African Journal of Biotechnology Vol. 6 (10), pp. 1217-1220, 16 May 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Occurrence of haemolysin antibodies among sickle cell anaemia patients within Calabar metropolis of Nigeria

R. A. Anyanwu[1]*, A. O. Emeribe[2], C. U. Igwe[3], I. Ajayi[4], J. Akpotuzor[2], K. C. Lele[1] and F. O. Emelike[1]

[1]Department of Medical Laboratory Science, Ambrose Alli University, Edo State, Nigeria.
[2]Department of Medical Laboratory Science, University of Calabar, Cross River State, Nigeria.
[3]Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria.
[4]Department of Physiology, University of Benin, Benin City, Nigeria.

Accepted 21 March, 2007

The role of alpha (α) and beta (β) haemolysins in blood transfusion has been well documented. However, the occurrence of haemolysins and its attending problems in sickle cell anaemia (SCA) patients has limited appearance in the literatures especially in black Africa. This study was therefore designed to investigate the occurrence of α and β haemolysins in SCA patients within Calabar metropolis. A total of 50 normal controls and 54 SCA patients attending the sickle cell clinic of the University of Calabar Teaching Hospital, Cross River State of Nigeria, were screened for haemolysins and quantitated using standard techniques. The distribution of haemolysins in the SCA patients was α (16.7), β (11.1) and α+β (16.7%). Their ABO blood groups were normally distributed in this order O>>A>B>AB (61.1, 20.4, 11.1 and 7.5%, respectively). The occurrence of α haemolysin among the O group was 15.2%, while β haemolysin was 12.1% and α+β haemolysin was 30.3%. Control samples gave non-significantly lower haemolysin values than in SCA patients (P>0.05), but in the same order (α, 9.0, β, 7.0, and α+β, 9.0%). The higher prevalence of haemolysins in SCA could be a major limiting factor in donation of blood, blood selection and transfusion into SCA patients. Consequently, greater care should be taken during cross match of blood for SCA patients especially in multiple transfusion procedures.

Key words: Haemolysin, sickle cell anaemia, blood transfusion, Calabar.

INTRODUCTION

Sickle cell disorder is a group of inherited disorders of haemoglobin. These disorders include sickle cell anaemia, sickle cell haemoglobin C disease (HbSC), and sickle cell thalassaemia (HbSBTHAL). Of these, sickle cell anaemia (SCA) is most prevalent with clinical manifestations attributed to the substitution of valine for glutamic acid at the sixth residue of the β-chain from the N-terminus of the sickle haemoglobin (HbS) protein chain.

Red blood cells, which contain HbS when exposed to reduce oxygen tension, assume a characteristic sickle shape. The sickle cell phenomenon is inherited as mendelian autosomal dominant characteristic. SCA is the homozygous state for the HbS gene (HbSS); the patient receiving one HbS gene from each parent, both of who show sickle cell trait (Bunn and Forget, 1986).

Haemolysin is a type of antibody that has the ability of combining with its specific antigen on the red blood cells and together with complement cause destruction of thecells. Haemolysin can be either IgG or IgM immunoglobulin. Hence its characteristic depends on the immunoglobulin class of the haemolysin antibody. It can be divided into alpha (α), beta (β) and alpha plus beta (α+β) haemolysin types. Alpha haemolysin may be detected in ABO blood group B and O individuals, while β haemolysin may be present in group A and O persons (Boorman et al., 1988). Immune haemolysin production may occur following ABO heterospecific pregnancy, incompatible blood transfusion or immunization with A or B substances (Usanga and Akwiwu, 1990). Alpha and β haemolysins

*Corresponding author. E-mail: roseyulunne@yahoo.com. Tel: +234 0803 777 9731.

Table 1. Influence of sex on development of haemolysin antibodies in the subjects.

Subjects	α (%)	β (%)	α+β (%)	Nil (%)	Total
Controls					
Male	4(8.6)	3(6.5)	6(13.0)	33(71.7)	46
Female	5(9.3)	4(7.4)	3(5.6)	42(77.7)	54
SCA Patients					
Male	8(23.5)	4(11.8)	4(11.8)	18(52.9)	34
Female	1(5.0)	2(10.0)	5(25.0)	12(60.0)	20

Percentages were calculated per row. α= Alpha haemolysin, β = beta haemolysin, α+β = alpha plus beta haemolysin, and Nil = absence of haemolysin.

occur more in blood group O donors than in other blood group donor types put together (Emeribe, 1990).

Haemolysin is important in blood group serology because of its incrimination in transfusion reactions and haemolytic disease of the newborn (HDN). Hence, this study was aimed at determining the occurrence of haemolysin antibodies in sickle cell anaemia patients in Calabar metropolis of Nigeria. These patients, because of the presence of HbSS are prone to intravascular haemolysis and associated crisis, and thus could be in danger as a result of transfusion reactions related to haemolysin activity.

MATERIALS AND METHODS

Subjects

The subjects were made up of 54 SCA patients attending sickle cell clinic of the University of Calabar Teaching Hospital, Calabar. The patients were age-matched with 100 apparently healthy (non-sickle cell patients and non-trait carriers) control subjects from Government School Obufa Esuk in University of Calabar, Cross River, Nigeria.

Ethical approval was obtained from the hospital Ethical committee and informed consent was obtained from all the subjects before sample collection.

Sample collection

Five milliliters (5 ml) of venous blood was obtained from each of the subjects. About 1 ml of the blood was dispensed into a plain container, allowed to clot and retract. Serum was separated from the whole blood after centrifugation and stored frozen at –20^{o}C for haemolysin analysis. The remaining venous blood was dispensed into EDTA container.

Haemoglobin electrophoresis

The anticoagulated blood was centrifuged, red blood cells separated and washed three times in physiological saline. Then to 1 volume of washed red cells, 2 volumes of Drabkins solution was added to lyse the cells. The lysed cells solution was mixed thoroughly and centrifuged at 3000 rpm for 5 min. The supernatant obtained was used as the haemolysate for the Hb electrophoresis. The Hb electrophoresis was carried out and interpreted as descrybed by Dacie and Lewis (Dacie and Lewis, 1994).

ABO and Rhesus blood grouping

Potent anti-A, anti-B, anti-A+B and anti-D sera were used to group the subjects' red cells as described by Dacie and Lewis (1994).

Haemolysin test and titration

Known A, B and O red cells were used to test the subjects' sera for agglutination and subsequent haemolysis of the red cells in the presence of haemolysin and complement in the fresh sera samples. Sera samples that showed evidence of haemolysis were selected for titration to determine their titer (Dacie and Lewis, 1994).

Statistical analysis

Chi-square test was used for the statistical analysis of data obtained. The 5% level of significance was adopted for significant findings.

RESULTS

ABO haemolysin antibodies were detected in the sera of 54 SCA patients consisting of 34 male and 20 female. The study of the influence of sex on development of haemolysin antibodies (Table 1) shows that male subjects had a significantly higher occurrence of α (23.5%) and β (11.8%) amongst the SCA patients and α+β (13.0%) amongst the male controls ($P<0.05$), but with non significantly lower occurrences of α+β (11.8%) amongst the SCA patients and α (8.6%) and β (6.5%) amongst the control subjects ($P>0.05$) when compared with their female counterparts. However, majority of both sexes had no haemolysins detected in their sera.

The effect of age on the development of haemolysin in SCA patients is presented in Table 2. It shows that age has a great influence over the production of haemolysin. There was a significant increase in haemolysin antibodies as age increased; age ranges ≤ 7 years (34.1%), 8 – 14 years (38.9%) and 15 - 21 years (37.0%) ($P<0.05$).

Table 2. Effect of age on development of haemolysin antibodies in the SCA patients.

Age range (years)	α (%)	β (%)	α+β (%)	Nil (%)	Total
1 – 7	0 (0.0)	1 (7.6)	0 (0.0)	12 (92.3)	13
8 – 14	6 (28.6)	2 (9.5)	3 (14.2)	10 (47.6)	21
15 – 21	3 (15.0)	3 (15.0)	6 (30.6)	8 (40.0)	20

Percentages were calculated per row. α = Alpha haemolysin, β = beta haemolysin, α+β = alpha plus beta haemolysin, and Nil = absence of haemolysin.

Table 3. Distribution of haemolysin antibodies in the various ABO blood groups among the subjects.

Subjects	Blood group				
	A	B	AB	O	Total
Number of SCA patients	11	6	4	33	54
SCA patients with haemolysin (%)	2 (8.3)	3 (12.5)	0 (0.0)	19 (79.2)	24 (44.4)
Number of control subjects	14	11	5	70	100
Controls with haemolysin (%)	1 (4.0)	1 (4.0)	0 (0.0)	23 (92.0)	25 (26.0)

Table 4. Prevalence of haemolysin antibodies among blood group O subjects.

Subjects	α (%)	β (%)	α+β (%)	Nil (%)	Total
Controls					
Male	3 (9.4)	3 (9.4)	6 (18.8)	20 (62.5)	32
Female	4 (10.5)	4 (10.5)	3 (7.9)	27 (71.1)	38
SCA patients					
Male	4 (19.0)	2 (9.5)	5 (23.8)	10 (47.6)	21
Female	1 (8.3)	2 (16.7)	5 (41.7)	4 (33.3)	12

Percentages were calculated per row. α = Alpha haemolysin, β = beta haemolysin, α+β = alpha plus beta haemolysin, and Nil = absence of haemolysin.

Table 3 presents the distribution of haemolysin in various ABO blood groups among the subjects. The results show that blood group O subjects had the highest occurrence of haemolysin (85.7%), followed by blood group B (8.2%), and then blood group A (6.1%) with blood group AB subjects having no detectable haemolysin presence. The distribution of haemolysin amongst the blood group O subjects is showed in Table 4. The frequency of occurrence of α (19.0%) haemolysin was higher in male blood group O SCA patients than the female blood group O SCA patients. On the other hand, there was no significant difference in the rates of occurrence of the haemolysin antibodies between the male and female blood group O control subjects ($P>0.05$).

Influence of ethnicity on development of haemolysin is shown in Table 5. It indicates that ethnicity has no influence on haemolysin antibody development.

DISCUSSION

Asian and black populations have been reported to have high frequency of strong haemolytic anti-A and anti-B haemolysins in comparison to Caucasians (Redman et al., 1990; Kulkarni et al., 1985; Adewuyi and Gwanzura, 2001). The higher frequency of haemolysins in these populations has been attributed to mosquito bites and intestinal parasitic infections, as well as a con-sequence of vaccinations or other antigens exposures (Mathai et al., 2003).

There is at present scarcity of information on SCA patients who are prone to crisis-associated intravascular haemolysis and hence may require blood and blood products transfusion frequently. High frequency of haemolysin antibodies was observed among the SCA patients in this study. This may be due to environmental factors as discussed by Mathai and colleagues (Mathai et al., 2003), or a consequence of previous blood transfusions. This is because these patients live in a malaria endemic area and about 90% of them have been transfused with blood at least once. This could be further buttressed by the fact that blood group O individuals are still falsely considered and used as universal donors in Nigeria (Olawumi and Olatunji, 2001). The risk associated with this is further enhanced by the highly significant occurrence of haemo-

Table 5. Influence of ethnicity on development of haemolysin.

Ethnic groups	α (%)	β (%)	α+β (%)	Nil (%)	Total
Controls					
Indigenes	4 (11.2)	2 (5.7)	3 (8.5)	26 (74.2)	35
Ibibios	1 (2.5)	5 (12.5)	4 (10.0)	30 (75.0)	40
Ibos & Yorubas	4 (16.0)	0 (0.0)	2 (8.0)	19 (76.0)	25
SCA patients					
Indigenes	6 (19.4)	2 (6.5)	6 (19.4)	17 (54.8)	31
Ibibios	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)	5
Ibos & Yorubas	3 (16.6)	4 (22.2)	3 (16.6)	8 (44.4)	18

Percentages were calculated per row. **α=** Alpha haemolysin, **β =** beta haemolysin, **α+β =** alpha plus beta haemolysin, and **Nil =** absence of haemolysin. The indigenes include all people from Cross River State of Nigeria.

lysin antibodies in the blood group O subjects in comparison with the other blood group classes. Thus, the use of blood group O for group A or B recipients have been advocated against (Emeribe, 1990). We wish to strongly reiterate this position given the high frequency of haemolysin antibodies observed among the blood groups A, B, and O SCA patients.

A study of the influence of sex, age and ethnicity on the frequency of haemolysin among the SCA patients showed no significant relationship. However, a comparison of the frequency of occurrence between the patients and control group with respect to their sex showed significant increase in haemolysin among the male than the female. This is in disagreement with previous reports of higher occurrence in girls than boys (Millison, 1979), and no relationship between sex and the prevalence of haemolysin (Olawumi and Olatunji, 2001). The effect of age on development of haemolysin antibodies in SCA patients showed that as age increased, haemolysin frequency tends to increase as well. The study shows that haemolysin development rate increased most between the ages of 8 to 14 years. This observation is in consonance with the finding that the maximum levels of haemolysin are attained at about the ages of 7 to 8 years (Millison, 1979). However, these differ with recent report of no relationship between age and haemolysin occurrence (Olawumi and Olatunji, 2001). On the other hand, a study of the effect of ethnicity on haemolysin occurrence among the SCA patients and control subjects showed no significant relationship. This could be seen to differ with the report of Kulkarni et al (1985) which stated that high frequency of these haemolysins occur among ethnic groups originating from the southern part of Nigeria. However, that report collaborates our observations of high frequency of haemolysin among these subjects who coincidentally are all from the southern part of Nigeria.

In conclusion, sickle cell anaemia patients have high frequency of α and β haemolysin, which increased significantly with age and occurrence of ABO blood groups O, B and A. The higher prevalence of haemo-lysins in SCA patients could be a major limiting factor in donation of blood, selection of blood and transfusion into the SCA patients. Thus greater care should be taken during cross-match of blood for SCA patients especially in multiple transfusion procedures.

REFERENCES

Adewuyi JO, Gwanzura C (2001). Racial difference between white and black Zimbabweans in the haemolytic activity of A,B,O antibodies. Afr. J. Med. Med. Sci. 30(1-2): 71-74.

Boorman KE, Dodd BE, Lincoln PJ (1988). Blood group serology, 6th edn. Churchill Livingstone, Edinburgh p. 22.

Bunn HF, Forget B (1986). Haemoglobin: Molecular, Genetics and Clinical Aspects. PA Saunders, Philadelphia, p. 86.

Dacie JV, Lewis SM (1994). Practical Haematology, 8th edn. Longman Group Ltd, Hong Kong, pp. 49-82.

Emeribe AO (1990). The status of alpha and beta haemolysins in Nigerian blood donors. East Afr. Med. J. 67(3): 205-208.

Kulkarni AG, Ibazebe R, Fleming AF (1985). High frequency of anti-A and anti-B haemolysins in certain ethnic groups of Nigeria. Vox Sang. 48(1): 39-41.

Mathai J, Sindhu PN, Sulochana PV, Sathyabhama S (2003). Haemolysin test for charaterisation of immune ABO antibodies. Indian J. Med. Res. 188: 125-128.

Millison PL (1979). Blood Transfusion in Clinical Medicine, 6th edn. Black Scientific Publications, Oxford, p. 89.

Olawumi HO, Olatunji PO (2001). Prevalence and Titre of Alpha and Beta Haemolysins in Blood Group 'O' Donors in Ilorin. Afr. J. Med. Med. Sci. 30(4): 319-321.

Redman M, Malde R, Contreras M (1990). Comparison of IgM and IgG anti-A and anti-B levels in Asian, Caucasian and Negro Donors in the Northwest Thames Region. Vox Sang. 59: 89-91.

Usanga EA, Akwiwu JO (1990). Prevalence and Titre of ABO Haemolysin Antibodies in Pregnant Nigerian Women. East Afr. Med. J. 67(6): 437-441.