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Assessing the Relationship between Plasma Von Willebrand Factor Antigen Levels, ABO and Rh (D) Blood Groups and Risk of Sickle Cell Anaemia Vaso – Occlusive Crisis

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Abstract

In sickle cell anaemia (SCA), continuous activation of the vascular endothelium by inflammatory cytokines leads to increased elaboration and secretion of von Willebrand Factor (vWF), a potent mediator of adhesive interactions involving the endothelium and circulating blood cells. Non-O blood groups are associated with the elevation of vWF concentration. Thus, SCA and non – O blood groups are determinants of increased levels of vWF, which plays a pivotal role in the pathophysiology of vaso-occlusive crisis (VOC). To determine the influence of plasma vWF:Ag levels, ABO and Rh (D) blood groups on the risk of occurrence of sickle cell vaso-occlusive crisis. We conducted a prospective study of frequencies of VOC with respect to plasma vWF:Ag levels, ABO and Rh (D) blood groups of 50 SCA patients. In comparison with blood group O, patients with non – O blood groups had significantly higher mean vWF concentration (4.17±3.16 IU/l vs 3.46±3.69 IU/l, $p< 0.001$), with a significantly higher mean number of VOC episodes per patient (3.2 vs 1.3, $p<0.001$). The relative risk of VOC for patients with non- O blood groups was 1.87 (95% confidence interval 1.5 - 2.2, $p<0.001$). However, the association of Rh (D) blood group of the patients and their plasma vWF:Ag levels on the risk of occurrence and frequency of VOC was not statistically significant ($P = 0.155$). SCA patients with non – O blood groups had more episodes and higher risk of VOC that were likely due to the effect of higher plasma vWF concentration. These results indicate that the non- O blood group is a risk factor for frequent VOC and an unfavourable prognostic marker in SCA. We hereby recommend that a large multicentre prospective study be carried out to definitely determine the impact of ABO, Rh and other blood groups on the overall clinical course of SCA.

Keywords: Sickle cell anaemia, von Willebrand Factor, O blood group, non – O blood group, Rh(D) blood group, vaso – occlusive crisis.

INTRODUCTION

Right from the seminal work in 1953 that demonstrated an association between gastric cancer and blood group A [1], there have been multiple studies on the plausible association or relationship of blood types to a variety of disease conditions, including infections, cancers, cardiovascular and thrombohaemorrhagic disorders [2-4]. For instance, individuals with the non – O blood groups have been shown to have a higher risk of thromboembolism and lower risk of haemorrhagic events compared to their counterparts with the O blood. The differences in thrombotic and heamorrhagic phenotypes have been attributed to higher levels of von Willebrand Factor (vWF) and Factor VIII (FVIII) in those with non – O groups [4-8]. Moreover, molecular analysis of the non – O blood groups revealed that the thromoembolic risk was relatively higher in homozygous than in heterozygous individuals owing to the dose-dependent effect of ABO alleles on vWF and FVIII levels, which portrayed that the risk was inversely related to H antigen expression [9]. The disparity in vWF concentration among the different ABO blood groups is due to intergroup variation in the rate of proteolytic clearance of vWF by ADAMTS – 13 [5, 8, 9]. This ABO – associated variability in vWF metabolism accounts for the shorter half-life of vWF in the plasma of blood group O in comparison with the non – O blood groups [8, 9]. Plasma vWF levels are

approximately 25 – 30% lower in group O persons than in non – O individuals [10].

The RhD blood group, on the contrary, has a less distinct relationship with disease entities. RhD status has majorly been linked to isoimmunization of pregnant women resulting in varying severity of anaemia in the fetus and newborn [11]. Aside from this effect, not much is known about its role in the pathogenesis of other conditions. The principal feature that distinguishes RhD – positive from RhD – negative blood group is the presence or absence of the RhD antigen on the red blood cell surface. However, individuals with or without RhD antigen possess the homologous RhCE protein and Rh – associated glycoprotein (RhAG) on their red cells. Hence, functions that should be carried out by RhD in RhD – negative individuals are likely undertaken by RhCE and RhAG, and this redundancy may somewhat explain the paucity of clinical conditions ascribed to the RhD status [9].

Sickle Cell Disease (SCD) is a heterogeneous group of haemoglobin disorders resulting from the inheritance of the sickle β – globin gene. The homozygous state for the HbS gene (HbSS or sickle cell anaemia) is the most common and severe form of the disease [12]. The haemoglobin S (HbS), which is a structural variant of the normal haemoglobin (HbA), arises because of a single nucleotide substitution (GTG for GAG) in the sixth codon of the β - globin gene on chromosome 11 where adenine is replaced by thymidine, resulting in the substitution of valine for glutamic acid in position 6 of the β - globin chain [13]. This genetic mutation confers upon HbS a considerable alteration in its physicochemical characteristics with attendant reduced solubility in the deoxygenated state [14]. The less common and milder forms of SCD are due to double heterozygosity involving the HbS gene and thalassaemia (HbSα/β+thal) and other haemoglobin variants such as C (HbSC), D (HbSD), O (HbSO) and E (HbSE) [12], all of which share a similar basic pathophysiologic mechanism marked by red cell sickling, haemolysis, leucocytosis, thrombocytosis and vasculopathies [15].

The clinical course of sickle cell anaemia (SCA) is typically characterized by variable episodes of painless steady state that are periodically interrupted by disabling vaso-occlusive crisis (VOC) owing to deoxygenation of HbS, red cell sickling, vaso-occlusion and tissue necrosis [14]. Red cell sickling, haemolysis and vaso-occlusion are also associated with the development of other vasculopathic morbidities like acute chest syndrome, priapism, stroke and retinopathy [14, 16]. The most distressing clinical manifestation of SCA is the episodic vaso-occlusive crisis [14]. The crisis occurs unpredictably throughout a life-time and it is the most common cause of hospital visits and admissions. The commonest acute vaso-occlusive morbidity in SCA is Bone Pain Crisis (BPC). BPC is defined as painful VOC involving one or more sites of bones in the absence of antecedent trauma or overt infection. The most frequently affected sites are the long bones of the limbs, lumbar spine and thoracic cage [17].

Studies have shown that the pathophysiology of VOC is intricately linked to adhesion of the blood cells, including sickled red cells, leucocytes and platelets to the vascular endothelium [14, 18]. In SCA, the vascular endothelium is in a constant state of activation by inflammatory cytokines resulting in increased production and secretion of vWF [19]. The elevated vWF levels in the plasma of SCA patients have also been attributed to increased levels of ultra-large vWF multimers and impaired processing by its cleaving protease ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) in the plasma of such patients [10, 19]. Moreover, the large vWF multimers are stabilized by extracellular haemoglobin, which renders them hyperadhesive and resistant to the degrading activity of ADAMTS-13 [19]. The clinical relevance of the raised levels of vWF in SCA is related to the enhancement of adhesion of the sickle red cells, leucocytes and platelets to the vascular endothelium, thereby provoking and propagating vaso-occlusive events and a myriad of other vasculopathic complications, which negatively affect the severity of SCA [12, 13, 16].

We infer that SCA patients with non – O blood groups would have higher plasma levels of vWF than those with O blood groups because of the concerted effect of SCA and the non – O blood groups, both of which are independently associated with the raised levels of vWF [5, 8, 9]. Therefore, we surmised that SCA patients with non – O blood groups would have greater increase in plasma vWF concentration and higher risk of VOC than those with blood group O. Considering the foregoing, SCA patients with non – O blood groups would have higher frequency of VOC than their counterparts with O blood group. To the best of our knowledge, the relationship between vWF, ABO and Rh blood groups and sickle cell VOC has not been previously evaluated in our environment. Hence, the objectives of this study were to determine the frequency and distribution of the ABO and Rh (D) blood groups as well as the plasma levels of vWF in SCA patients and to ascertain if there is any association between the frequencies of VOC and vWF, ABO and Rh (D) blood groups as seen in our centre.

METHODS

Study Design

This was a prospective, cross-sectional cohort hospital-based study.

Study Population

Fifty SCA patients in BPC were recruited for the study. The patients were recruited successively from the Haematology Clinic (HDCU), Medical Out-patient Clinic (MOPC) and Accident and Emergency Unit of the University of Uyo Teaching Hospital, Uyo, between January – December, 2021. Fifty healthy non – sickle cell persons served as the control group.

Inclusion Criteria

SCA patients who presented in BPC and healthy HbAA controls were enrolled in the study after a written informed consent had been obtained from them.

Exclusion Criteria

SCA patients who had other forms of sickle cell crisis such as acute chest syndrome, aplastic crisis, sequestration crisis, priapism among others and those with chronic infections like hepatitis, HIV infection and tuberculosis were excluded from the study. Patients who were on long-term transfusion regimen including those taking hydroxyurea and those with chronic renal failure as well as female patients who were pregnant or taking oral contraceptives were excluded. Patients with other types of SCD such as HbSC, HbSD, HbS/β-thalassaemia, HbS/α-thalassaemia were also excluded from the study.

Ethical Consideration

Ethical approval was obtained from the Health Research Ethics Committee of the hospital before the commencement of the study.

Specimen Collection/Analytical Procedure

After obtaining an informed consent, eight millilitres of free flowing venous blood was collected from each of the participants. Three millilitres of this blood was dispensed into ethylene diamine tetra-acetic and (EDTA) vacutainer tubes. These samples were used to determine the complete blood count (CBC), and ABO and Rh(D) blood groups of the subjects. CBC was determined using the sysmex KX31 Haematology auto-analyzer. Analysis was done within 2 hours of sample collection. Standard tube method as described by Bain and Lewis [20] was used for the determination of ABO and Rh(D) blood groups using antisera obtained from Biotec laboratory, United Kingdom.

The remaining 5ml of blood was dispensed into trisodium citrate bottles 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 vWF: Ag levels within a week. The plasma levels of vWF: Ag of patients and equal number of age-matched and sex-matched healthy HbAA volunteers were determined by sandwich enzyme – linked immunosorbent assay technique using Assay Max human von Willebrand Factor ELISA Kit manufactured by Assay Pro, St. Charles, MO, USA.

Prospective Analysis of Vaso-Occlusive Crisis and Case Definition of Vaso-occlusive Crisis

The medical history of each patient was obtained to enumerate the number of episodes of VOC in the past 1 year. An episode of VOC was diagnosed if the patient presented at the clinic or emergency room with history of pain involving the extremities, chest, abdomen, back, head or neck regions that occurred at least 2 hours prior to presentation and could not be ascribed to conditions other than SCD.

Data Collation and Statistical Analysis

Data on demographic and haemogram parameters, and vWF: Ag levels of the patients were documented for each ABO blood group.

The mean values of the parameters studied were compared between the non – O and O blood groups using T-test, with a P-value of less than 0.05 taken as significant. In addition, the relative risk of VOC among patients with the non – O blood group was determined based on logistic regression analysis model. Value of relative risk was adjudged to be statistically significant if its range of 95% confidence interval did not include 1.0 with a P-value of less than 0.05. Statistical analyses were carried out using SPSS version 23.0 and the results were presented in simple tables.

RESULTS

A total of 50 patients with SCA were studied. The mean age of the SCA patients was 28.30 (±7.33) years with a range of between 18 – 58 years while that of the control group was 27.62 (±6.38) years with a range of between 18 – 56 years. There were more female patients than males (60% and 40% respectively). Also, there were more females than males in the control group (54% and 46% respectively) Table 1.

The proportions of patients and controls in the ABO and Rh(D) blood groups are shown in Table 2. The mean levels of vWF: Ag among the SCA patients with blood group O and the non – O blood groups were higher (3.46 ± 3.69 IU/l and 4.17 ± 3.16 IU/l respectively) than those of the HbAA controls (1.41 ± 0.40 IU/l and 1.91 ± 0.39 IU/l respectively) Table 3.

Table 4 compared the demographic profile, haemogram parameters, vWF: Ag levels and frequencies of VOC as found in SCA patients with the blood group O ad non – O blood groups; there were significant differences between patients with blood group O and those with non – O blood groups with regard to mean values of haematocrit, WBC count, platelet count, frequencies of VOC ($P<0.05$) but not with mean age and sex ratio ($P>0.05$). However, in comparison with blood group O, patients with non – O blood groups had significantly higher mean value of vWF level (4.17 ± 3.16 IU/l vs 3.46 ± 3.69 IU/l, $P<0.001$) and higher mean number of VOC episodes per patient (3.2 vs 1.3, $P<0.001$). In a regression model

adjusting for age, sex and vWF: Ag levels as co-variables, the relative risk of VOC for patients with the non – O blood groups was 1.87 (95% confidence interval 1.5 – 2.2, P<0.001). A comparison of the vWF: Ag levels and ABO and Rh(D) blood groups of the patients showed no statistically significant difference (P = 0.155) Table 5.

Table 1: Age and Gender Distribution of Subjects and Controls

	HbAA Control		Subjects (SCA)	
Age (yr)	**Male**	**Female**	**Male**	**Female**
≤ 20	1	3	0	2
21 – 25	7	8	10	12
26 – 30	5	7	7	10
31 – 30	4	4	1	2
36 – 40	2	3	1	1
41 – 45	1	1	0	1
46 – 50	2	1	1	1
≥ 51	1	0	0	1
Total	**23**	**27**	**20**	**30**
Mean	**27.62±6.38**		**28.30±7.33**	

Table 2: Distribution of ABO and Rh(D) Blood Groups among SCA Patients and Controls

Blood Groups	Controls		SCA Patients	
	Frequency	%	Frequency	%
A^-	4	8.0	0	0
A^+	18	36.0	11	22.0
B^+	3	6.0	1	2.0
AB^+	1	2.0	1	2.0
O^-	4	8.0	3	6.0
O^+	20	40.0	34	68.0

Table 3: Comparison of the Mean von Willebrand Factor Antigen Levels between Subjects and Controls

Variable	Controls Mean (SD)		SCA Patients Mean (SD)	
	Blood Group O	Non – O Blood Group	Blood Group O	Non – O Blood Group
vWF: Ag	1.41(0.40)	1.91(0.39)	3.46(3.69)	4.17(3.16)
t-test	4.478		0.619	
P-value	0.539		0.000	

Table 4: Demographic Profile, Haemogram Parameters, von Willebrand Factor Antigen Levels and Frequencies of Vaso-Occlusive Crisis among Sickle Cell Anaemia Patients in relation to ABO Blood Groups

Parameters	Blood Group O (n = 37)	Non – O Blood Group (A + B + AB) (n = 13)	P-value
Age (mean ± SD) (years)	28.92 ± 7.53	26.54 ± 6.68	0.528
Sex ratio (male/female)	0.42(11/26)	0.63(5/8)	0.260
Haematocrit (mean ± SD) (l/l)	19.60 ± 1.92	16.43 ± 2.50	0.000
WBC count (mean ± SD) ($x10^9$/l)	16.43 ± 2.49	13.20 ± 2.51	0.000
Platelet count (mean ± SD) ($x10^9$/l)	357.56 ± 46.69	352.80 ± 50.54	0.000
vWF level (mean ± SD) (IU/l)	3.46 ± 3.69	4.17 ± 3.16	0.000
Mean number of VOC per patient	1.3	3.2	0.000

Table 5: Correlation between the Plasma vWF: Ag Concentration and the ABO and Rh(D) Blood Groups of the SCA Patients

Blood Group	vWF: Ag Mean (SD)	F	P-value
A^+	3.15(2.10)	1.75	0.155
B^+	10.34(0.00)		
AB^+	9.30(0.00)		
O^-	2.65(0.20)		
O^+	3.53(3.85)		

DISCUSSION

The frequency and distribution of the ABO and Rh (D) blood groups of patients and controls were consistent with those of the general population in our sub-region [5,8]. The demographic profile and haematological parameters of the patients in each blood group revealed similar findings though with subtle differences. The haematological parameters showed that the patients had anaemia, leucocytosis and thrombocytosis, all of which are in consonance with earlier reports [14,17,18].

In this study, the SCA patients had significantly higher vWF:Ag levels than the HbAA controls. This observation reasserts the notion that the vascular endothelaim of patients with SCA is in a constant state of stimulation by inflammatory cytokines, leading to increased elaboration and secretion of vWF[18]. Our finding of raised plasma levels of vWF in patients with SCA is also in keeping with the results of previous studies [16,18,19]. This work further revealed that in comparison with the subjects with the blood group O, those with the non- O blood groups had significantly higher plasma concentration of vWF with attendant higher frequencies of VOC as exemplified by higher mean number of VOC episodes per patient. In addition, the finding of a relative risk of 1.87 indicates that patients with the non-O blood groups were about two times more likely to experience VOC than patients with blood group O. These results are consistent with reports from other studies which have demonstrated the pivotal role of vWF as a mediator of adhesion of sickle red cells, leucocytes and platelets to the vascular endothelium in the pathogenesis of VOC [14, 18, 19]. Similarly, the finding of elevated plasma levels of vWF in individuals with the non-O blood groups agrees with the study by Dentali *et al*., [21] who reported high plasma concentration of vWF and FVIII in the same category of subjects, the latter observation being explained by the fact that vWF serves as a carrier of FVIII and its protector from proteolysis in the plasma [19]. The aforementioned scenarios underscore the profound influence the ABO blood group has on haemostatis as well as rate the non-O blood groups as the commonest genetic risk factors for venous thromboembolism [7, 21]. It can therefore be surmised that SCA patients with the non- O blood groups would be particularly predisposed to frequent and severe VOC owing to the concerted effects of raised plasma levels of vWF and FVIII, resulting in adhesion of blood cells to the endothelium thereby contributing to the occurrence of vaso-occlusive events and thrombosis within the vasculature [16, 18, 19].

Vaso-occlusive crisis is the most common and harrowing clinical manifestation experienced by SCA patients. It can be triggered by psychological, physical or infective factors [22]. Patients with SCA have impaired immune function and are prone to develop recurrent infections by a variety of pathogens that include encapsulated bacteria, viruses, mycobacteria and parasites [23]. Infection is the most frequent precipitating factor for VOC and malaria infection has been shown to be the topmost trigger of VOC given the endemic nature of the infection in countries, including ours, where majority of the SCA patients reside [24, 25].

Furthermore, some studies have demonstrated a direct correlation between the non- O blood groups and the risk of severe malaria [26-28]. The mechanisms underlying variations in susceptibility to severe malaria in individuals with different ABO Blood groups have not been fully elucidated. However, several proximate mechanisms have been portrayed to relate to these associations, notably an affinity for Anopheles species, shared ABO antigens with P. falciparum, impairment of merozoite penetration of RBCs, cytoadherence, endothelial activation, and rosetting of parasitized erythrocytes [28, 29]. The phenomenon of rosetting is the most widely documented [26, 28, 29]. Rosetting plays a role in the pathogenesis of severe malaria by obstructing microvascular blood flow [29]. Individuals with blood group O display reduced erythrocyte rosetting with marked humoral and phagocytic antimalarial immune response, resulting in clinically non severe malaria. In constrast, their counterparts with the non- O blood groups exhibit increased red erythrocyte rosetting with relatively reduced humoral and phagocytic antimalarial immune response, culminating in clinically severe malaria [26-29]. It is, therefore, believed that blood group O may be a protective factor against severe malaria [29].

Malaria parasites can precipitate VOC through a number of pathways, which include disruption of red cell metabolism, invasion of the red cells of all ages and the erythroid precursors in the bone marrow, leading to sickling and the formation of histidine – rich protein knobs on red cell membrane that mediate adhesion of sickle red cells to the endothelial living of the blood vessels with resultant haemolysis, vaso-occlusion and local hypoxia [30, 31]. Moreover, some authors have reported reductions in ADAMTS-13 activity in severe malaria, which would further exacerbate plasma vWF levels and increase the risk of VOC in SCA patients [32, 33]. Based on the current evidence, it seems likely that any episode of severe malaria among SCA patients with non – O blood groups would aggravate their pre-existing heightened risk of VOC due to elevated plasma concentration of vWF. It can therefore be opined that antimalaria prophylaxis would be an all-important preventive strategy for patients with the non- O blood groups in whom the risk of severe infection is significantly higher [26-31].

In relation to Rh status, the result of this study did not show any statistically significant association between the Rh (D) blood group of the patients and their plasma vWF.Ag concentration. It is interesting to

note that this study has established for the first time in our country that Rh (D) phenotype is not a modulator of plasma vWF:Ag level in SCA patients. However, further studies with larger sample size is needed to examine this relationship.

The clinical relevance of our results is hinged upon the fact that the frequency of VOC is of immense prognostic importance in SCA [24, 31]. Increased frequency of VOC is associated with severe disease characterized by multi-organ dysfunction and failure, overall poor prognosis and untimely death in patients with SCA [22, 31]. Thus, the findings from the present study, would indicate that the non- O blood groups are associated with recurrent and frequent episodes of vaso-occlusive crisis, which are unfavourable prognostic factors in SCA.

CONCLUSION

Subjects with the non- O blood groups have higher plasma concentration of vWF:Ag and experience more episodes of sickle cell VOC compared to those with O blood group. However, the association of Rh (D) blood group of the subjects and their plasma vWF:Ag levels on the risk of occurrence and frequency of VOC was not statistically significant. The Non- O blood group is therefore an important risk factor for frequent VOC and an adverse prognostic index in SCA patients. Large-scale studies are required to determine the impact of ABO, Rh and other blood groups on the overall clinical course of SCA.

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