Is there any Relationship between Rh(D) Blood Group and Von Willebrand Factor Antigen Concentration?

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Abstract: *Background:* Several reports have documented the influence of ABO blood group on plasma von Willebrand Factor Antigen (vWF:Ag) levels. However, a thorough search through the literature has not revealed any study on the relationship between Rh blood group and plasma von Willebrand Factor antigen levels.

Objective: The aim of this study was to determine the relationship between plasma vWF:Ag levels and Rh(D) blood group among apparently healthy Nigerian subjects.

Methods: This was a cohort study of 100 Blood Donors attending the Blood Bank Unit at University of Uyo Teaching Hospital, Uyo, Nigeria. Plasma vWF:Ag levels were determined by ELISA method while the ABO and Rh(D) blood group phenotypes were determined using the standard tube method. The data were analyzed with SPSS version 16.0. Chi square was used for test of significance.

Results: The study population consisted of 63 (63%) males and 37 (37%) females with a mean age of 31.7 ± 6.39 years. The frequency of Rh(D) positive and Rh(D) negative blood groups were 95% and 5%, respectively. The mean plasma vWF:Ag concentration of the subjects was 1.38 ± 1.02 IU/ml. Group B Rh(D) positive subjects had the highest mean vWF:Ag level (2.27 ± 1.57 IU/ml), followed by group O Rh (D) negative (2.00 ± 1.04 IU/ml), group AB Rh(D) positive (1.69 ± 1.06 IU/ml), group B Rh(D) negative (1.53 ± 0.57 IU/ml), group O Rh(D) positive (1.24 ± 1.00 IU/ml) and group A Rh(D) positive (1.08 ± 0.40 IU/ml) having the lowest level.

Conclusion: There was no statistically significant association between the plasma vWF:Ag levels and Rh(D) blood groups of study subjects (P = 0.1546). However, further research with larger sample size is required to determine the relationship between plasma vWF:Ag levels and Rh blood group in general before reaching definite conclusion about the lack of influence of Rh blood group on the plasma level of vWF:Ag

Keywords: Von willebrand factor antigen, ABO blood group, Rh(D)blood group, ELISA, Uyo.

INTRODUCTION

The Rh blood group system, formerly referred to as Rhesus blood group system, was discovered in 1940 by Karl Landsteiner and A. S. Wiener [1]. It is unarguably the most polymorphic protein-based blood group system. With more than 60 antigens so far described, it is the largest blood group known in humans. The usually large number of Rh antigens is attributable to its intricate genetic basis [2]. These antigens (Rh D and Rh CE determinants) consist of complex protein molecules expressed in the membrane of red blood cells and their immediate precursors [3]. Thus, Rh matching is crucial in blood transfusion and stem cell transplantation [4].

The Rh blood group is second in its clinical importance only to the ABO blood group in the field of transfusion medicine [5]. It is of utmost relevance in Obstetrics, being the major cause of haemolytic disease of the newborn (HDN) [6]. Its significance is related to the fact that the Rh antigens, particularly the D antigen, are highly immunogenic. In the case of the D antigen, individuals who do not express D antigen on their red cells will produce anti-D antibodies if they encounter the D antigen on transfused RBCs (causing a haemolytic transfusion reaction, HTR) or on fetal RBCs (causing haemolytic disease of the newborn, HDN) [6, 7]. Aside from its importance in blood transfusion practice, the Rh blood group is useful in population genetic studies, researching population migration patterns as well as resolving certain medico-legal issues, especially of disputed paternity cases [8, 9]. Also, its relation to myeloid diseases such as acute or chronic myeloid leukaemia, myeloid metaplasia, polycythaemia vera and myelofibrosis has been reported [10]. However, studies on the role of Rh blood group in haemostasis are few and far between. Furthermore, there are no published reports on the relationship between Rh blood group and von Willebrand Factor Antigen.

Von Willebrand Factor is named after Dr. Erik von willebrand, a Finnish Paediatrician who first described it in 1926. It is a large multimeric protein produced in the endothelial cells and megakaryocytes. It mediates adhesion and aggregation of platelets in blood vessels

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and also serves as a carrier molecule for Factor VIII (FVIII) protein in plasma [11]. Deficiency of vWF:Ag results in von Willebrand Disease, the most common inherited bleeding disorder in humans [12].

Several factors are known to affect the plasma concentration of vWF:Ag. About 60% of the variations are caused by genetic factors, with ABO blood group accounting for about 30% while 20% of the variability can be traced to the vWF gene [13, 14]. Environmental Factors such as age, stress, exercise, pregnancy and drugs account for the remainder [15].

Multiple studies have consistently reported that ABO blood group has a profound effect on the plasma levels of vWF:Ag [13,14,16,17]. However, as earlier highlighted, there is no study addressing the effect of Rh blood group on vWF:Ag in the literature. Thus, this study seeks to examine the relationship between Rh blood group and plasma vWF:Ag level. It is envisaged that information from this work would add to existing body of knowledge as well as serve as a template for further research

MATERIALS AND METHODS

Study Site/Design

This was a cross-sectional descriptive study of 100 blood donors who attended the blood bank unit of the University of Uyo Teaching Hospital (UUTH), a tertiary referral hospital in Uyo, Akwa Ibom State, South-south Nigeria. The hospital provides specialized healthcare services to the indigenes of Uyo and its environs with a population of 4 million people [18].

Inclusion and Exclusion Criteria

Apparently healthy, consenting subjects, between the ages of 18 and 65 years were included in the study. Non consenting subjects and those on medications such as anticoagulants, contraceptives, anti-platelet drugs and herbal concoctions were excluded.

Procedure

10ml of free-flowing venous blood was obtained from each subject under aseptic condition. Half (5ml) of this was dispensed into ethylene-diamine – tetracetate (EDTA) bottles for full blood count (FBC), ABO and RhD blood grouping. The second aliquot of 5ml of blood was dispensed into a trisodium citrate specimen bottle, and centrifuged at 3000g for 10minutes at 4^0C within 30 minutes of collection and stored in aliquots at – 80^0C 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 Dacie and Lewis [19] 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.

Data Analysis

The data were collated, analyzed using statistical package for social sciences (SPSS) windows version 16.0 and presented in simple frequency tables. The comparisons were carried out with chi-square test as appropriate and the p-value of less than 0.05 was used to determine the level of statistical significance.

Ethical Consideration

This study was conducted after receiving appropriate approval from Ethics and Research committee of our hospital. Informed consent was obtained in writing from all subjects.

RESULTS

A total of 100 blood donors, aged 21 – 53years (mean age 31.7 + 6.39years; 63 males, 37 females) were included in the study. Age and sex distribution of the donors are summarized in Table **1**.

Donors with blood group O^+ were the majority with a total of 47 subjects (47%) and mean vWF:Ag level of 1.24 ± 1.00 IU/ml while group B^- had the least with 2 subjects (2%) with mean vWF:Ag level of 1.53 ± 0.57 IU/ml. Subjects with blood group A^+ were a total of 31 (31%) and had a vWF:Ag level of 1.08 ± 0.40 IU/ml, while subjects with B^+ accounted for 14 (14%) with mean vWF:Ag level of 2.27 ± 1.57 IU/ml. Blood groups O^- and AB^+ accounted for 3 (3%) each and had mean

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
40-59	10	10
50-59	5	5
60-69	-	-
70^{+}	-	-
Total	**100**	**100**

Sex		
Male	63	63
Female	37	37
Total	**100**	**100**

vWF:Ag levels of 2.00 ± 1.04 IU/ml and 1.67 ± 1.06 IU/ml, respectively. Table **2a** shows the distribution of vWF:Ag levels among the various ABO and Rh(D) blood groups.

Results showed that subjects with non-O blood group had a plasma vWF:Ag level significantly higher than those of group O (Table **2b**)

Table 2: Distribution of von-Willebrand Factor Antigen Concentration (vWF:Ag, IU/ml) among the ABO and Rh(D) 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

A comparison of the mean vWF:Ag levels of the various ABO blood groups using 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 the donors while Table **4** shows the relationship between mean plasma vWF:Ag concentrations and ABO and Rh blood groups (P < 0.05). However, there was no statistically significant difference between the plasma vWF:Ag concentration and the Rh (D) blood group (Table **5**).

Table 3: Relationship between Mean Plasma vWF:Ag Concentration (IU/ml) and ABO Blood Groups of 100 Donors of UUTH, Uyo

ABO Blood Group	Frequency (n)	Kruskal Wallis
A	31	1394.00
AB	3	184.00
B	16	1161.50
O	50	2310.50
Chi-square = 11,918 with 3df, P = 0.0077		

Table 4: Relationship between Plasma vWF:Ag Concentration IU/ml and ABO and Rh (D) Blood Groups of Donors at UUTH, Uyo

ABO Blood Group	Frequency (n)	Kruskal Wallis Rank Sum
O^{+}	47	2102.00
A^{+}	31	1394.00
B^{+}	14	1027.50
AB^{+}	3	184.00
O^{-}	3	208.50
B^{-}	2	134.00
Chi-square = 14,060 with 5df, P = 0.0152		

Table 5: Relationship between Plasma vWF:Ag Concentration IU/ml and Rh(D) Blood Groups of the Blood Donors

Blood Group	Observation (n)	Rank Sum	Z^{-} Value	p
RhD^{+}	95	4707.5	1.44	0.1546
RhD^{-}	5	342.5		

n = no of observations

DISCUSSION

The main objective of this study was to investigate whether there is any relationship between Rh(D) blood group and plasma von Willebrand Factor Antigen level using a cohort of 100 blood donors in a Southern Nigerian population. The literature is replete with publications of an association of plasma von Willebrand Factor Antigen level with many biologic factors. Von

Willebrand Factor antigen has been known to vary widely in healthy individuals [16, 17, 20]. Both environmental and genetic factors contribute to this variation. Increased levels of vWF:Ag are associated with stress, exercise, advancing age, pregnancy and when oestrogen levels are raised [15]. The environmental factors are known to stimulate the endothelial cells to secrete vWF, whereas the genetic factors play a dominant role in determining the baseline level of vWF. Genetic factors are thought to account for 70% of the variability, of which approximately 30% is due to the effect of ABO blood group [16]. Several studies have documented the influence of ABO blood group on plasma vWF levels [13, 14, 16, 17]. Despite the prodigious body of evidence which affirms the effect of ABO blood group on plasma levels of vWF:Ag, the role of Rhblood group has remained elusive, as previous studies have primarily focused on the former blood group. It is in the context of the foregoing that we considered it imperative to undertake this study.

In the present study, the influence of ABO blood group on plasma von Willebrand Factor antigen level was also evident. We observed a statistically significant association between ABO blood group and plasma vWF:Ag levels (Tables **3** and **4**). Also, vWF:Ag levels were found to be lower in blood group O subjects compared to non-O subjects (Table **2b**). This is consistent with earlier reports [14, 16, 17]. The plausible explanation for this observation involves the effect of the ABO locus and altered susceptibility of the different ABH determinants to cleavage by ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type – 1 repeats – 13) metalloprotease. vWF is one of the few non- erythrocyte proteins that expresses ABH antigens and ABH oligosaccharide structures have been identified on the N-linked oligosaacharide chains of vWF. These side chains contain A and B blood group antigens which are encoded by the ABO blood group gene, located on the long arm of chromosome 9. The presence of A and B antigens leads to decreased susceptibility of vWF to cleavage by ADAMTS13. Thus, individuals with blood groups A, B and AB (non – O blood groups have approximately 25% higher vWF:Ag levels than individuals with blood group O [21]. A similar finding has been reported in a study done by kumara and associates [22].

Furthermore, Bowen *et al.* [23] reported that increased vWF clearance is significantly faster for group O compared to non-group O vWF persons in the following order: O ≥ B > A≥ AB. The mechanism by which ABO blood group influences the catabolism of vWF is not understood fully, 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). Therefore, the oligosaccharide chain composition may be responsible for stabilizing the conformation of this vWF region, such that the removal of terminal sugar permits the A2 domain to adopt a configuration more permissive for ADAMTS13 proteolysis [24].

In relation to Rh status, the result of this study did not show any statistically significant association between the Rh(D) blood group and the plasma vWF:Ag concentration. Interestingly though, this study has established for the first time that Rh(D) phenotype is not a modulator of plasma vWF:Ag level. However, further studies with larger sample size are needed to examine the effects, if any, of the other Rh phenotypes or genotypes.

CONCLUSION

This study has shown that plasma vWF:Ag levels in healthy Nigerian subjects vary widely. It is also evident from this work that ABO blood group but not Rh(D) blood group has a significant influence on plasma vWF:Ag levels and needs to be taken into cognizance when establishing reference range for vWF:Ag. However, we recommend that further studies including community – based survey with larger sample size should be conducted in other parts of the world to determine the relationship between the Rh blood group in general and plasma vWF:Ag concentration for the purpose of validating the present observation. It is hoped that this work will form the basis for a more extensive prospective study in the nearest future.

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