

Beta- Thalassaemia Trait Screening Using Capillary Electrophoresis Among Voluntary Blood Donor in North Central Nigeria

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Abstract

Beta-thalassaemia(β -thalassaemia) carriers exists in malaria endemic zones of sub-Sahara Africa including Nigeria. Studies on β -thalassaemia in Nigeria are few in the literature. The high variation in the prevalence of β -thalassaemia in Nigeria as reported in previous studies raises concern. Newer screening techniques such as capillary electrophoresis is now being used in clinical laboratories. This study screened for β -Thalassaemia carriers among voluntary blood donors using capillary electrophoresis.

A descriptive cross-sectional study was conducted among 100 voluntary blood donors among students of Kwara State Polytechnic, Ilorin, Nigeria with age range of 18-34years. The participants consist of 55 males (55%) and 45 females (45%). Capillary electrophoresis using the Minicap system was used for determining the Haemoglobin A2 and F quantification in alkaline buffer (PH 9.4). Data analysis was done using SPSS and p-value<0.05 was taken as the level of significance

The mean age of the participants was 22.23 ± 3.3 SD years(mean age of males 23 ± 3.3 and females 20 ± 2.8) The mean HbA2 among the participants was 3.49 ± 0.9 SD (Range 0.8-5.30%). The mean HbF was 1.43 ± 0.57 SD (Range 0.80-2.50%). Three (3/100) of the subjects had both elevated HbA2 > 3.9% and HbF >1%, giving a prevalence of 3% for β thalassaemia carriers among blood donors in this study.

The prevalence of β thalassaemia carrier was 3% in our study and found among female folks. Data from this study will be useful in raising awareness and genetic counseling especially among female blood donors.

Key words: Prevalence, Beta-thalassaemia, Capillary Electrophoresis.

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Introduction

Quantitative abnormalities affecting the polypeptide chain of the globin are inherited genetically. These haemoglobin abnormalities are due to a reduction in globin chain synthesis and may affect one or more globin chains as seen in thalassaemias. Among the most commonly inherited genetic disorders worldwide is haemoglobin disorder which accounts for about 7% of the population of the world (approximately 269 million people) said to be carriers.^{1,2}

Beta-thalassemia is most common in Mediterranean countries, the central part of Asia and the Mid- Eastern countries. Other countries/region with a high prevalence of β thalassaemia includes; India, Southern Far East, and China. However, it is not uncommon for β thalassaemia to occur sporadically.³

About 270 million people all over the world are carriers of abnormal haemoglobin. About 80million people worldwide are β thalassaemia carriers with a prevalence rate of 1.5%.³ An earlier study done in Nigeria reported the prevalence rate of β thalassaemia carrier to be 0.8%.⁴ However, a high prevalence rate of 25% was reported for β thalassaemia carrier by another study in south-western Nigeria.⁵ The variations in the prevalence rate of β thalassaemia carrier in Africa and Nigeria appears largely to be affected by the method used in the analysis of Hb A2 as well as Hb F and also the defining criteria used in the diagnosis of β thalassaemia carrier. Most of the previous studies focused on the use of densitometry scanning after cellulose acetate electrophoresis for Hb A2 quantification in which its precision and accuracy have been reported to be unsatisfactory.¹ The use of Isoelectric focusing (IEF) was said to be accurate and has excellent resolution, but it is less effective and tedious.⁶

In order to improve the precision and accuracy of Hb A2 quantification for the detection of β -thalassaemia carriers and for diagnosis of other haemoglobinopathies, capillary electrophoresis (CE) is currently being used in the analysis of haemoglobin in most clinical laboratories. It is important to note that, for the qualitative as well as numerical analysis of haemoglobin, the superlative similarity between the use of capillary electrophoresis and usage of high-performance cation-exchange chromatography

(HPLC) has been established and reported in several previous studies.^{7,8,9} Blood donors represent a significant category of the population that is easily accessible for β -thalassaemia carrier screening as this will help create awareness of the haemoglobinopathies. Countries with a high prevalence of β -thalassaemia have also adopted different strategies aimed at preventing the disease which include premarital screening programmes as well as advocacy for donor screening. Studies on β -thalassaemia in Nigeria are few in literature. The high variation in the prevalence of β -thalassaemia in Nigeria as reported in previous studies raises concern. This study screened for β -thalassaemia carrier among blood donors using the capillary electrophoresis method.

Materials and Methods

This was a descriptive cross-sectional study conducted among students of Kwara State Polytechnic, Ilorin. Permission from the management of the institution was obtained and ethical approval from the Ethics and Research Committee of the University of Ilorin was also obtained before the study was commenced. Written informed consent was also obtained from each participant after counselling them on the importance of voluntary blood donation and the study. Participation was voluntary. One hundred (100) prospective blood donors who met the donor selection criteria were recruited. They were male and female students of Kwara State Polytechnic, Ilorin who were aged between 18 and 34 yrs. Those that did not give consent were excluded from the study. This study determined the quantification of haemoglobin A2 and F levels among voluntary blood donors in the Institution. Materials and instrument used included tri-potassium ethylene diamine tetra-acetic acid (K3EDTA) tubes, the H6500CAP analyzer, the kit containing Use stock reagents, Reagents cups, filters, haemolysing reagent, B2 buffer solution, wash solution, distilled water, A2 control, Hb AFSC control, capiclean, centrifuge

Blood donors were recruited through the convenient sampling method which is a non-probability sampling method where participants are enrolled based on their willingness to donate blood and their fitness. Four milliliters (4 mls) of blood was collected by venipuncture from the ante-cubital vein after cleaning the site of venipuncture with methylated spirits. The blood sample collected was put into labelled K3EDTA tubes. Samples collected from donors were kept at 2–8 °C. Analysis of the samples was done in the laboratory within 24 hours of blood collection.

4mls of Donor blood taken in EDTA bottle was put in a barcode labeled plastic tube. This was then spun for 7mins at the 5000 rates per minute. To prevent pre-analytical error, maximum volume of plasma was removed. It was then vortexed for 5 seconds. Two

hundred microliters of packed red cells were subsequently introduced into a nesting cup. The H6500CAP open analyser was started as stated in the company's manual. A 10ml tube filled with haemolysing reagent was placed in position.¹⁰ The door was closed and the button OK was clicked. A minimum of 200 μ l of the control sample was placed in a nesting cup and inserted into the labeled haemoglobin A2 control tube. It was then placed in position numbered.¹¹ The door was closed and ok was clicked. An indicator appears to input the lot # appears and analysis was done by selecting 1 run. when the control has proceeded, the assembled samples were put in the rotating wheel (carousel) from locations identified as 1-26 and close the door. In this method, the normal haemoglobin was separated by electrophoretic mobility of the charged molecules into A, F and A2 via electrophoresis in an alkaline buffer at pH 9.4

Capillary electrophoresis using the minicap system was used to analyse the normal haemoglobin. The normal Hb were separated into A, F and A2. The silica capillaries of the minicap system function effectively in parallel enabling two concurrent analysis of quantifying Hb. After the separation of Hb in silica made capillaries, direct and specific detection of the separated Hb was done at 415nm absorbance wavelength. The analysis was commenced after clicking ok. The instrument was now left to run the samples. Analysis of the results was done as stated in the company's manual

Data analysis

Relevant information was collected using structured questionnaires and data from the study was analyzed using SPSS version 20. The means and standard deviation of the age, and each of the haemoglobin A2 and F were established using the independent t-test while the comparison of proportion was done using Chi square. The test of significance was put at values of $p < 0.05$.

Results

In our study of one hundred (100) donors, the proportions of male participants were 55(55%) and females were 45 (45 %). Fig 1.0 The mean age of the participants was 22.23 ± 3.3 SD years (mean age of males 23 ± 3.3 and females 20 ± 2.8). 60 (60%) of the participants were aged 20–25. (Fig 2.0). The mean HbA2 among the participants was 3.49 ± 0.9 SD and the range was between 0.8-5.30%. The mean HbF was 1.43 ± 0.57 SD with a range between 0.80-2.50%. 29 (29%) of the blood donors had both Hb A2 $\geq 4\%$ and Hb F $< 1\%$. Three (3/100) of the female participants had both elevated HbA2 $> 3.9\%$ and HbF $> 1\%$. (Table 1.0).

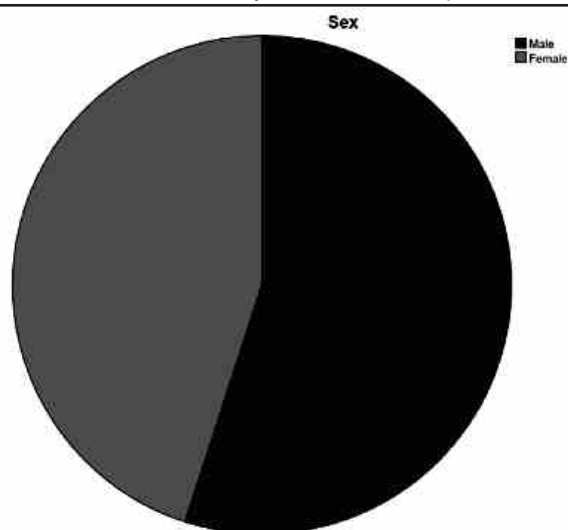


Fig 1.0: Pie chart showing the gender distribution of the blood donors

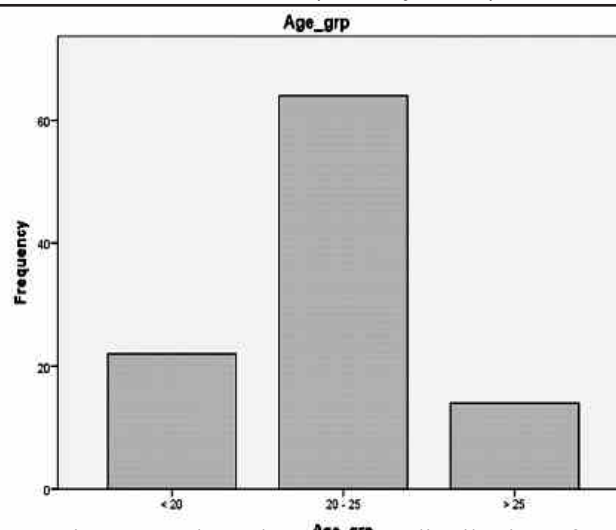


Fig2.0: Bar chart showing Age distribution of blood donors

Table:1.0 Distribution of Hb A2 and Hb F among Blood donors.

VARIABLES	PROPORTION	PERCENTAGES
Hb A2<4% + HbF<1%	56	56%
Hb A2 <4% + Hb F>1%	10	10%
Hb A2 =4% + Hb F<1%	29	29%
Hb A2 =4% + Hb F>1%	3	3%

Hb A2: HaemoglobinA2, Hb F: Haemoglobin F

Discussion

We found the prevalence of β thalassaemia carrier among voluntary blood donors to be 3%. This prevalence rate in our study is higher than 0.8% reported by Esan et al. (4) The difference could be because Hb A2 quantification was done on all our samples while haemolysates with raised levels of HbA2 were considered on visual assessment in the report of the study done by Esan et al. (4) Report from a study from Liberia shows a higher value of 9% but only elevated Hb A2 above 3.8% was used as criteria for β thalassaemia carrier identification whereas Hb A2 >3.9% coexisting with Hb F > 1% was used as criteria for identifying β thalassaemia carriers in our study.^{12,13}

The use of the capillary electrophoresis method, when compared to well-accepted chromatographic methods, has been shown to be accurate, time-saving and easy to use.⁷ Various methods have been used for the determination of HbA2, ranging from the old capillary isoelectric focusing (which is less suitable for routine use), automated HPLC anion-exchange chromatography in microcolumns.^{7,8} The newer capillary electrophoresis method used in our study for quantifying levels of Hb A2 and Hb F has been reported to compare well with anion-exchange HPLC

and anion-exchange chromatography in microcolumns.⁷

Report of previous work done by Pandya and Kumar et al with the use of HPLC in analysing samples from donors in India revealed the prevalence of β thalassaemia trait to be 2.0% and 3.3% respectively. In our study all the β thalassaemia traits were found among females even though males and females are said to have similar rate of disease. Although there are differences in the geographical distribution of the β thalassaemia globally, the report on the prevalence of β thalassaemia in our study appears to be similar to the report of Pandya et al and Kumar et al.^{14,15}

Conclusion

The prevalence of 3% was recorded among female voluntary donors in this study. Findings in screening tests for β thalassaemia carriers in blood donors can be used for raising awareness and genetic counseling since most of the voluntary blood donor falls within the premarital age group. Thus, the period of blood donation can help prospective blood donors to know their carrier status early and can help them make informed future decisions. Present premarital screening programmes in Nigeria focus mainly on

haemoglobin phenotype determination which may be inadequate. β -thalassaemia screening with newer techniques may be valuable in the detection of β -thalassaemia carriers.

Acknowledgment: The research was supported by TETFUND National Research Fund

References

1. World Health Organisation 2008, "Management of haemoglobin disorders," in Proceedings of the Report of Joint WHO-TIF Meeting, Nicosia, Cyprus, November 2007.
2. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. *Bulletin of the World Health Organization*, 2001, 79:704–712.
3. De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, M. β -Thalassemia Distribution in the Old World: an Ancient Disease Seen from a Historical Standpoint. *Mediterr J Hematol Infect Dis*. 2017; 9(1): e2017018
4. Esan GJF. The thalassaemia syndromes in Nigeria. *British Journal of Haematology*. 1970; 19: 47-56.
5. Kotila TR, Adeyemo AA, Mewoyeka OO, Wuraola A, Shokunbi WA. Beta thalassaemia trait in western Nigeria. *Afr Health Sci*. 2009;9(1):46–48.
6. Lafferty J. College of America Pathologists haemoglobinopathy survey HG-B. Chicago, IL; College of American Pathologists; 1999.
7. Cotton F, Lin C, Fontaine B, Gulbis B, Janssens J, Vertongen F. Evaluation of a Capillary Electrophoresis Method for Routine Determination of Hemoglobins A2 and F. *Clinical Chemistry*. 1999; 45:2 237–243.
8. Mario N, Baudin B, Aussel C, Giboudeau J. Capillary isoelectric focusing and high-performance cation-exchange chromatography compared for qualitative and quantitative analysis of hemoglobin variants. *Clin Chem*. 1997;43:2137–42.
9. Delft van P, Lenters E, Bakker-Verweij M, et al. Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in Multi-ethnic populations. *International Journal of Laboratory Hematology*. 2009;31(5):484–495
10. Zaccacheus AJ. Abnormal haemoglobin variants, ABO and Rh blood groups among students of African descent in Port Harcourt, Nigeria. *J Afr Health Sci*. 2006;6(3):177–81.
11. World Health Organisation. Sick Cell Anaemia Report by the Secretariat. Fifty ninth world Health Assembly. 2006.
12. British Committee for Standardization in Haematology. Guidelines for investigation of the α and β thalassaemia trait. *Journal of Clinical Pathology*. 1994; 47: 289-295.
13. Willcox M.C. Thalassaemia in northern Liberia: A survey in the Mount Nimba area. *Journal of Medical Genetics*. 1975; 12:55-63.
14. Pandya M, Parmar C, Chiplonkar S, Singh M. Pathology Screening of hemoglobinopathies in blood donors (A study of 800 cases). *Int J of Biomed and Adv Res*. 2018; 9(10): 345-352.
15. Kumar R, Gupta S, Jindal A, Kakkar S, Kaur A. Screening of β -thalassaemia trait and other hemoglobinopathies among blood donors in Punjab. *Int J Med Public Health*. 2015; 5:106-9.