Sudan Journal of Medical Sciences
Volume 16, Issue no. 1, DOI 10.18502/sjms.v16i1.8935
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Research Article

Determination of Normal and Variant Hemoglobin using Capillary Electrophoresis among Voluntary Blood Donors in North Central Nigeria: Implications on Blood Transfusion Services

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Received 2 January 2021
Accepted 16 March 2021
Published 31 March 2021

Production and Hosting by Knowledge E

Editor-in-Chief:
Prof. Mohammad A. M. Ibnouf

Abstract

Background: Voluntary non-remunerated blood donation is a strategy adopted by World Health Organization aimed at ensuring safety and adequacy of blood supply. Sub-Saharan Africa has a high prevalence of hemoglobin disorders and therefore needs to adopt stringent measures in donor selection to ensure safety for the recipient of blood transfusion. This study aimed to analyze normal and variant hemoglobin among voluntary blood donors.

Methods: In this descriptive cross-sectional study, 100 prospective blood donors including 55 (55%) males and 45 (45%) females, aged 18–34 years were recruited. Capillary electrophoresis using the Minicap system was used for determining the hemoglobin variants in alkaline buffer (PH 9.4). Data analysis was done using SPSS version 20 and p-value < 0.05 was considered as the level of significance

Results: The mean age of the participants was 22.23 $\pm$ 3.3 SD years. The proportion of participants with genotype AA was 67 (67%), those with AS were 17 (22 %), while those with AC were 11 (11 %). While Hb A $\geq$ 90% was noted in 67 (67%) blood donors, Hb S was seen in 22 (22%) and Hb A2 > 3.5% in 57 (57%). Hb F > 2% was observed in 3% of the studied participants

Conclusion: Variant hemoglobin is common among blood donors and this should be taken into consideration whenever blood is being crossmatched for recipients of blood transfusion. Data from this study will be useful in raising awareness and genetic counseling.

OPEN ACCESS

Keywords: prevalence, hemoglobin variants, capillary electrophoresis

1. Introduction

Structural abnormalities affecting the polypeptide chain of the globin molecule are disorders inherited genetically as occurs in hemoglobin (Hb) C, Hb S, Hb E. Hemoglobin disorder is one of the most common inherited genetic disorder worldwide and accounts for about 7% of the world population (approximately 269 million people) said to be carriers [1, 2].

The emotional and economic burden of managing hemoglobin disorders is quite enormous especially in Africa with limited resources. The sub-Saharan part of Africa has a prevalence of 10–45% for carriers of sickle cell [3, 4] and about 20–30% of the Nigerian population is reported to be carriers of the mutant S gene [5, 6].

A previous study done among people living in the south western part of Nigeria reported the prevalence of Hb AC (Hb C trait) to be 6% [7].

About 270 million people all over the world are carriers of abnormal hemoglobin [8]. Hemoglobin electrophoretic analysis of hemoglobin variants in previous studies in Nigeria and other part of sub-Saharan Africa was largely carried out with the use of cellulose acetate method at alkaline pH [7, 9]. Analyzing Hb with the use of capillary electrophoresis has been identified with increased accuracy. Of note, for the numerical and qualitative analysis of hemoglobin, superlative similarity between use of capillary electrophoresis and high-performance cation-exchange chromatography (HPLC) has been established and reported in several previous studies [10–12].

The task of ensuring safe, continuous, and adequate supply of blood as instructed by World Health Organisation (WHO) will remain sacrosanct. Blood transfusion safety entails blood collection from voluntary, non-remunerated, risk-free donors with strict adherence to procedures for selecting donors [13]. The suitability of blood donated by donors with sickle cell trait has limitations for use in some clinical situations, although present blood donation criteria allow them to donate blood [13, 14].

In neonatal exchange transfusion as well as intrauterine transfusion, donated blood from sickle cell carriers may not be appropriate due to possible worsening of hypoxia especially when this blood has been stored for couples of days. Transfusing patients with sickle cell anemia who had acute chest syndrome and those with stroke who are on chronic transfusion with blood donated by Hb S carrier may worsen patient's outcome since such blood are not tagged or separated from other blood units [15]. Blood transfusion in patient with sickle cell anemia who had stroke is intended to lower the proportion of Hb S to value <30%, thus blood donated by SCT will not be appropriate for this category of patients [15, 16].

This study intends to determine hemoglobin variants among blood donors using capillary electrophoresis method.

2. Materials and Methods

2.1. Study design

This study is a descriptive cross-sectional study. It assesses the Hb variants among voluntary blood donors.

2.2. Study participants

The recruited participants included 100 prospective blood donors who met the required criteria for blood donor selection. The participants were male and female students of Kwara State Polytechnic, Ilorin who were aged between 18 and 34 years. Only those that granted consent were included in the study.

2.3. Materials and tools

Materials and tools used included the H6500CAP analyser, tri-potassium ethylene diamine tetra-acetic acid (K3EDTA) tubes, reagents cups and filters, centrifuge, and capiclean.

The prepared solutions and reagents used included wash solution, hemolyzing reagent, the kit containing use stock reagents, distilled water, B2 buffer solution, A2 control, and AFSC control.

2.4. Sampling method

Convenient sampling method was adopted for the recruitment of blood donors. The studied participants were enrolled via a non-probability method of sampling. They were recruited based on their readiness to donate blood.

2.5. Sample collection

Four milliliters of blood were collected via venipuncture from the ante-cubital vein and was subsequently put into labelled K3EDTA tubes. The collected samples were kept

at 2–8°C. Sample analysis was carried out in the laboratory within a period of 24 hr of blood collection.

2.6. Principle and procedure

In capillary electrophoresis, normal hemoglobin was separated via electrophoretic mobility of the charged molecules into hemoglobin A, A2, and F. The common hemoglobin variants including S, C, E, or D were detected via electrophoresis in alkaline buffer at pH 9.4. The surface charge of the hemoglobin variants are different and thus their electrophoretic mobility in free solution is also different. The electro osmotic flow and pH of the electrolytes also determine the separation of the charged molecules.

Capillary electrophoresis using the Minicap system was used to analyze the variants of hemoglobin. The analyzer directly detected the Hb variants S, C, E, and D while the normal Hb were separated into A, F, and A2.

The Minicap system with its silica capillaries works efficiently in parallel allowing two concurrent analysis of Hb. At 415-nm wavelength of the absorbance, specific and direct detection of the separated Hb were done.

The procedure was carried out according to the company's manual. 4 ml of the collected donor blood sample in EDTA bottle was put in a plastic tube (labeled). With the use of a centrifuge, the blood in the plastic tube was then spinned at a rated of 5000 per minute for a total of 7 mins.

With a micropipette, 200 hundred microliters of packed red cell were subsequently introduced into a nesting cup. The open analyzer (H6500CAP) was started and a 10-ml solution of the hemolyzing reagent was put inside the tube placed in position 27. The door was closed, and the OK button was clicked. The analyzer then requested for the control in position 28. 200-µl of the control reagent was subsequently placed in a nesting cup and placed into the labeled hemoglobin A2 control tube at position number 28. The door was then closed, and the OK button was clicked. An indicator appeared requesting to input the lot # and analysis was done after selecting 1 run button. The assembled test samples were then put inside the rotating wheel (carousel) of the analyzer from locations numbered 1–26 and the door was closed. The instrument was now left to run the samples. Analysis of the results was done as stated in the company's manual.

2.7. Data analysis and ethical consideration

Relevant information was collected using structured questionnaires and data generated from the study was subsequently analyzed using SPSS version 20. The proportion and percentages of hemoglobin variant of blood donors were determined. The means and standard deviation of the age, gender, and each of the normal hemoglobin analyzed were established using the independent *t*-test while comparison of proportion was done using Chi-square. Test of significance was put at values of $p < 0.05$.

3. Results

In this study of 100 donors, 55 (55%) were males and 45 (45 %) females. The mean age of the participants was 22.23 ± 3.3 SD years. Fifty-seven (57%) participants were aged 20–24 years and were found to have the highest donation (Table 1).

The proportion of participants with phenotype AA was 67 (67%), those with AS was 17 (22 %), while those with AC was 11 (11 %). Among participants with phenotype AA, the proportions of males was 38 (38 %) while that of females was 29 (29%). For participants with phenotype AS, the proportions of males was 10 (10%) and that of females 12 (12%). Seven (7%) and four (4%) females were of phenotype AC (Table 2).

Hb A ≥ 90% was noted in 67 (67%) blood donors, Hb S was seen in 22 (22%), while Hb A2 > 3.5% was seen in 57 (57%). Hb F > 2% was observed in 3% of the studied participants (Table 3).

Table 1: Gender and age group distribution among voluntary blood donors.

Variables	Frequency	Percentage (%)
Gender		
Male	55	55
Female	45	45
Total	100	100
Age group (yrs)		
15–19	22	22
20–24	57	57
25–29	19	19
30–34	2	2
Total	100	100

Table 2: Prevalence of hemoglobin phenotypes among voluntary blood donors.

Hb Variants	Gender		Proportion	Percentage
	Male	Female		
AA	**38 (38%)**	**29 (29%)**	**67**	**67%**
AC	**7 (7%)**	**4 (4%)**	**11**	**11%**
AS	**10 (10%)**	**12 (12%)**	**22**	**22%**
Total	**55 (55%)**	**45 (45%)**		

Table 3: Proportion of donors with normal and specific hemoglobin variants.

Hemoglobin	Frequency	Percentage (%)
Hb A	100	100.0
Hb A ≥ 90.0%		
Yes	67	67.0
No	33	33.0
Hb A_2	100	100.0
Hb A_2 > 3.5%		
Yes	53	53.0
No	47	47.0
Hb S	22	22.0
Hb F	16	16.0
Hb F > 2.0% (n = 16)		
Yes	3	18.8
No	13	81.3
Hb F with Hb S	3	3.0
Hb F > 2.0% and Hb S (n = 3)		
Yes	1	33.3
No	2	66.7
Hb C	11	2
Hb A2 > 3.9% and Hb F > 1%	2	11

On the hemoglobin variants among the donors, about a quarter had HbS. Only 3% had both HbS and HbF.

4. Discussion

Blood transfusion is a clinical procedure aimed at correcting anemia, replacing losses from hemorrhages, supporting patients on chemotherapy, and so on. It is thus a lifesaving procedure. Careful and selective processes are undertaken by blood bank service providers to ensure that donated blood is safe for the recipients of this blood. Safe blood donation practice must be given very important consideration to ensure availability of safe blood without risk to the recipient. Despite stringent measures adopted in blood donor selection criteria, quite a number of blood donors who did not know their carrier

status still find their way into blood donor population and this poses a risk to some category of blood transfusion recipients.

Our study reported a prevalence of HbAA to be 67%, HbAS was found to be 22%, while Hb AC was 11%. These findings were consistent with the results of previous works by Garba *et al.* and Omisakin *et al.*, in which the prevalence rates for Hb AA were reported as 66.9% and 72.4%, respectively [17, 18]. The frequency of Hb AA in our study falls within the normal range of 55–75%, earlier reported among Blacks [19]. The prevalence of Hb AS in our study was 22% and is consistent with the results of Akhigbe *et al.* who reported 22.19% as prevalence of Hb AS in blood donors among students and Zaccheaus *et al*. with a finding of 19.68% [20, 21]. Omisakin *et al.* reported 26.1% for Hb S [18]. High prevalence rate of SCT was noted in children of African descent with up to 10-40% being carriers of the S gene, as reported by WHO in 2006 [17]. The prevalence of carriers of mutant S gene among blood donors as revealed in our study is similar to findings from other parts of Nigeria [17]. Thus, the prevalence rate of Hb S trait is high in Nigeria. The prevalence of Hb C trait in our study was 11%, which was higher than the prevalence of 6% reported in a previous study [7]. Further studies with a larger cohort will be needed to determine the prevalence rate of Hb C trait in our environment. Our study showed Hb A2 > 3.5% in 57 (57%) blood donors which raises the suspicion of a possibility of β-Thalassemia among blood donors in our environment.

5. Conclusion

Although blood donors with Hb S trait are allowed to donate their blood if they satisfy the donor selection criteria and screening test for TTIs, the use of blood containing Hb S trait is subject to limitations. The prevalence of Hb S trait is high in Nigeria. Blood banking service providers must ensure screening of donated blood for hemoglobin variants determination before crossmatching blood for recipients who have sickle cell crisis or stroke whenever blood transfusion is indicated. Findings in research studies on Hb S carriers in blood donors can be used for raising awareness and genetic counseling since most voluntary blood donors fall within premarital age group. Thus, blood donation can help prospective blood donors to know their carrier status early and can help them make informed future decisions.

Acknowledgement

The authors want to appreciate all voluntary blood donors that participated in this study.

Ethical considerations

Ethical approval from the Ethical Committee of the University of Ilorin was obtained prior to the study. Written informed consent was also obtained and participation was voluntary.

Competing interests

None declared.

Availability of data and material

The study materials are available with the author upon request.

Funding

The current research was supported by TETFUND National Research Fund.

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