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Measurement of Von Willebrand Factor Antigen Levels in a Nigerian Population: Relative Effects of ABO Blood Group, Age, Gender and Ethnic Differences

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ABSTRACT

Several studies have shown that von Willebrand Factor Antigen (vWF: Ag) levels are influenced by some factors such as ABO blood group, age, gender and ethnic differences, however, similar reports in our environment are non-existent. Therefore the objective of this study was to determine the relationships between the plasma vWF: Ag levels and these factors among apparently healthy Nigerian subjects. Using the blood samples of 100 blood donors at the Blood Bank Unit, University of Uyo Teaching Hospital, Uyo, Nigeria, the ABO blood group phenotypes were determined by standard tube method while vWF: Ag levels were determined by enzyme-linked immunosorbent assay(ELISA) method. The age, sex and ethnic origins of the respondents were also considered. There were 63 male subjects and 37 female subjects with a mean age of 31.7+ 1.02IU/ml with blood group A having the lowest mean vWF:Ag level (1.087+ 0.402), group O 1.2896 + 1.005), group AB (1.687+ 1.061) and group B having the highest level (2.176+ 1.487). The association of the ABO blood groups with the vWF: Ag levels was statistically significant (P = 0.0077). vWF: Ag levels were significantly higher among the female subjects compared to their male counterparts (1.71+1.31 vs 1.19+ 0.75, p = 0.0126). There was no significant correlation with age and ethnic group (P = 0.9664 and P = 0.6058, respectively). The association of vWF: Ag levels with the ABO blood groups and gender but not age and ethnic origin was statistically significant. Interestingly, the study has reaffirmed the time-honoured notion that ABO blood group remains an important modulator of circulating vWF: Ag levels.

INTRODUCTION

Von Willebrand Factor (vWF) is a large adhesive sialoglycoprotein synthesized by endothelial cells and megakaryocytes that circulates in the plasma as a series of heterogeneous multimers[1]-[3]. vWF is best known for its crucial hemostatic role in mediating platelet – subendothelium adhesion and platelet-to- platelet interactions following vascular injury under conditions of high shear stress. In addition, vWF serves as chaperone for coagulation factor VIII and protects it from proteolytic degradation, prolonging its half-life in circulation and efficiently confining it to the site of injury[4].

Quantitative or qualitative defects of vWF result in von Willebrand Disease (vWD), the most common congenital bleeding disorder in humans, estimated to affect up to 1% of the population[5]-[7]. The definitive diagnosis of this disease requires the presence of a mutation in the vWF gene. This mutation accounts for the presence of reduced amounts of vWF or abnormal forms of vWF in the circulation, which in turn are the central features of all forms of vWD[5]. However, several lines of evidence have shown that other gene loci, as well as non-genetic factors, exert major effects on the plasma levels of vWF. It is well established that plasma vWF levels vary according to ABO blood group, age, race, ethnicity and gender[8]-[11]. This is of utmost clinical interest because selection of normal ranges based on these variables may influence the diagnosis of von Willebrand Disease. It does actually help to segregate patients at risk of bleeding or really assist in the identification of true von WillebrandDisease[12].

Genetic factors are known to account for 70%[13] of the variation in plasmavWF: Ag levels, with ABO blood group being the major contributor. Studies have shown that 30%[14]and 20%[5] of plasma vWF level variability are due to the ABO blood group and vWF gene, respectively. Jenkins et al.[15], in their study reported that the vWF levels are approximately 25 – 30% lower in blood group O individuals compared to non -O blood group individuals, which correlates with the increased risk of bleeding of the former. Other authors have consistently corroborated these findings[16]-[19]. The plausible explanations for this observation range from the effect of ABO blood group on the rate of synthesis and secretion of vWF, to an effect on the survival of vWF and its clearance from the plasma by the protease ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin type – 1 repeats – 13) metalloprotease[20]. Results of multimeric analysis and collagen – binding assays have

demonstrated that proteolysis is significantly faster for group O vWF individuals compared to non – OvWF individuals in the following order 0≥ B > A ≥ AB[21].

Payne et al.[22] and Zhou et al.[23] in their studies on the effects of gender, race and ethnic differences on the plasma levels of vWF, found that the mean levels of vWF were significantly higher in females than in males and in African-Americans than in Caucasians. This agrees with previous studies in other populations of African descent in Britain[24], Brazil[25], and South Africa[10]. Linear increase in vWF with advancing age has been observed by many authors[26]-[28]. Plasma levels of vWF are known to increase by approximately 0.17 and 0.15 IU/ml per decade in a normal population[24]. Indeed older age is associated with lower ADAMTS13 activity[29] and this explains the higher vWF levels observed with increasing age.

Most of the studies on the effects of the aforementioned factors on the plasma levels of vWF have been done in the Oriental and Western world and there is paucity of information on these associations in Nigeria and several other African countries. Given the widely known perception that there are many ethnicity-specific variations in physiologic processes[30], we sought to determine the influence of ABO blood group, age, gender and ethnic differences on the plasma levels of vWF among apparently healthy subjects in our center. It is our fervent hope that our findings will add to the existing body of knowledge and serve as a template for further research particularly in our environment where there is no report on this subject.

MATERIALS AND METHODS

This was a cross-sectional descriptive study. A total of 100 blood donors who attended the Blood Bank Unit of the University of Uyo Teaching Hospital (UUTH), a 500 – bed tertiary referral hospital in Uyo, Akwa Ibom State, South-south Nigeria, were recruited into the study. Apparently healthy, consenting subjects aged 18 to 65years were included in the study while those excluded were non-consenting subjects and those on medications such as anticoagulants, contraceptives, anti-platelet drugs like aspirin and herbal – based concoctions.

A well- structured questionnaire was used to obtain information on demographic variables such as age, sex and ethnic origin of each participant.

10ml of free-flowing venous blood was obtained from each subject under aseptic condition. Half (5ml) of the sample was dispensed into ethylene –diamine-tetraacetate (EDTA) bottles

for full blood count (FBC) and ABO grouping. The second aliquot of 5ml of blood was dispensed into trisodium citrate specimen bottles, and centrifuged at 3000g for 10minutes at 4ºC within 30 minutes of collection and stored in aliquots at – 80ºC for use in the determination of baseline prothrombin time (PT), activated partial thromboplastin time (APTT) and vWF: Ag within a week. All samples collected were labeled with a serial number allotted to each subject.

The full blood count (FBC) was carried out using the Sysmex Haematology Analyzer. Standard tube as described by Bain and Lewis[31]was used for the determination of ABO blood group using antisera obtained from Biotec Laboratory, United Kingdom. Plasma concentration of vWF: Ag was estimated using a commercial assay kit-Assay max human von Willebrand Factor (vWF) ELISA kit manufactured by Assay Pro, St. Charles, MO, USA. The PT/APTT time was determined using standard commercial PT/APTT reagents manufactured by Diagnostic Reagent Ltd, Thames, Oxon, United Kingdom. Adequate controls were included in all tests carried out.

The results were collated, analyzed and presented as simple proportion tables and the comparisons carried out with chi-square test as appropriate. The level of significance was set at 5% ($p < 0.05$).

Ethical approval for this study was obtained from Ethics and Research committee of UUTH, Uyo. A written consent was sought from participants after a thorough explanation of the study protocols. Results and records were strictly kept confidential.

RESULTS

A total of 100 blood donors, aged 21-53 years (mean age 31.7±6.39 years; 63males, 37 females) participated in this study. Table 1 shows the socio-demographic characteristics of the blood donors.

Donors with blood group O constituted the majority with a total of 50 subjects (50%) and mean vWFAg level of 1.2896 ± 1.005 while group AB had the least with 3 subjects (3%) with mean vWF: Ag level of 1.687 ±1.061. Subjects with blood group A were a total of 31(31%) and had the lowest vWF: Ag concentration (1.082 ± 0.402), while subjects with blood group B were 16 (16%) and had the highest vWF: Ag concentration (2.176±1.487). table 2a shows the distribution of vWF: Ag levels among the various ABO blood groups.

The results showed that the plasma level of vWF: Ag in subjects with non- O blood group was significantly higher than that in group O subjects (table 2b).

A comparison of the mean vWF: Ag levels of the various ABO blood groups using the Kruskal Wallis rank test showed that the difference between their means was statistically significant (P = 0.0077). Table 3 shows the relationship between mean plasma vWF: Ag concentrations and ABO blood groups of Donors in UUTH, Uyo.

vWF levels were higher in the female subjects (1.71±1.31IU/ml) compared to their male counterparts (1.19±0.75IU/ml). A comparison of the mean values between the two sexes showed a statistically significant relationship (P = 0.0126). Table 4 shows the relationship between mean plasma vWF: Ag levels and the gender of the blood donors.

Majority (58%) of the participants were Ibibio, followed by Annang (23%), Igbo (13%), Yoruba (4%) and Oro (2%). There was no statistically significant association between the plasma vWF: Ag concentrations and the ethnic origins of the donors (P = 0.6058, table 5).

Table 6 presents the relationship between the plasma vWF: Ag concentration lU/ml and the age of the blood donors. There was no statistically significant correlation between the two variables (P = 0.9664).

Table 1: Socio-demographic characteristics of Blood Donors in UUTH

(a) Age (years)	**Frequency**	**Percentage**
20 – 29	25	25
30 – 39	60	60
40 – 49	10	10
50 – 59	5	5
60 – 69	-	-
Total	**100**	**100**
Mean Age	31.7	-
Standard Deviation(SD)	6.39	-
(b) Sex		
Male	63	63
Female	37	37
Total	**100**	**100**

(c) Population groups		
Ibibio	58	58
Annang	23	23
Igbo	13	13
Yoruba	4	4
Oro	2	2
Total	**100**	**100**

Table 2: Distribution of von Willebrand Factor antigen concentration (vWF: Ag, lU/ml) among the ABO blood groups of blood donors in UUTH

(a) Blood Group	**Means**	**Standard Deviation**
A	1.082	0.402
B	2.176	1.487
AB	1.687	1.061
O	1.2896	1.005
	1.38	**1.02**
(b) Non – O	1.468	1.0406
O	1.289	1.005
	1.38	**1.02**

Table 3: Relationship between mean plasma vWF: Ag concentration (lu/ml) and ABO Blood Groups of the Blood Donors

ABO Blood Group	**Frequency (n)**	**Kruskal Wallis**
A	31	1394.00
B	16	1161.50
AB	3	184.00
O	50	2310.50

Chi-square = 11.918 with 3df, P = 0.0077

Table 4: Relationship between plasma vWF: Ag concentration lU/ml and sex (Gender) of Blood Donors

Gender	**Frequency (n)**	**Means**	**Standard Deviation**
Male	63	1.19	0.75
Female	37	1.71	1.31

P = 0.0126, P < 0.05

Table 5: Relationship between plasma vWF: Ag concentration (lU/ml) and the ethnic groups of the Blood Donors

Ethnic Group	Frequency	Kruskal Wallis rank
Ibibio	58	3037.50
Annang	23	1095.00
Oro	2	102.00
Igbo	13	697.50
Yoruba	4	118.00

Chi-square = 2.72 with 4df, P = 0.6058

Table 6: Relationship between plasma vWF: Ag concentration lU/ml and the age of the Blood Donors

Age Group(years)	Observation	Kruskal Wallis rank	Statistical test and values
20 – 29	50	2525.00	X^2 – 0.265
30 – 39	35	1767.50	DF = 3
40 – 49	15	717.00	P = 0.9664
50 – 59	1	40.50	
60 – 69	-	-	-

DISCUSSION

Studies have shown that a wide variety of factors influence the circulating vWF: Ag levels. These include but not limited to the following: stress, exercise, advancing age, pregnancy, race, gender, ABO blood group, cancer, surgery, trauma and certain medical conditions[11]-[32]. In the present study, we evaluated the influence of ABO blood group, age, gender and ethnic differences on the plasma levels of vWF: Ag. To the best of our knowledge, this is the first report in our population. The ABO blood group has been known to exert a significant effect on hemostasis, being a major modulator of circulating vWF. Seventy[13] percent of the variation in plasma levels of vWF: Ag is genetically determined and 30%[14] of this has been attributed to the ABO blood group. Our study has clearly demonstrated that the ABO blood group has an obvious effect on the vWF: Ag plasma levels. vWF: Ag level was found to be significantly lower in O blood group than non-O blood group (A, B and AB). Similar results have been reported in other populations in USA[33], Australia[34], Norway[14], Japan[29], Brazil[19],Italy[18], and Canada[35]. The explanation for the observed difference in the two ABO blood types is that blood group O possesses the greatest amount of H substance and

expression of H gene is inversely related to Vwf[36]. However, in the study done by Coppola etal[37], there were no significant differences in vWFlevels between group O and non-O in healthy Italian centenarians, although they still found a clear distinction in younger controls.

The mechanism by which ABO blood affects the plasma vWF: Ag levels is poorly elucidated, though it is thought to be due to increased susceptibility to cleavage by the vWF-cleaving protease ADAMTS13 which is significantly faster for group OvWF compared to non-group OvWF persons [21]. This is consistent with other reports[29]-[39]. Ostavik and associates[14] in their study to assess the association of vWF, FVIII, FIX and ABO blood group observed that the effect of the ABO blood group on the coagulation proteins can be traced to the ABO locus. vWF is one of the few non-erythrocyte proteins that expresses ABH antigens and ABH oligosaccharide structures have been identified on the N-linked oligosaccharide chains of vWF. These N-linked oligosaccharide chains on vWF molecules contain A and B blood group antigens which are encoded by the ABO blood group gene, which is located on the long arm of chromosome 9. The gene for ADAMTS13 is located on chromosome 9q approximately 140, 000 nucleotides from the ABO locus. The ABO blood group, therefore, influences the susceptibility to proteolysis by ADAMTS13 because two of its potential N-linked glycosylation sites (asparagines 1515 and 1574) are located in close proximity to the ADAMTS13 cleavage site which is the peptide bond between tyrosine and methionine (Tyr 1605-Met 1606) in the central A2 domain of vWF. Furthermore, the oligosaccharide chain composition may be involved in stabilizing the conformation of the vWF region, such that the removal of terminal sugar allows the A2 domain to adopt a conformation more permissive for cleavage by ADAMTS13[18]-[39].

Our results showed that individuals with blood group B had the highest plasma vWF: Ag levels while those with blood group A had the lowest levels. These findings are however in dissonance with previous reports. Bowen et al.[40] reported that group B vWF was more susceptible to ADAMTS13 – induced proteolysis than group A vWF, whereas Gill et al.[8] observed the highest plasma vWF: Ag levels in AB subjects and the lowest levels in O subjects. The findings of high vWF: Ag levels in different ABO blood groups may pose a daunting diagnostic challenge as individuals with the blood groups in question with genetic defects of vWF may have the diagnosis of vWD in them disregarded because their vWF: Ag levels are elevated. Pertinent to these observations, therefore, we may adduce that there is an exigent need for blood group – specific reference ranges to be established in all populations.

Reports from previous studies[12]-[35] have shown the vWF significantly increased with age. This may account for the higher incidence of thrombosis in older individuals, as vWF alongside other procoagulant proteins such as fibrinogen and FVIII are three recognized risk factors for thrombosis[25],[28].Peng Jiang et al.[41] in their work demonstrated that the effect of age on plasma vWF levels was greater than that of ABO blood type. The mean vWF: Ag was found to be approximately 20.1% lower in O than in non-O carriers and approximately 32.1% lower in individuals younger than 40 years than those older than 50 years. Kadir et al.[24] observed that vWF: Ag increased by an average of 0.17 (equivalent to 17%) for each 10- year increase in age. However, in our study, we did not observe any age-related differences in the vWF: Ag levels. This finding is comparable with the results reported by Belch and associates[42]. Our result perhaps may be due to the small sample size of patients and the lack of elderly subjects in this study, thus, a larger study that deliberately targets the elderly i.e. 60 years and above is required before reaching definite conclusion. Some studies[3],[43],[44] have identified age 60years and above as a factor that significantly influences the plasma vWF: Ag levels. The discrepancies could also be due to differences in the testing methodologies (antigen or activity) and geographical locations.

A systematic review of published literature revealed varying reports on the influence of gender on the circulating concentration of vWF: Ag. Conlan et al.[11] in their study to evaluate risk factors for the development of atherosclerotic diseases, reported that vWF levels were significantly higher in females than in males, whereas Campos and associates[45] in their work documented that the mean ± SD vWF antigen level for males (112.9%±42.7%) was significantly higher than that for females (111.19±42.4%). However, majority of the studies reported no variation in plasma vWF:Ag levels in relation to gender[23],[34],[41],[46],[47]. Our result showed that the female subjects had a higher vWF: Ag level (1.71±1.32) than their male counterparts (1.19±0.75). The reason for the higher level in the female respondents could be due to estrogen-induced synthesis of vWF: Ag[33][48].

Ethnic and racial origins of individuals have been shown to be an important determinant of their plasma levels of vWF: Ag[10],[22],[23],[24],[25]. Sukhu et al. [10] reported that the concentration of vWF: Ag in Africans was significantly higher than that of the Indians and Caucasians. In addition, they found that there were no significant differences in the values between the Indians and caucasians. This finding is in agreement with the study done by Platt and associates[33] who reported that caucasian women have significantly lower figures

compared to the African – American women. Similar observations have been made in other populations of African descent in Brazil[25] and Britain[24], suggesting that this is part of the genetic heterogeneity that typify racial groups. Conversely, in the present study, the ethnic origins of the subjects did not have any significant influence on their vWF: Ag levels. This is consistent with an earlier study done in Northern Italy by Werner and associates[49] in which vWF: Ag activity was determined in 600 healthy children, aged 2 to 18 years. A total of 315 subjects were Caucasians, 212 Black, 16 Hispanic, 10 were from other ethnic groups and 4 were biracial. They reported no significant differences in vWF: Ag activity by ethnicity. However, our finding may not be a true reflection of the influence the ethnic origins of the respondents have on their vWF: Ag levels because of the small sample size used in this study and by extension under-representation of some of the ethnic groups in the study population.

CONCLUSION

This study has shown that the gender and ABO blood group but not age and ethnic origin of an individual have significant influence on his circulating levels of vWF: Ag and need to be taken into cognizance when establishing reference ranges for vWF: Ag. The major limitation of this study is the small sample size of the subjects. We hereby recommend that a large multicentre study including community-based survey be conducted in Nigeria and other countries in Africa for the purpose of validating our results. It is envisioned that this work will form a template for a more extensive prospective study in the nearest future.

REFERENCES

1. Ruggeri ZM, Zimmerman TS. The complex multimeric composition of Factor VIII/vWF. Blood 1981; 57:1140-1143.
2. Sadler JE. Von Willebrand Factor. Ann Rev Biochem 1998; 67:395-424.
3. Ruggeri ZM. Structure of von Willebrand Factor and its function in Platelet adhesion and thrombus formation. Best Pract. Res Clinical Haematol 2001; 14: 257-259.
4. Volt AJ, Koppelman SJ, Bouma BN, Sixma JJ. Factor VIII and von Willebrand Factor. Thromb Haemost 1998; 79:456-465.
5. Sadler JE, Mannucci PM, Berntop E. Impact, diagnosis and treatment of von Willebrand disease. Thromb Haemost. 2000; 84:160-14.
6. Sadler JE. For the Subcommittee on von Willebrand Factor of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. A revised classification of von Willebrand disease. Thromb Haemost 1994; 71:520-525.
7. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. Blood 198; 69:454 – 459.
8. Gill JC, Endres – Brooks J, Bauer PJ. The effect of ABO blood groups and von Willebrand Factor. Br J Haematol. 11989; 69:1691 – 1695
9. Franchini M. Hemostasis and aging, Crit Rev OncolHemat. 2006; 60:144-51

10. Sukhu K, Poovalingam V, Mahomed R, Giangrande PL. Ethnic variation in von Willebrand Factor levels can influence the diagnosis of von Willebrand disease. International Journal of Laboratory Hematology. 2003; 25:247-249.
11. Conlan MG, Folsom AR, Finch A, Davis C, Sorlie P, Marcucci G, Wuk. Associations of Factor VIII and von Willebrand Factor with age, race, sex and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) study. Thrombosis and haemostasis 1993, 0: 380 – 385.
12. Favaloro EJ, Lilicrap D, Lazzari MA. vonWillebrand disorder laboratory aspects to diagnosis and treatment. Hemophilia, 2004; 10:164-168.
13. De Lange M, Kluft C. Genetic influences on Fibronogen, tissue plasminogen activator – antigen and von Willebrand Factor in males and females. Thromb Haemost 2006; 95:414 – 419.
14. Orstavik KH, Magnus P, Reisner H, Berg K, Graham JB. Factor VIII and Factor IX in a twin population; evidence for a major effect of ABO locus on factor VIII level. AM J Hum Genet 1985; 3:89-101.
15. Jenkins P, O' Donnell J. ABO blood group determines plasma von Willebrand Factor levels: A biologic function after all. Transfusion 2006; 46:1836 – 44.
16. Franchini M, Mannucci PM. ABO blood group and thrombotic vascular disease. Thrombosis and Haemostasis 2014; 112:1103-1109
17. Liumbruno GM, Franchini M. Beyond immunohaematology: the role of the ABO blood group in human diseases. Blood Transfusion 2013; 11:491 – 499.
18. Gallinaro L, Cattini MG, Pontara E, Pagnan A, Casonato A, Daldone V, Sartorello F. A shorter von Willebrand Factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand Factor Blood 2008; 111:3540 – 3545.
19. Rios DRS, Fernandes AP, Gomes KB, Dasse LMS, Carvalho MG. Relationship between ABO blood groups and von Willebrand Factor, ADAMTS13 and Factor VIII in patients undergoing hemodialysis. Journal of Thrombosis and Thrombolysis 2012; 33:416-421.
20. Casari C, Lenting P, Wohner N, Christophe O, Denis C. Clearance of von Willebrand Factor. Journal of Thrombosis and Haemostasis 2013; 11:202-211.
21. Matsui T., Titani K, Mizuochi T. Structures of the asparagine-linked Oligosaccharide chains of human vWF. Occurrence of blood group A, B and H (O) structures. J. Biol Chem. 1992; 267:8723-31.
22. Payne AB, Miller CH, Hooper WC, Lally C, Austin HD. High Factor VIII, von Willebrand Factor, and fibrinogen levels and risk of venous thromboembolism in blacks and whites. Ethnicity and Disease 2014; 24:169-14.
23. Zhou Z, Yu F, Buchanan A, Fu Y, Campos M, Wukk, Dong JF, possible race and gender divergence in association of genetic variations with plasma von Willebrand Factor: a study of ARIC and 1000 genome chohorts. PLOS ONE 2014;9:e84810
24. Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Variations in coagulation factors in women: effects of age, ethnicity, menstrual cycle and combined oral contraceptive Thromb Haemost 1999; 82:1456-61.
25. Paludo CA, Manfroi WC, Roisenberg 1, Ghisleni GL. Association studies between – 1185A/G von Willebrand Factor gene polymorphism and Coronary artery disease. Braz J Med Biol Res 2003; 36:709-714.
26. Cohen W, Castelli C, Alessi MC, Aillaud MF, Bouvet S, Saut N, Brunet D. ABO blood group and von Willebrand Factor levels partially explained the incomplete penetrance of congenital thrombophilia. Arteriosclerosis, Thrombosis, and Vascular 2012, 32: 2021-2028.
27. Cowman J, Dunne E, Oglesby I, Byrne B, Ralph A, Voisin B, Kenny D. Age-related changes in platelet function are more profound in women than in men. Scientific Reports 2015; 5:12235.
28. Iushikawa S, Kario K, Nago N, Kayaba K, Goto T, Tsutsumi A, Nakamura Y. Factor VII and fibrinogen levels examined by age, sex, and other atherosclerotic risk factors in a Japanese population. The Jichi Medical School Cohort Study. Thrombosis and Haemostasis 1997; 77:890-893.
29. Kokame K, Sakata T, Kobubo Y, Miyata T. von Willebrand Factor-to-ADAMTS13 ratio increases with age in a Japanese population. J Thromb Haemost. 2011;9:1426-8.
30. Song J, Chen F, Campus M, Bolgiano D, Houakk Wu KK, Boerwinkle, Couper D. Quantitative influence of ABO blood groups on Factor VIII and its ratio to von Willebrand Factor, novel observations from an ARIC study of 11, 673 subjects. PLOS ONE 2015; 10: e0132626.

31. Bain BJ, Lewis SM, Bates I. Basic Haematological Techniques. In: SM Lewis, BJ Bain, I Bates (Eds). Dacie and Lewis Practical Haematology 10th ed. Philladelphia Churchill Livingstone. 2006; 36-39.
32. Laffan M, Brown SA, Collins PW. The diagnosis of von Willebrand disease. A guideline from the UK Haemophilia Centre Doctors Organization Haemophilia 2004; 10:199-217.
33. Miller C, Haff E, Platt S, Rawlins P, Drews C, Dilley A, Evatt B. Measurement of von Willebrand Factor activity: relative effects of ABO blood type and race. Journal of Thrombosis and Haemostasis 2003; 1:2197.
34. Favaloro EJ, Soltani S, Mc Donald J, Grezchnik E, Easton L, Favaloro JW. Reassessment of ABO blood group, sex, and age on laboratory parameters used to diagnose von Willebrand disorder. American Journal of Clinical Pathology 2005; 124:910-916.
35. Albanez S, Ogiwara K, Michels A, Hopman W, Grabell J, James P, Lillicrap D. Aging and ABO blood type influence vWF and FVIII levels through interrelated mechanisms. Journal of Thrombosis and Haemostasis 2016; 14:953-963.
36. O'Donnell J, Boulton FE, Manning RA, Laffan MA. Amount of H antigen expressed on circulating von Willebrand Factor is modified by ABO blood group genotype and is a major determinant of plasma von Willebrand Factor antigen levels. ArteriosclerThrombVascBiol 2002; 22:335-41.
37. Coppola R, Mari D, Lattuada A, Franceshi C. von Willebrand Factor in Italian Centenarians. Haematologica 2003; 88:39-43.
38. Feys HB, Canciani MT, Peyvandi F, Deckmyn H, Vanhoorelloeke K. ADAMTS13 activity to antigen ratio in physiological and pathological Conditions associated with an increased risk of thrombosis. British Journal of Haematology 2007; 138:534-540.
39. Mannucci PM, Capoferri C, Canciani MT. Plasma levels of von Willebrand Factor regulate ADAMTS13 its major cleaving protease. British Journal of Haematology 2004; 126:213-218.
40. Bowen DJ. An influence of ABO blood group on the rate of proteolysis of von Willebrand Factor by ADAMTS13. J Thromb Haemost 2003; 1:33-40.
41. Peng J, Rong Z, Fangshae L, Pans, Harjun C. Influences of ABO blood group, age and gender on plasma coagulation factor VIII, fibrinogen, von Willebrand Factor and ADAMTS13 levels in a Chinese population. Peer J 2017; 5: e3156.
42. Belch J, Douglas V, Nuki G. Macrovascular Disease and Systemic Sclerosis. Annals of Rheumatic Disease 200;59:1-5.
43. Merkel S, Schellerer V. The Clinical value of von Willebrand Factor in colorectal carcinomas. AMJ Transl Res 2011; 3:445-453.
44. Zuo L, Han J, Wang J, Pan S. Increased active von Willebrand Factor during disease development in the aging diabetic patient population. Age (Dordr) 2011; 20:1-5.
45. Campos M, Sun W. Genetic determinants of Plasma von Willebrand Factor Antigen Levels: target gene SNP and haplotype analysis of ARIC cohort. Blood Journal 2011; 117:5224-5230.
46. Blann AD. Normal levels of von Willebrand Factor antigen in human body fluids. Biologicals 1990; 18:351-353.
47. Asuquo I, Okafor I, Usanga A, Isong I. von Willebrand Factor antigen levels in different ABO blood groups in a Nigerian population. International Journal of Biomedical Laboratory Science 2014; 1:24-8.
48. Harrison RL, Mckee PA. Estrogen stimulates von Willebrand Factor production by cultured endothelial cells. Blood Journal 1984; 63:657-664.
49. Werner EJ, Broxson EH, Tucker EI. Prevalence of von Willebrand disease in children: a multiethnic study. J. Pediatr 1993; 123:893-8.