrhage is a clinical problem of indisputable concern both to pregnant women and clinicians. Major obstetric haemorrhage (MOH) refers to any kind of excessive bleeding in a parturient and can occur during the antepartum period (20-24wks after gestation to the onset of delivery), delivery period or postpartum period (<24hrs – 6wks after birth)

The findings of this research agrees with the work and findings of Brenner et al 1998 and further establish the fact that an absolute deficiency of gamma-glutamyl carboxylase can be associated with an absolute deficiency of clotting factors and bleeding tendencies as the results indicates a weak positive correlation (r=0.06) between gamma-glutamyl carboxylase enzyme and prothrombin time (PT) though a negative correlation (r = −0.093) between gamma-glutamyl carboxylase enzyme and activated partial thromboplastin time (APTT). The reason for this difference between PT and APTT in relation to GGCX is not known and is therefore subject to further studies.

More so, the findings of this research show that there is no statistical difference in the means between the group of the factors for PT, APTT, and GGCX in the first trimester, second trimester, and third trimester. This finding agrees with the earlier studies conducted by Szecsi*et. al.* (2010) that despite the changes that occur to the coagulation system, the standard coagulation tests such as prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (APTT) do not change during pregnancy or are slightly decreased.

About 26.1% of the patients produced ≤ 2 ng of gamma glutamayl carboxylase enzyme and this produced the same effect on the clotting factor mechanism as those who produced 20-70 ng of the enzyme (3.0% of patients), hence, the effect of gamma-glutamyl carboxylase enzyme is based on all or non-principle which states that the strength of the response is not dependent upon the strength of the stimulus and that response above the threshold is the same.

Previous studies by Stafford *et al. (*2005), Thomas and Charles (2013) suggest that carboxylation by the enzyme is dependent upon the rate of carboxylation and dissociation rate constant. The rate of carboxylation is controlled by reduced vitamin K while the dissociation rate constant is dependent upon both the propeptide and Gla domain of the substrate.

However, since all patients sampled did not show an absolute deficiency in gamma-glutamyl carboxylase enzyme (GGCX), the observed prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT) in some of the patients may be due to other factors like circulating anticoagulants, Factor VIII antibodies, autoimmune disorders, rheumatoid arthritis or single factor deficiency as earlier reported in previous studies by Joel *et al.* (2020). Circulating anticoagulants are glycosaminoglycans with heparin-like anticoagulant activity arising from their ability to increase antithrombin III activity. They cause bleeding by binding prothrombin not by neutralizing clotting activity.

According to the findings of Ajenifuga*et al.* (2010) in Nigeria on postpartum haemorrhage, the leading cause of postpartum haemorrhage was identified to be retained placenta which accounted for about 78.57% followed by uterine atony which accounted for about 10.71%. However, while the work of Ajenifuga*et al* estimates actual (visual) blood loss, this study x-rays predisposing factors. In this study, about 83(46.1%) of subjects showed a high risk of bleeding with prothrombin time (PT) $\geq$ 15.0 sec and with APTT of $\geq$ 37.0, 23(12.8%) of the patients have a significant risk of bleeding and are prone to postpartum haemorrhage.

The work of Ajenifuga*et al.* (2010) reported about 6 deaths from postpartum haemorrhage and globally according to the World Health Organization obstetric haemorrhage causes about 127,000 deaths per annum and is the leading cause of maternal mortality. Since the risk of dying from postpartum haemorrhage does not only depend on the rate of blood loss but also on the health status of the woman, taking into consideration other risk factors as shown in this research, can drastically reduce these death rates.

Over the years, antenatal care (ANC) for pregnant women in Nigeria does not always include a clotting profile as a routine screening test except in a few special cases such as those with a history of bleeding or undergoing warfarin therapy. The risks for postpartum haemorrhage have been based on prolonged labor, retained placenta, oxytocin induction/augmentation of labor, instrumental vaginal deliveries, cesarean section, genital tract laceration, poorly managed third-stage labor, etc. It is therefore pertinent to note that about half (46.1%) of the women in this study showed a high risk of bleeding with prolonged prothrombin time; and since postpartum haemorrhage can occur in the absence of any identifiable risk factor as earlier reported by Kinikanwo*et al,(*2015) its anticipation should not be constrained or limited to theater and moments of delivery but adequate diagnostic examinations aided by clotting profile test before delivery will promptly aid in the intervention of postpartum haemorrhage and thereby reducing the associated mortality and morbidity.

More so, most clinical studies on postpartum haemorrhage based their assessment on visual estimation of blood loss. This method is often biased and inaccurate, grossly subjective, and in most cases underestimated and this makes the exact prevalence of primary postpartum haemorrhage difficult to determine. This study has therefore provided a diagnostic approach and foresight to knowing those who might be predisposed to bleeding before the time of delivery which is paramount to prompt intervention and adequate preparation to mitigate the foreseen bleeding. The maternal mortality rate at UPTH from the 2011 annual report was at 792.1/100,000 deliveries with primary postpartum haemorrhage accounting for 17.24% while a study carried out in Ilorin, North-central Nigeria showed that postpartum haemorrhage accounted for about 34% of maternal mortality.

Even though primary postpartum haemorrhage is difficult to determine, deaths from postpartum haemorrhage are preventable. Its management should therefore start with identifying the risk factors, and adequate patient care management supported by laboratory diagnosis. This is likely to aid in reducing the incidence and occurrence of postpartum haemorrhage and its associated morbidity and/or mortality.

CONCLUSION

Following the findings of his research, it is therefore concluded that assessment of GGCX, prothrombin time, and activated partial thromboplastin time might be very useful in identifying patients at risk of postpartum bleeding and thereby reducing the incidents of maternal mortality and morbidity due to postpartum haemorrhage.

RECOMMENDATIONS

We, therefore, recommend that:

1. Further study of gamma-glutamyl carboxylase (GGCX) levels be conducted on patients with known bleeding history or conditions to further establish its role in bleeding risk.
2. Gamma-glutamyl carboxylase to be assayed in patients with prolonged prothrombin time since it shows a stronger positive correlation.
3. Gamma-glutamyl carboxylase and Prothrombin time to be included in the antenatal screening test for pregnant women.

ACKNOWLEDGMENT

I sincerely wish to acknowledge the commitment, guidance, and support of my amiable supervisors, Prof. Aisha KuliaGwarzo and Mr. NuraGarba, they are the best supervisors any student can have.

I also wish to appreciate Dr. Aisha Abdurrahaman the HOD ANC department FMC, Katsina, YandutseYunusa Mahmood Director Medical Laboratory Services (DMLS) FMC Katsina, Yusuf Ado Director Medical Laboratory Services (DMLS) NOFIC Katsina for their immense support and encouragement in the course of this work. Great thanks also to all my colleagues, technicians, and assistants who assisted in one way or the other.

Most importantly many thanks also to the HOD Medical Laboratory Department Dr. Jamilu Abubakar Bala and the entire lecturers of the department, the journey so far was made possible by their coherent tutelage and support.

REFERENCES

Ajenifuga K.O., Adepiti C.A., OgunniyiS.O.,(2010). Postpartum haemorrharhage in a teaching hospital in Nigeria: a 5-year experience. Africa Health Science 10 (1):71-81

Brenner, B., Sánchez, V., B., Wu, S.M., Lanir, N., Stafford, D.W., Solera, J. (1998). A missense mutation in the gamma-glutamyl carboxylase gene causes a combined deficiency of all vitamin K-dependent blood coagulation factors. *Blood. 92:4554–4559.*

Chee, Y.L., Crawford, J.C., Watson, H.G., Greaves, M. (2008). Guidelines on the assessment of bleeding risk before surgery or invasive procedures: British Committee for Standards in Haematology. *British Journal of Haematology.* 140: 496–504

Collis, R.E. and Collins, P.W. (2015). Haemostatic management of obstetric haemorrhage Anesthesia .70 (Suppl. 1): 78–e28

Derek, G.W., Anthony, P. S. (2018). Medical Pharmacology and Therapeutics, 5th edition, *Elsevier*, 11;175-190

Dutton, R.P., Lee, L.A., Stephens, L.S., Posner, K.L., Davies, J.M. Domino, K.B. (2014) Massive hemorrhage: a report from the anesthesia closed claims project. Anesthesiology 121: 450–8

Elbourn, D.R., Pendriville, W.J., Caroli, G., Wood, J. and McDonald S. (2001) prophylactic use of oxytocin in the third stage of labor. Cochrane Database System Review. (4) CD001808.

Institute of International Studies. iis.berkeley.edu.Retrived 2021-03-07

Joel L.M (2020). Uncommon hereditary coagulation disorders. Baylor College of Medicine. MSD manual

Kathleen L.B. (2000). The Vitamin-K dependent carboxylase. *The Journal of Nutrition, Volume 130, Issue 8, August 2000*

Khan, KSW. (2006). WHO analysis of causes of maternal death: A systemic Review. *Lancet. 367:1006-1074.*

Kinikanwo, I.G., John D.O., Mmom C.F. (2015). Primary Postpartum haemorrhage at University of Port Harcourt Teaching Hospital: Prevalence and risk factors. *The Nigerian Health Journal. 15(3):111-117*

Lavery I. and Ingram P. 9 (2005). Venepunture: best practice. *Nursing standard. 19(49): 55-65*

Monica C. (2006). District laboratory practices in tropical countries. Part 2, second editon Cambridge university press. Pp 340 – 347.

Presnell, S.R., Stafford, D.W (2002). "The vitamin K-dependent carboxylase" *Thrombosis and Haemostasis. 87 (6): 937–46.*

Randal, J. Kaufman, and Laura, Popolo. (2018). Haematology 7th edition *Elsevier Inc.* 5:45-48

Stafford D.W (2005) The Vitamin K cycle. Journal of thrombosis and haemostasis2005;3:1873-8

Suleiman, L., Négrier, C. and Boukerche, H. (2013). Protein S: A multifunctional anticoagulant vitamin K-dependent protein at the crossroads of coagulation, inflammation, angiogenesis, and cancer. *Critical Reviews in Oncology/ Hematology.88:637–654.*

Szecsi, P.B., Jørgensen, M., Klajnbard, A., Andersen, M.R., Colov, N.P., Stender, S. (2010). Haemostatic reference intervals in pregnancy. *Journal of Thrombosis and Haemostasis 103: 718–27*

Tie, J.K. and Stafford, D.W. (2016). Structural and functional insights into enzymes of the vitamin K cycle. *Journal of Thrombosis and Haemostasis*. 14:236–247.

Thomas, C. A and Charles, S.A. (2013). Transfusion medicine and haemostasis. Elsevier Inc. 16:747.

World Health Organization, author. Reducing the global burden: postpartum haemorrhage 200. [5 December 2008].