

ABO blood group status and Von Willebrand Factor antigen levels in a cohort of 100 blood donors in an African population**Akpan, IS* and Essien, EM***Lecturer, Department of Haematology, College of Health Sciences, University of Uyo, Akwa Ibom State, Nigeria****Correspondence Info:**

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Abstract

Von Willebrand Factor (vWF) antigen levels are known to vary widely in healthy individuals. Approximately 30% of the variation in plasma vWF is due to the effect of ABO blood group. The aim of this study was to determine the plasma vWF levels in different ABO blood groups in an African population. Using blood samples of 100 blood donors at the Blood Bank Unit, University of Uyo Teaching Hospital, Uyo, Nigeria, the ABO blood group phenotype was determined by standard tube method while von Willebrand factor antigen (vWF: Ag) was determined by ELISA Method. There were 63 male subjects and 37 female subjects with a mean age of 31.7 ± 6.39 years. The frequency of O, A, B and AB blood groups were 50%, 31%, 16% and 3%, respectively. The mean plasma vWF: Ag concentration of the subjects was 1.38 ± 1.02 IU/ml with blood group A having the lowest mean vWF: Ag level (1.087 ± 0.402) followed by group O (1.2896 ± 1.005), followed by group AB (1.687 ± 1.061), followed by group B (2.176 ± 1.487) having the highest level. This study reaffirms the well-known notion that ABO blood group influences the plasma level of vWF: Ag and also demonstrates that vWF levels in group O individuals are lower than non-group O individuals in Nigeria, Africa.

Keywords: von Willebrand Factor, von Willebrand Factor Antigen, ELISA, ABO blood group, Blood Bank Unit.

1. Introduction

The ABO blood group system, arguably the best known, and yet the most functionally mysterious, genetic polymorphism in humans [4, 5], was first described by the Austrian Nobel Prize winner, Karl Landsteiner in 1900.[4] The antigens of the ABO blood group system (A, B and H determinants) consist of complex carbohydrate molecules expressed on the extracellular surface of the red blood cell (RBC) membranes. However, the ABH antigens are not confined to RBCs, but are widely expressed in a variety of human cells and tissues including the epithelium, sensory neurons, platelets and the vascular endothelium.[6] Thus, ABO matching is critical not only in blood transfusion but also in cell, tissue and organ transplantation.[7]

The clinical significance of the ABO blood group extends beyond the traditional boundaries of immunohaematology and transfusion medicine, to the pathophysiology of a wide range of human diseases, such as cancers, infections, cardiovascular and thrombo-haemorrhagic disorders.[8] Studies indicate that the ABO blood group also exerts a profound influence on haemostasis, documented by the close relationship between ABO blood type and von Willbrand Factor (vWF).[2, 3, 9]

Von Willebrand Factor was first described by Eric Adolf von Willebrand, a Finnish pediatrician, in 1926.[10] It is a large multimeric glycoprotein produced in the megakaryocytes and endothelial cells. It mediates adhesion and aggregation of platelets in blood vessels and also serves as a carrier for factor VIII (FVIII) protein in plasma.[10] Deficiency of vWF results in von Willebrand Disease, the most common inherited bleeding disorder in humans.[11]

Although the risk of bleeding segregates with phenotypic extremes, plasma vWF levels are highly variable and present as a broad continuum within the general population, typically ranging between 50% and 200% of the population average among phenotypically normal individuals. The true impact of this variability on disease risk can be difficult to determine, particularly when distinguishing between individuals with vWF levels within lower range of 'normal' and upper range of those seen in type I vWD (20-50% of the mean).[12] About 60% of the variations are caused by genetic factors, with ABO blood group accounting for about 30%. [2, 3] In a study by Gill *et al*, von Willebrand factor levels in a normal population is known to be 25-35% lower in group O individuals than non-group O. Therefore

blood group specific reference ranges have been widely advocated for the diagnosis of von Willebrand Disease.[13] Other environmental or circumstantial factors contributing to VWF levels include age, stress, drugs and hormonal status.[14]

Most of the studies on the effect of ABO blood group on the plasma levels of vWF have been carried out in Oriental and Western populations and there is paucity of information on this relationship in Nigeria and several other African countries.[3, 6, 13, 15] The aim of this study, therefore, is to determine the influence of ABO blood group on plasma level of VWF antigen in a Nigerian population and to compare the results to reports in other climes.

2. Materials and methods

This was a cross-sectional analytical study. A total of 100 blood donors attending the Blood Bank Unit of the University of Uyo Teaching Hospital (UUTH), Uyo, Nigeria were recruited into the study. Apparently healthy, consenting subjects, between the ages of 18 and 65 years were included in the study while those excluded were non consenting subjects and those on medications such as anticoagulants, contraceptives, anti-platelet drugs such as aspirin and herbal remedies.

Under aseptic condition, 10 ml of free-flowing venous blood was obtained from each subject. Half (5 ml) of this was dispensed into ethylene-diamine-tetraacetate (EDTA) bottles for full blood count (FBC), ABO and Rh D blood grouping. The second aliquot of 5 mls of blood was dispensed into a trisodium citrate specimen bottle, and centrifuged at 3000g for 10 minutes at 4°C within 30 minutes of collection and stored in aliquots at -80°C 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 method as described by Bain and Lewis [16] 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 were 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 before

commencement of the study. Informed consent was sought and obtained in writing from subjects included in the study.

3. Results

A total of 100 blood donors participated in this study, 63 (63%) were males and 37 (37%) were females with a male to female ratio of 1.7:1. The age range was 21-53 years with a mean of 31.7 ± 6.39 years. Table 1 show the age and sex distribution of these donors.

Donors with blood group O were the majority with a total of 50 subjects (50%) and mean vWF: Ag level of $1.2896 \pm 1.0.05$ 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 a vWF: Ag level of 1.082 ± 0.402 , while subjects with blood group B accounted for 16 (16%) with mean vWF: Ag level of 2.176 ± 1.487 . Table 2a shows the distribution of vWF: Ag levels among the various ABO blood groups.

Results showed that subjects with non-O blood group had a level significantly higher than those of group O. Table 2b shows the distribution of vWF: Ag levels among non-O and O blood group donors in UUTH, Uyo.

A comparison of the mean vWF: Ag levels of the various ABO blood groups using the kruskal Wallis rank test showed that the differences between their means were statistically significant ($p < 0.05$). Table 3 shows the relationship between mean plasma vWF: Ag concentrations and ABO blood groups of donors at UUTH, Uyo. There was no statistically significant difference between the plasma vWF: Ag concentration and the RhD positive and RhD negative blood groups (Table 4).

Male subjects in the study presented with a mean vWF: Ag level of 1.19 ± 0.75 IU/ml while female subjects had a mean vWF: Ag level of 1.71 ± 1.3 IU/ml. A comparison of the mean values between the males and females showed a statistically significant relationship ($p < 0.0126$). Table 4 shows the relationship between mean plasma vWF: Ag concentrations and the sex/gender of blood donors in UUTH, Uyo.

Table 1: Age and sex distribution of 100 blood donors in UUTH, Uyo

Age group(years)	Frequency(n)	Percentage (%)
20-29	25	25
30-39	60	60
40-49	10	10
50-59	5	5
60-69	-	-
70+	-	-
Total	100	100
Sex		
Male	63	63
Female	37	37
Total	100	100

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

(a) Blood Group	Mean	Standard Deviation
A+	1.08	0.402
AB+	1.686	1.0608
B+	2.27	1.57
B ⁻	1.53 ⁻	0.57
O+	1.24	1.00
O-	2.00	1.04
	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 (IU/ml) and ABO Blood Groups of 100 Donors at UUTH, Uyo

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

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

Table 4: Relationship between Plasma vWF:Ag concentration IU/ml and RhD blood groups of the Blood Donors.

Blood type	Observation (n)	Rank sum	Z - value	P
Rh D+	95	4707.5	1.44	0.1546
Rh D-	5	342.5		

n= no of observations

Table 5: Relationship between plasma vWF: Ag concentration IU/ml and Sex (Gender) of Blood Donors at UUTH, Uyo

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

P= 0.0126, P<0.05

4. Discussion

The ABO blood group has been known to exert a major quantitative effect on circulating vWF levels. Studies have shown that 60% of the variation observed in plasma vWF levels is genetically determined while 30% of the total variation is explained by the effect of the ABO blood group. Ostavik[2] and associates clearly demonstrated that the effect of the ABO blood group on plasma vWF level is the result of a primary effect of the ABO locus. von Willebrand Factor is one of the few non-erythrocyte proteins that expresses ABH

antigens and ABH oligosaccharide structure has been identified on the N-linked oligosaccharide chains of vWF. The N-linked oligosaccharide side chains on VWF molecules contain A and B blood group antigens which are encoded by the blood group gene (ABO), which is located on the long arm of chromosome 9. The presence of A and B antigens leads to a decreased vWF clearance[17] hence individuals with blood group A, B, or AB (non-O blood groups) have approximately 25% higher vWF: Ag level than individuals with blood group O[13]. Similar findings have been reported in other studies.[2, 3]

The reduced vWF: Ag levels in blood group O individuals involves a decreased survival due to increased susceptibility to cleavage by ADAMTS13 which is significantly faster for group O vWF compared to non-group O vWF persons in the following order: $0 \geq B > A \geq AB$. [17] The mechanism by which ABO blood group influences the susceptibility to proteolysis by ADAMTS13 is still elusive, but two N-linked potential glycosylation sites (asparagines 1515 and 1574) are located in close proximity to the ADAMTS13 cleavage site (Tyr 1605 – Met 1606 bond within the A2 domain of vWF). Thus, the oligosaccharide chain composition may be involved in stabilizing the conformation of this vWF region, such that the removal of terminal sugar allows the A2 domain to adopt a conformation more permissive for ADAMTS13 cleavage.[17]

In this study, vWF: Ag levels were found to be lower in blood group O subjects than in non-O subjects, consistent with previous reports. Individuals with blood group B had the highest plasma vWF: Ag levels while the blood group A subjects had the lowest levels. However, in the study done by Bowen *et al*[18] group B vWF was reported to be more susceptible to ADAMTS13-induced proteolysis than group A vWF. Our findings are also at variance with those of Gill *et al*[13] who reported the highest plasma vWF: Ag levels in AB subjects and the lowest levels in O subjects. The findings of high plasma vWF: Ag levels in AB subjects in the study done by Gill *et al*[13] are of immense clinical relevance as individuals of the blood groups in question with genetic defects of vWF may have the diagnosis of vWD overlooked because vWF: Ag levels are elevated.

In relation to Rh status, there is no demonstrable effect of Rh blood group on plasma vWF: Ag level in the literature. The result of this study did not also show any statistically significant association between the RhD blood group in general and the plasma vWF: Ag concentration (P= 0.1546; Table 4). Further studies with larger sample size are required to examine the effects, if any, of the other Rh phenotypes on the plasma vWF: Ag levels.

The mean plasma vWF: Ag levels of the female subjects (1.71 ± 1.32) was significantly greater than that of male subjects (1.19 ± 0.75). This could be due to estrogen-induced synthesis of vWF: Ag in female respondents.[19] These results were not consistent with the findings of Campos

[20] and associates, who reported that the mean vWF: Ag level for males ($112.99 \pm 42.4\%$) was significantly higher than that for females ($111.1\% \pm 42.4\%$). Other workers such as Bland [21] and Easton [22] and their associates, however reported no variation in plasma vWF: Ag levels in relation to gender.

5. Conclusion

We report our findings from this study which shows that among apparently healthy Nigerians in Uyo, Akwa Ibom State, vWF: Ag levels are lower in group O than non-group O individuals with higher levels occurring in females than males. Owing to the known heterogeneity within the plasma vWF: Ag levels of different ABO blood group phenotypes, we recommend that further studies should be performed in other regions of Nigeria and sub-Saharan Africa to establish the ABO group specific vWF: Ag reference ranges.

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