Original Article

Von Willebrand Factor Antigen Levels in Different ABO Blood Groups in a Nigerian Population

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Von Willebrand factor is a glycoprotein produced in the endothelium and megakaryocytes. Its levels are known to vary between individuals. About 60% of the variations are caused by genetic factors with ABO blood group accounting for about 30%. This research was carried out to determine von Willebrand factor antigen (vWf:Ag) levels in different ABO blood groups in a Nigerian population. Blood samples were collected from 207 apparently healthy residents of Calabar, Nigeria, for the determination of ABO blood group phenotypes and vWf:Ag levels. The subjects were aged between ten and seventy years. Both male and female subjects were involved. ABO blood group phenotype was determined by standard tube method while vWf:Ag was determined by ELISA method. The result showed that 48 (23.2%) of the subjects were of blood group A, 48 (23.2%) belonged to group B, 5 (2.4%) were of group AB, while group O subjects constituted 106 (51.2%) of the subjects. The mean vWf:Ag for all the subjects was 93.79 ±26.2%. Group A had mean value of 101.38 ± 24.26%, group B had 110.63 ± 21.36%, group AB had a level of 104.60 ± 24.17% and group O had a mean value of 82.23 ± 26.28%. A comparison of the mean levels between group O and non-group O individuals showed that non-group O had significantly higher levels than group O ($p<0.05$). The male in this study had mean vWf:Ag level of 91.43 ± 24.85% while the female subjects had a value of 96.09 ± 27.54%. The subjects aged 10-30 years had vWf:Ag level of 92.81 ± 25.90%, those between 31-50 years had mean value of 93.2 ± 27.64% while subjects aged 51-70 years of age had mean vWf:Ag level of 108.73±18.18%. This study has shown that vWf:Ag is higher in non-group O than blood group O individuals and that sex does not affect the level of vWf:Ag.

Key words: Von Willebrand factor, von Willebrand factor antigen, ABO blood groups, Nigeria.

Introduction

The ABO blood group system was discovered by an Austrian scientist Carl Landsteiner in 1900. ABO is arguably the best known and yet the most functionally mysterious genetic polymorphism in humans. In clinical practice, ABO is the most important system for blood group compatibility [(1)].

In the century since their discovery ABO antigen association with infections and other diseases have been the subject of many publications [(2)]. The occurrence and severity of diseases like malaria, carcinoma of the stomach, peptic ulcer, pancreatic cancer and ovarian cancer have been attributed to ABO blood type.

Von Willebrand factor (vWf) is a large multimeric glycoprotein produced in the megakaryocytes and sub endothelial tissues [(3,4)]. The gene for vWf is located on the short arm of chromosome 12. von Willebrand factor

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expresses ABO antigen [5], and ABO blood group influences the level of von Willebrand factor in the blood [6]. Von Willebrand factor is named after Erick Adolf von Willebrand, a Finnish pediatrician who first described it in 1926 [7]. Von Willebrand factor antigen (vWf:Ag) or factor VIII-related protein is a plasma protein found in circulation combined by non-covalent interactions with factor VIII coagulant protein (FVIII:C).

Von Willebrand factor has two major functions in haemostasis. First it is essential for platelet sub endothelial adhesion and platelet to platelet aggregation. Second, von Willebrand factor is a specific carrier for factor VIII in the plasma and protects it from proteolytic degradation, prolonging its half-life in circulation and efficiently localizing it to the site of vascular injury [8]. Without von Willebrand factor the *in vivo* half-life of factor VIII is shortened from 10-12 hours to few minutes [9].

Deficiency of von Willebrand factor is responsible for von Willebrand disease while increase levels predisposes to thrombotic diseases [10]. The plasma level of vWf varies significantly between and within individuals. Within individuals it is known to vary between repeated examinations [11]. Variations have been associated with ABO blood type, oestrogen levels, age, race, pregnancy and smoking [12]. About 60% of the variations are caused by genetic factors with ABO blood group accounting for about 30% [13].

In the normal population, von Willebrand factor level is known to be 25% - 35% lower in group O individuals than non-group O [14]. Studies have shown that individuals carrying one O allele (AO or BO) have significantly lower plasma levels of vWf than those carrying no O allele (AA, AB and BB) [15]. The reduced levels of the factor in group O is attributed to increased clearance of vWf via a fucose mediated mechanism[4]. Bowen [16] subjected vWf of different ABO blood groups to proteolysis by A disintegrin and metalloproteinase with thrombospondin 1 motif 13 (ADAMTS 13) and concluded that there was greater rate of proteolysis for group O vWf than for non-group O vWf.

Blood group specific reference ranges have been advocated for the diagnosis of von Willebrand disease [17]. But Favaloro et al., [18] concluded that the use of blood group specific reference ranges, though scientifically sound, lacks clinical usefulness as it does not assist in identifying people at increased risk of bleeding.

Most of the published works on effect of ABO blood group on the levels of von Willebrand factor have been carried out on the Caucasians. Very little work on this subject has been done on Nigerians. Since there is a well known difference in the physiological system and disease pattern between the two races, there is need for studies to be carried out on Nigerian population so that inferences drawn from such studies can be appropriately applied to the local population. This study is aimed at ascertaining if the reported influence of ABO blood groups on plasma levels of von Willebrand factor antigen reported in other centres is the same in our environment.

Materials and methods

A total of two hundred and seven apparently healthy residents of Calabar metropolis in south south geo-political region of Nigeria were used for the study. The subjects were between ten (10) and seventy (70) years of age. Both male and female subjects participated in the study. Informed consent was obtained from all the subjects.

Six and a half millilitre of blood was collected from each subject by clean venous puncture into two sample containers; 2ml was delivered into plain container for the determination of ABO blood group while 4.5ml was placed in a bottle containing 0.5ml of 3.8% trisodium citrate for the estimation of vWf:Ag. The samples for the determination of vWf:Ag were centrifuged immediately at 2500rpm for 5 minutes and plasma extracted into sterile plain bottles. Estimation of vWf:Ag was carried out immediately using Enzyme Linked Immunosorbent Assay (ELISA) method with kit obtained from Helena Bioscience, United Kingdom (Lot 10979885). Standard tube method as described by Dacie and Lewis [19] was used for the determination of ABO blood group. All the antisera used were obtained from Biotec Laboratory, United Kingdom. Adequate controls were included.

Statistical Analysis

One way analysis of variance (ANOVA) was used to compare the mean ± SD of vWf:Ag levels between the various ABO blood group phenotypes. Student t-test was used to compare the mean vWf:Ag levels between the male and female subjects. Turkey test was used in a multiple comparison of the mean vWf:Ag of all the blood groups. One way ANOVA was used to compare mean ±SD vWf:Ag levels between three age groups.

Results

Table 1 shows the distribution of vWf:Ag levels among the various ABO blood groups. Group A had a total of 48 subjects (23.2%) and mean vWf:Ag level of 101.38±24.26%, group B accounted for 48 subjects (23.2%) with mean vWf:Ag level of 110.63±21.36%, group AB had 5 subjects (2.4%) with mean vWf:Ag level of 104.60±24.17%, while group O constituted 106 (51.2%) of the total subjects with mean vWf:Ag of 82.23±23.69%. A comparison of the mean vWf:Ag levels of the various ABO groups using one way ANOVA showed that the differences between their means were statistically significant ($p<0.05$).

Table 2 is a multiple comparison of the mean vWf:Ag of all the blood groups using Turkey test. The result confirmed that all the non-O blood groups had levels significantly higher than that of group O while the differences between the non-O blood groups were not statistically significant ($p>0.5$).

Table 3 shows gender distribution of vWf:Ag. Male subjects in the study were 102 (49.3%) and presented with a mean vWf:Ag of 91.43±24.85% while the female subjects constituted 105 (50.7%) with mean level of 96.09±27.54. A comparison of the mean values between the males and females did not indicate any significant difference ($p>0.05$).

Table 4 compares mean vWf:Ag of three age groups. Subjects between 10-30 years had mean value of 92.81±25.91%, those between 31-50 years presented with mean value of 93.23±27.64% while those in the age range of 51-70 years had value of 108.73±18.18%. The differences in their mean levels using one way ANOVA were not statistically significant ($p>0.05$).

Table 1 The distribution of vWf:Ag levels among the various ABO blood group phenotypes

ABO group	No	%	Mean vWf:Ag (%)
A	48	23.2	101.38 ± 24.26
B	48	23.2	110.63 ± 21.36
AB	5	2.4	104.60 ± 24.17
O	106	51.2	82.23 ± 23.69
Total	207	100	93.79 ± 26.28

P<0.05

Table 2 vWf:Ag levels between the various ABO groups

ABO group	diff.	SEM	p	Remark
B vs O	28.39	4.07	.00	S
B vs A	9.25	4.78	.05	N/S
B vs AB	6.02	11.00	.58	N/S
AB vs O	22.37	10.71	.03	S
AB vs A	3.23	11.00	.77	N/S
A vs O	19.15	19.15	.00	S

O<AB≤A≤B S=Significant NS=Non-significant

Table 3 The distribution of vWf:Ag levels between male and female subjects

Subjects	No	%	mean vWf:Ag (%)
Male	102	49.3	91.43 ± 24.85
Female	105	50.7	96.09 ± 27.54
Total	207	100	93.79 ± 26.29

p>0.05

Table 4 The distribution of mean vWf:Ag levels among three age groups.

Age (yrs)	No	%	vWf:Ag(%)
10-30	128	61.8	92.81 ± 25.91
31-50	68	32.9	93.2 ± 27.64
51-70	11	5.3	108.73 ± 18.18
Total	207	100	93.79 ± 26.28

p>0.05

Discussion

Von Willebrand factor is one of the few non-erythrocytic proteins that express ABO antigens. ABH oligosaccharide structure have been identified on the N-linked oligosaccharide chains of vWf located on the A_1 domain which contains the binding site for platelet glycoprotein [20].

This study has shown that vWf:Ag is significantly lower in individuals of blood group O than non-group O individuals. This finding agrees with that of earlier studies [13], [14], [18]. This difference may be due to the increased production of the factor in non group O individuals or increased clearance of the factor in group O individuals.

The possibility that the difference is due to increased synthesis in non-group O was rejected by earlier study by Brown et al.,[21]. The other possible mechanism of increased clearance is still controversial. O'Donnell et al.,[4] postulated that the increased clearance is facilitated by hepatic receptor that has affinity for H antigen, but findings that individuals with Bombay phenotype that also lack H antigen have lower levels of the factor than blood group OO genotype does not support such claim.

It is most likely that the nature of carbohydrate in ABO antigen determines the rate of proteolysis. Individuals of non-group O (AB, A and B) have more complex carbohydrate antigens and so have slower rate of proteolysis while individuals of Bombay phenotype with the least carbohydrate antigen content presents with the fastest rate of proteolysis. It is common knowledge that the nature of blood group antigen influences the vulnerability or resistance to certain diseases. For example, it requires the presence of Duffy antigen for infection with plasmodium *vivax* to occur[22], while the presence of blood group O reduces the severity of plasmodium falciparum infection[23].

A comparison of the levels of vWf:Ag between the male and female subjects did not show significant difference. The result agrees with earlier findings by Davies et al., [24] that sex has no effect on vWf:Ag.

Orstavik et al., [13] reported that age has influence on the level of von Willebrand factor. Our results show slight non significant increase with age and this coupled with the low number of individuals in the oldest group (11 as against 128 for the younger age group), we cannot confirm if age affects the level of von Willebrand factor in our subjects.

In conclusion, this study has emphasized that vWf:Ag level is higher in non-group O than in group O individuals and that sex does not affect the level of vWf:Ag. We recommend that separate reference ranges of vWf:Ag should be established for the various ABO blood group phenotypes and also in interpreting vWf:Ag results, ABO blood type should be taken into consideration.

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