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RESEARCH PAPER

RHESUS D DISTRIBUTION IN BLOOD GROUP OF INDIVIDUALS AND THE RELATIONSHIP WITH BLEEDING TIME

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ABSTRACT

Relationship between Rhesus factor and bleeding time in blood group O subjects selected randomly from Niger Delta University, Bayelsa State, Nigeria was investigated, using standard antisera and Duke's method. Group O^+ subjects had shorter bleeding time (1.32 ± 0.41min) than O^- (1.33 ± 0.37 min). Gender-wise, group O^+ females had shorter bleeding time (1.30 ± 0.40 min) than male O^+ (1.34 ± 0.42 min), and males with O^- blood type had shorter bleeding time (1.20 ± 0.39 min) than female O^- (1.39 ± 0.37 min). Comparison within same gender shows that group O^+ males had longer bleeding time (1.34 ± 0.42 min) than O^- males (1.20 ± 0.39 min), and the females with blood type O^+ had shorter bleeding time (1.30 ± 0.40 min) than O^- females (1.39 ± 0.37 min). All these observed differences were statistically significant at $p< 0.05$. In conclusion, the current study suggests that generally, blood group O^+ individuals have shorter bleeding time than O^-. Bleeding time appear prolonged in O^+ males than O^+ females and prolonged in O^- females than O^- males. Within gender, O^+ males appear to have longer bleeding time than O^- males, while O^+ females have shorter bleeding time than O^- females.

Key words: Rhesus factor, Blood group O, Bleeding time, male, female.

INTRODUCTION

The ABO and Rhesus blood group system have been recognized to be the most important clinically (Seeley et al, 1998), due to their significance in the avoidance of transfusion reactions and death (Honig and Bore, 1980). The distribution of ABO Rhesus blood types varies in different populations of the world. Blood group O has been reported as the most prevalent of ABO blood type in many studies; In Argentina Quiroga et al, 1988 reported 53.8% blood group O subjects in a study population. Peon-Hidalgo et al, 2002 reported 58.5% prevalence of group O among Argentina populace. In Nigeria, study by Kulgami et al,1985 shows the distribution is O (46.6%), A (23.1%), B (26%), AB (4.3%).

Recent studies showing how ABO Rhesus blood groups may make us prone to lethal diseases or even protect us from them can have profound life-saving implications for preventive medicine and in the development of new therapies. Flegr *et al*, 2015; Nejat, 2010). For instance, it is reported that the risk of developing gastric cancer was much higher for people with blood type A than those of blood group O. But people with blood group O had a greater risk of peptic ulcers (Gustaf *et al*, 2011). Furthermore, Nejat (2010) reported that women with blood type A may have higher chances of becoming pregnant, while those of blood type O have twice as high chances than blood group A. Women with blood type AB have double or triple risk of developing preeclampsia and those of blood type AB or B were reported to have high risk of developing ovarian cancer. Meanwhile women with blood group O are vulnerable to nonovirus which can cause vomiting and diarrhea. Recently, people with blood group O are reported to have lower risk of heart attacks but more common occurrence of epistaxis than other ABO blood groups (Reddy *et al*, 2008).

Epistaxis and other hemorrhagic disorders have been associated with Von Willebrand factors found on blood vessel walls and platelets; facilitating platelet adhesion, platelet aggregation, and regulation of circulating levels of factor VIII (anti-hemophilic factor A), (Ganong, 2012). Some research suggests interaction of genes, like gene locus of ABO blood group on the chromosome 9q34 with the Von Willebrand gene (Qureshi and Bhatti, 2003). Meanwhile it is not elucidated whether Rhesus D antigen has any interaction with Von Willebrand factors. This preliminary study was therefore to find out the distribution of Rhesus D antigen (Rhesus factor) in blood group O individuals and the relationship with bleeding time.

MATERIAL AND METHOD

STUDY AREA: The study was done among student's population in Niger Delta University, Wiberforce Island, Amassoma, Bayelsa State of Nigeria.

SAMPLE COLLECTION: Blood samples of 236 subjects randomly selected, were collected by finger prick under aseptic conditions for blood grouping. and bleeding time studies.

STUDY DESIGN: The study was designed to determine ABO blood groups of subjects with particular consideration of group O subjects among study samples drawn from the population and further investigation of the presence of Rhesus D Antigen among the group O subjects. Then determination of bleeding time and comparison of bleeding time with the Rhesus Antigen D distribution in the group O subjects along various categories; General comparison, between and within gender comparisons.

INCLUSION AND EXCLUSION CRITERIA: Students consent was sought before inclusion and those who were healthy and within age range of 18 to 32 years were included. Those who were hypertensive, diabetic previously or currently, and subjects below 18 and above 32 years were excluded. Only subjects who tested blood group O (positive or negative) were then involved in the bleeding time studies.

ETHICAL CLEARANCE: The study was conducted in compliance with the Declaration on the Right of the Patient (WMA, 2000) and the approval of the Niger Delta University Ethical Committee on Research involving human subjects.

SAMPLE ANALYSIS: Samples for blood grouping were mixed with normal saline to prepare red cell suspension. The suspension was then mixed with antisera anti-A, anti-B, Anti-D and covered with petri dish for 8 – 10 minutes. Blood group and Rhesus factor was determined by observing the presence or absence of agglutination under low-power objective microscope (Ghai, 2007). Bleeding time was estimated by Duke's method in 113 students who tested blood group O and either Rhesus D positive or negative. Under aseptic conditions, a deep finger prick was made and the length of time taken for bleeding to stop was recorded by blotting the drop of blood coming out of the incision every 30 second, using blotting paper (Schafer and Loscalzo, 2003). Then bleeding time was calculated by multiplying the number of spots on the filter paper and 30 seconds. Meanwhile, normal range is 1 -5minutes by Duke's method.

STATISTICAL ANALYSIS: The SPSS Version 16 was used for statistical analysis and the Student's t-Test was applied to compare separately, the relationship between the blood group O Rhesus D positive (O^+) and blood group O Rhesus D negative (O^-) with bleeding time. This comparison was also done between and within gender.

RESULTS

The result of the preliminary test for prevalence of ABO blood group as shown in Table 1 indicates that blood group O (47.9%) is most predominant among the study population, followed by B (25.4%) > A (23.7%) > AB (3%).

Table 1: Prevalence of ABO blood group in students of Niger Delta University

Blood group	A	B	AB	O
Frequency	56	60	7	113
Percentage	23.7%	25.4%	3%	47.9%

Difference is significant at $p < 0.05$

Rhesus factor distribution among the blood group O subjects is shown in table 2, where Rhesus D antigen is present (O^+) in 87.6% and absent (O^-) in 12.4%. Also, comparison of bleeding time between these O^+ and O^- individuals show it was shorter in O^+ than O^-.

Table 2: Distribution of Rhesus factor in blood group O individuals and relationship with bleeding time

Blood group O	Frequency	Percentage %	Mean bleeding time ± SD (min)
O^+	99	87.6	1.32 ± 0.41
O^-	14	12.4	1.33 ± 0.37

Difference is significant at $p < 0.05$

Result in table 3 shows that gender-wise, bleeding time was shorter in O^+ females than O^+ males.

Table 3: Distribution of blood group O^+ between gender and relationship with bleeding time

Blood group O^+	Frequency	Percentage %	Mean bleeding time ± SD (min)
Male	34	34.3	1.34 ± 0.42
Female	65	65.7	1.30 ± 0.40

Difference is significant at $p < 0.05$

The result in table 4 indicates that bleeding time was shorter in blood group O^- male subjects than O^- female subjects.

Table 4: Distribution of blood group O^- between gender and relationship with bleeding time

Blood group O^-	Frequency	Percentage %	Mean bleeding time ± SD (min)
Male	4	28.6	1.20 ± 0.39
Female	10	71.4	1.39 ± 0.37

Difference is significant at $p < 0.05$

Within the male subjects, bleeding time was longer in O^+ males than O^- males (Table 5).

Table 5: Distribution of Rhesus factor within blood group O Males and relationship with bleeding time

Male	Frequency	Percentage %	Mean bleeding time ± SD (min)
O^+	34	89.5	1.34 ± 0.42
O^-	4	10.5	1.20 ± 0.39

Difference is significant at $p < 0.05$

Within the female subjects, bleeding time was shorter in O^+ females than O^- females as shown in table 6 below

Table 6: Distribution of Rhesus factor within blood group O Females and relationship with bleeding time

Female	Frequency	Percentage %	Mean bleeding time ± SD (min)
O^+	65	86.7	1.30 ± 0.40
O^-	10	13.3	1.39 ± 0.37

Difference is significant at $p < 0.05$

DISCUSSION

The preliminary study was done with 236 students aged 18 – 32 years among whom 113 were blood group O. Among this blood group O subjects, those with Rhesus D antigen (O^+) was predominant in the general population (87.6%) and within gender; male (89.5%) and female (86.7%). Bleeding time was found to be shorter in O^+ subjects than O^-. Gender-wise; between genders bleeding time was shorter in O^+ females than O^+ males and shorter in O^- males than O^- females. Within gender, O^+ females had shorter bleeding time than O^- females but O^+ males had longer bleeding time than O^- males. All the observations were significant at $p < 0.05$.

Our preliminary investigation showing that blood group O was most abundant supports the reports of Kulgami *et al*, 1985; Mirda and Jena, 2016. The prevalence order of O > B > A > AB is similar to that in Pakistan (Egezie *et al*, 2008). The presence of Antigen D in people with blood group O appears to be associated with shorter bleeding time as shown in the study population from the comparison in general and within female gender. However, within male gender the presence of

Antigen D did not show same trend of reduced bleeding time. And in the absence of Antigen D, bleeding time was shorter in males than females.

Research groups have found that epistaxis is more common in blood group O compared to other ABO blood groups, may be due to lower expression of von Willebrand factor (vWF) in them (Reddy et al, 2008). Blood vessel walls and platelets contain vWF which helps in platelet adhesion and platelet aggregation, besides regulating circulating levels of factor VIII or anti-hemophilic factor A (Ganong, 2012). It is also reported that hemorrhagic disorders are due to deficiency of vWF, since it plays an important role in temporary hemostatic plug formation and activates clotting mechanism that leads to definite clot formation (Ruggeri, 2001; Ruggeri and Zimmerman, 1981). Furthermore, some research has shown that the location of gene coding for the A and B antigens is on chromosomes 9 and 19 (Schleef *et al*, 2004) and that other genes like gene locus of ABO blood group on the chromosome 9q34 have influence on the vWF gene (Reddy *et al*, 2008).

From the current study population, the presence of antigen D in blood group O subjects seem to reduce bleeding time when all O^+ and O^- subjects irrespective of gender were compared. This was same, when the O^+ and O^- females were compared. There may be a link between the antigen D and von Willebrand factor, perhaps at the genetic levels which is not elucidated; such that in the presence of antigen D, the expression of vWF may have accentuated to facilitate clotting mechanism and thus shorter bleeding duration/time. Although this trend was not consistent in the comparison of the O^+ and O^- males, in which case the males without antigen D had shorter bleeding time than O^+ males. This perhaps may be due to some other overriding factors not elucidated; which may also explain why in the absence of antigen D, males in this study population had shorter bleeding time than the females.

Conclusion and Recommendation

In conclusion, the presence of antigen D (Rhesus factor) in blood group O individuals appears to be associated with reduced bleeding time compared to blood group O people without Rhesus factor. However, in males the present investigation did not show same trend. We recommend further studies to elucidate these observations: Elaborate general and gender-based study on possible genetic interaction between the VwF and antigen D.

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AUTHORS CONTRIBUTION

All three authors participated in the study. Erigbali, P.P initiated the experimental design and other aspects of the work. Potts-Johnson, G was involved in the collection and collation of data, while Koikoibo, W did the statistical analysis and contributed in the discussions.